

# A comparison of the efficiency of DNA barcoding regions in a small and a large genus

by

Paula Spies

Thesis presented in order to qualify for the degree  
*Philosophiae Doctor* in the Faculty of Natural and  
Agricultural Sciences (Department of Genetics) at the  
University of the Free State

December 2013

Promoter:  
Co-promoter:

Professor J.J. Spies  
Professor J.P. Grobler

A thesis presented on the efficiency of barcoding regions in the small genus *Clivia* (family Amaryllidaceae) compared to a large genus *Lachenalia* (family Asparagaceae).

## TABLE OF CONTENTS

|                            |      |
|----------------------------|------|
| List of Abbreviations..... | v    |
| List of Figures.....       | viii |
| List of Tables.....        | x    |
| Acknowledgements.....      | xii  |

### PART A: Introduction and Methods

|                                                                                     |           |
|-------------------------------------------------------------------------------------|-----------|
| <b>Chapter 1: General introduction .....</b>                                        | <b>1</b>  |
| 1.1 Introduction to the genus <i>Clivia</i> Lindl.....                              | 2         |
| 1.1.1 Statement of the taxonomic problem in <i>Clivia</i> and its cultivars.....    | 2         |
| 1.2 Introduction to the genus <i>Lachenalia</i> .....                               | 3         |
| 1.2.1 Statement of the taxonomic problem in <i>Lachenalia</i> .....                 | 4         |
| 1.3 Comparison between <i>Clivia</i> and <i>Lachenalia</i> .....                    | 5         |
| 1.4 Plant DNA-barcoding.....                                                        | 6         |
| 1.4.1 History of DNA-barcoding .....                                                | 9         |
| 1.4.2 Challenge of recently diverged organisms and DNA-barcoding in<br>general..... | 12        |
| 1.4.3 Plastid and nuclear genomes .....                                             | 13        |
| 1.4.4 Evaluation of some of the core chloroplast coding regions .....               | 13        |
| 1.4.5 Evaluating some chloroplast non-coding regions .....                          | 15        |
| 1.4.6 The nuclear <i>ITS</i> region .....                                           | 18        |
| 1.4.7 Why implement DNA-barcoding?.....                                             | 19        |
| 1.5 Aims and objectives .....                                                       | 21        |
| 1.6 Structure of the thesis.....                                                    | 21        |
| <b>Chapter 2: Barcoding Techniques .....</b>                                        | <b>23</b> |
| 2.1 DNA-barcoding data analyses.....                                                | 24        |
| 2.1.1 Similarity methods/pairwise distance methods.....                             | 24        |
| 2.1.2 Tree-based methods .....                                                      | 28        |
| 2.1.3 Character-based and diagnostic methods.....                                   | 29        |
| 2.2 Reference databases.....                                                        | 30        |
| 2.2.1 GenBank.....                                                                  | 31        |
| 2.2.2 The Barcode of Life Data System (BOLD).....                                   | 32        |
| 2.3 DNA-barcode data standards .....                                                | 34        |
| 2.4 Methods used during this study .....                                            | 35        |
| 2.4.1 DNA extraction .....                                                          | 36        |
| 2.4.2 PCR .....                                                                     | 37        |
| 2.4.3 Qualification and quantification of DNA and PCR products .....                | 38        |
| 2.4.4 Sequencing.....                                                               | 39        |
| 2.4.5 2C measurements.....                                                          | 40        |
| 2.4.6 Data analyses .....                                                           | 41        |
| 2.4.6.1 Editing and alignment of sequences .....                                    | 41        |
| 2.4.6.2 Tree-based analysis .....                                                   | 41        |
| 2.4.6.3 Character-based barcoding analysis .....                                    | 42        |

|                                                           |    |
|-----------------------------------------------------------|----|
| 2.4.6.4 Evolutionary distances and the barcoding gap..... | 43 |
| 2.4.6.5 Data analyses .....                               | 43 |
| 2.4.6.6 Phylogeography and specimen mapping.....          | 44 |
| 2.4.6.7 SpeciesIdentifier analyses .....                  | 44 |
| 2.4.6.8 Networks .....                                    | 45 |

## PART B: *Clivia*

### Chapter 3: A review of phylogenetic relationships in the genus *Clivia* ..... 46

|                                                                              |    |
|------------------------------------------------------------------------------|----|
| Abstract .....                                                               | 47 |
| Preface .....                                                                | 47 |
| 3.1 Introduction.....                                                        | 48 |
| 3.2 Cytogenetic studies .....                                                | 49 |
| 3.2.1 Chomosome numbers .....                                                | 49 |
| 3.2.2 Karyotype analysis .....                                               | 50 |
| 3.3 Phylogenetic studies .....                                               | 52 |
| 3.3.1 Position of <i>Clivia</i> in Amaryllidaceae.....                       | 52 |
| 3.3.2 Chromosome and genome evolution in the tribe Haemantheae .....         | 54 |
| 3.3.3 Phylogeography.....                                                    | 58 |
| 3.3.4 Phylogenetic relationships within the genus <i>Clivia</i> .....        | 61 |
| 3.3.5 Phylogeny vs DNA content (2C) of <i>Clivia</i> .....                   | 63 |
| 3.3.6 The implications of the 2C value of <i>Clivia</i> .....                | 65 |
| 3.4 Survival threats.....                                                    | 67 |
| 3.5 Biochemical compositions and medicinal properties of <i>Clivia</i> ..... | 68 |
| 3.6 Pollen and pollination .....                                             | 73 |
| 3.7 Conclusion .....                                                         | 75 |
| 3.8 Statement of research questions .....                                    | 76 |

### Chapter 4: DNA-barcoding in the genus *Clivia* ..... 78

|                                                                                |     |
|--------------------------------------------------------------------------------|-----|
| Abstract .....                                                                 | 79  |
| Preface .....                                                                  | 79  |
| 4.1 Introduction.....                                                          | 80  |
| 4.1.1 The need for DNA-barcoding in <i>Clivia</i> .....                        | 80  |
| 4.1.2 Aims and objectives.....                                                 | 81  |
| 4.2 Materials and Methods.....                                                 | 82  |
| 4.2.1 Collection and Material .....                                            | 82  |
| 4.2.2 Methods.....                                                             | 85  |
| 4.3 Results and Discussion .....                                               | 86  |
| 4.3.1 Extraction, amplification and sequencing .....                           | 86  |
| 4.3.2 Pre-screening for candidate DNA-barcoding regions in <i>Clivia</i> ..... | 88  |
| 4.3.3 Assessing the analysis methods for selecting a barcode .....             | 90  |
| 4.3.3.1 Tree-based analysis .....                                              | 90  |
| 4.3.3.2 Distance-based analysis.....                                           | 98  |
| 4.3.3.3 Character-based analysis.....                                          | 101 |
| 4.3.3.4 Comparing the tree, distance and character based<br>analyses.....      | 104 |
| 4.3.4 Combining datasets.....                                                  | 105 |

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| 4.3.5 Additional applications of barcoding in <i>Clivia</i> ..... | 110 |
| 4.3.6 Recommendations for future studies .....                    | 110 |
| 4.4 Conclusions .....                                             | 114 |

## **PART C: *Lachenalia***

### **Chapter 5: Cytogenetic and phylogenetic review of the genus *Lachenalia* 117**

|                                                                                |     |
|--------------------------------------------------------------------------------|-----|
| Abstract .....                                                                 | 118 |
| Preface .....                                                                  | 118 |
| 5.1 Introduction .....                                                         | 119 |
| 5.2 Cytogenetic studies .....                                                  | 123 |
| 5.2.1 Chromosome counts .....                                                  | 123 |
| 5.2.2 Chromosome morphology .....                                              | 128 |
| 5.2.3 Basic chromosome numbers and polyploidy .....                            | 129 |
| 5.2.4 Meiotic studies .....                                                    | 132 |
| 5.3 Phylogenetic studies .....                                                 | 133 |
| 5.3.1 The phylogenetic position of <i>Lachenalia</i> .....                     | 133 |
| 5.3.2 Phylogeny within the genus .....                                         | 134 |
| 5.4 Cross-ability in <i>Lachenalia</i> .....                                   | 135 |
| 5.5 Comparison between cross-ability, cytogenetic and molecular data ....      | 136 |
| 5.5.1 Basic chromosome numbers and cladograms .....                            | 137 |
| 5.5.2 Basic chromosome numbers and cross-ability .....                         | 139 |
| 5.5.3 Evolution and relatedness of different basic chromosome<br>numbers ..... | 140 |
| 5.5.4 Existence of different basic chromosome numbers .....                    | 147 |
| 5.5.5 Existence of hybrid species .....                                        | 148 |
| 5.6 Conclusion .....                                                           | 149 |
| 5.7 Statement of research questions .....                                      | 151 |

### **Chapter 6: A comparison of the efficiency of DNA-barcoding regions in *Lachenalia* ..... 152**

|                                                                        |     |
|------------------------------------------------------------------------|-----|
| Abstract .....                                                         | 153 |
| Preface .....                                                          | 153 |
| 6.1 Introduction .....                                                 | 153 |
| 6.1.1 The need for DNA-barcoding in <i>Lachenalia</i> .....            | 153 |
| 6.1.2 Aims and objectives .....                                        | 157 |
| 6.2 Materials and Methods .....                                        | 157 |
| 6.2.1 Materials .....                                                  | 157 |
| 6.2.2 Methods .....                                                    | 158 |
| 6.3 Results and Discussion .....                                       | 164 |
| 6.3.1 Sample identification .....                                      | 164 |
| 6.3.2 Amplification and sequencing .....                               | 164 |
| 6.3.3 Assessing the analysis methods for selecting a barcode .....     | 165 |
| 6.3.3.1 Tree-based analysis .....                                      | 165 |
| 6.3.3.2 Distance-based analysis .....                                  | 171 |
| 6.3.3.3 Character-based analysis .....                                 | 174 |
| 6.3.3.4 Comparing the tree, distance and character based analyses .... | 177 |

|                                                                                                                                                             |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 6.3.4 DNA-barcoding analyses of concatenated data of focus species.....                                                                                     | 178 |
| 6.3.5 The effect of wrong identification, hybridization and samples<br>from a broad geographical range on the interpretation of<br>barcoding analyses ..... | 180 |
| 6.3.6 Future studies on <i>Lachenalia</i> and recommendations.....                                                                                          | 185 |
| 6.4 Conclusions.....                                                                                                                                        | 186 |

## **PART D: DNA barcode comparison**

### **Chapter 7: Comparison between *Clivia* (small genus) and**

#### ***Lachenalia* (large genus) ..... 188**

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| Abstract .....                                                    | 189 |
| Preface .....                                                     | 189 |
| 7.1 Introduction.....                                             | 189 |
| 7.2 Comparing gene regions .....                                  | 190 |
| 7.3 Evaluating the core barcodes in a small and large genus ..... | 190 |
| 7.4 Most effective regions for the small and large genus .....    | 191 |
| 7.5 Conclusion .....                                              | 194 |

|                      |            |
|----------------------|------------|
| <b>Summary .....</b> | <b>196</b> |
|----------------------|------------|

|                       |            |
|-----------------------|------------|
| <b>Opsomming.....</b> | <b>199</b> |
|-----------------------|------------|

|                         |            |
|-------------------------|------------|
| <b>References .....</b> | <b>202</b> |
|-------------------------|------------|

### **Appendices (on CD at back of thesis)..... 235**

|                                                                                              |     |
|----------------------------------------------------------------------------------------------|-----|
| <b>Appendix A:</b> Distribution of the sequences deposited in public<br>databases.....       | 236 |
| <b>Appendix B:</b> Aligned <i>Clivia</i> sequences.....                                      | 244 |
| <b>Appendix C:</b> <i>Clivia</i> cladograms .....                                            | 332 |
| <b>Appendix D:</b> Cut-off values with the defined <i>Clivia</i> MOTUs generated .           | 342 |
| <b>Appendix E:</b> Mean interspecific and intraspecific distances .....                      | 358 |
| <b>Appendix F:</b> Aligned <i>Lachenalia</i> sequences .....                                 | 360 |
| <b>Appendix G:</b> Cladograms for different gene regions in <i>Lachenalia</i> .....          | 424 |
| <b>Appendix H:</b> Cut-off values with the defined <i>Lachenalia</i> MOTUs<br>generated..... | 434 |

## List of abbreviations

---

|             |                                                       |
|-------------|-------------------------------------------------------|
| 2n          | Somatic chromosome number                             |
| 5S rDNA     | 15S ribosomal DNA                                     |
| 18S rDNA    | 18S ribosomal DNA                                     |
| μl          | Micro Litre                                           |
| ABI         | Applied Biosystems                                    |
| aff.        | Affinis (related)                                     |
| AFLP        | Amplified Fragment Length Polymorphism                |
| APE         | Analysis of Phylogenetics and Evolution               |
| APG         | Angiosperm Phylogeny Group                            |
| ARC         | Agricultural Research Council                         |
| <i>atpB</i> | ATPase beta chain                                     |
| ABGD        | Automatic Barcode Gap Discovery                       |
| <i>B</i>    | Barcode quality index                                 |
| BDP         | Barcode of Life Data Portal                           |
| BI          | Bayesian Inference                                    |
| BLAST       | Basic Local Alignment Search Tool                     |
| BLOG        | Barcoding with LOGic formulas                         |
| BOLD        | The Barcode of Life Data System                       |
| bp          | Base Pair                                             |
| BRONX       | Barcode Recognition Obtained with Nucleotide eXpose's |
| CI          | Consistency Index                                     |
| CAOS        | Characteristic Attribute Organization System          |
| CBC         | Compensatory Base Change                              |
| CBOL        | Consortium for the Barcode of Life                    |
| CCDB        | Canadian Centre for DNA Barcoding                     |
| CNI         | Close-Neighbor-Interchange                            |
| cpDNA       | Chloroplast DNA                                       |
| csv         | Comma Seperated Values                                |
| CTAB        | Cetyltrimethylammonium Bromide                        |
| DAPI        | 4',6-diamidino-2-phenylindole                         |
| dATP        | Deoxyadenosine Triphosphate                           |
| DBWG        | Database Working Group                                |
| dCTP        | Deoxycytidine Triphosphate                            |
| dGTP        | Deoxyguanosine Triphosphate                           |
| DDBJ        | DNA Data Bank of Japan                                |
| DIECA       | Diethyldithiocarbamic Acid                            |
| DMSO        | Dimethyl Sulfoxide                                    |
| DNA         | Deoxyribonucleic Acid                                 |
| dNTP        | Deoxynucleotide Triphosphate                          |
| dTTP        | Deoxythymidine Triphosphate                           |
| EDTA        | Ethylene Diamintetra Acetic Acid                      |
| ENA         | European Nucleotide Archive                           |
| EtOH        | Ethyl-alcohol (ethanol)                               |
| EMBL        | European Molecular Biology Laboratory                 |
| FISH        | Fluorescent <i>in situ</i> Hybridization              |

|                                              |                                                                            |
|----------------------------------------------|----------------------------------------------------------------------------|
| GISH                                         | Genomic <i>in situ</i> Hybridisation                                       |
| HCl                                          | Hydrochloric acid                                                          |
| HKY85                                        | Hasegawa-Kishino-Yano                                                      |
| HMM                                          | Hidden Markov Model                                                        |
| IDS                                          | Identification System                                                      |
| INDEL(S)                                     | Insertion(s)/Deletion(s)                                                   |
| INSDC                                        | International Nucleotide Sequence Database Collaboration                   |
| <i>ITS1</i>                                  | Internal Transcribed Spacer 1                                              |
| <i>ITS2</i>                                  | Internal Transcribed Spacer 2                                              |
| <i>ITS1-2</i>                                | Internal Transcribed Spacer 1, 5.8S rRNA and Internal Transcribed Spacer 2 |
| <i>g</i>                                     | Gravitational force                                                        |
| IGS                                          | Inter Genic Spacer                                                         |
| jMOTU                                        | Java program to identify Molecular Operational Taxonomic Units             |
| K <sub>2</sub> S <sub>2</sub> O <sub>5</sub> | Potassium Bisulfide                                                        |
| MAS                                          | Management and Analysis System                                             |
| MCMC                                         | Markov chain Monte Carlo                                                   |
| MEGA                                         | Molecular Evolutionary Genetics Analysis                                   |
| mg/ml                                        | Miligram per Millilitre                                                    |
| mM                                           | Milimolar                                                                  |
| ML                                           | Maximum Likelihood                                                         |
| MOTUs                                        | Molecular Operational Taxonomic Units                                      |
| MP                                           | Maximum Parsimony                                                          |
| m/v                                          | Mass per Volume                                                            |
| N                                            | Normal                                                                     |
| <i>n</i>                                     | Gametic chromosome number                                                  |
| NaCl                                         | Sodium chloride                                                            |
| NBI                                          | National Botanical Institute                                               |
| NCBI                                         | National Center for Biotechnology Information                              |
| ng                                           | Nanogram                                                                   |
| NH <sub>4</sub> OH                           | Ammonia acetate                                                            |
| NJ                                           | Neighbor-Joining                                                           |
| NLM                                          | National Library of Medicine                                               |
| NNI                                          | Nearest-Neighbor-Interchange                                               |
| nrDNA                                        | Nuclear DNA                                                                |
| OD                                           | Optical Density                                                            |
| <i>P</i>                                     | Approximate maximum prior intraspecific distance                           |
| PAUP                                         | Phylogenetic Analysis Using Parsimony                                      |
| PCR                                          | Polymerase Chain Reaction                                                  |
| pmol/μl                                      | Picomole per Microlitre                                                    |
| PVP                                          | Polyvinylpyrrolidone                                                       |
| PWG                                          | Plant Working Group                                                        |
| RAPD                                         | Random Amplified Polymorphic DNA                                           |
| RAxML                                        | Randomized Axelerated Maximum Likelihood                                   |
| SPIDER                                       | SPeCies IDentity and Evolution in R                                        |
| <i>rbcL</i>                                  | Ribulose bisophosphate carboxylase (large)                                 |
| RC                                           | Rescaled Consistency Index                                                 |
| rDNA                                         | Ribosomal DNA                                                              |

|                |                                                     |
|----------------|-----------------------------------------------------|
| rRNA           | Ribosomal RNA                                       |
| RI             | Retention Index                                     |
| RNA            | Ribonucleic Acid                                    |
| RNase          | Ribonuclease A                                      |
| SANBI          | South African National Biodiversity Institute       |
| SAP            | Statistical Assignment Package                      |
| SDS            | Sodium Dodecyl Sulphate                             |
| SNL            | Signal to Noise                                     |
| SNP            | Single Nucleotide Polymorphism                      |
| TAE            | Tris; Acetic Acid; EDTA                             |
| Taq. Pol.      | <i>Thermus aquaticus</i> Super Therm DNA Polymerase |
| TBR            | Tree-Bisection-Reconnection                         |
| T <sub>m</sub> | Melting temperature                                 |
| TRIS           | 2-amino-2-(hydroxymethyl )-1,3-propanediol          |
| <i>trnL</i>    | Transfer RNA gene for Leucine                       |
| <i>trnF</i>    | Transfer RNA gene for Phenylalanine                 |
| UPGMA          | Unweighted Pair Group Method with Arithmetic Mean   |
| UV             | Ultra Violet                                        |
| V              | Volts                                               |
| VOPI           | Vegetable and Ornamental Plant Institute            |
| v/v            | Volume per Volume                                   |
| x              | Basic chromosome number                             |



## List of Figures

---

|                                                                                                                        |     |
|------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Figure 1.1</b> The <i>matK</i> chloroplast coding region .....                                                      | 14  |
| <b>Figure 1.2</b> The chloroplast coding region <i>rbcl</i> .....                                                      | 15  |
| <b>Figure 1.3</b> The <i>trnT-L-F</i> cistron .....                                                                    | 17  |
| <b>Figure 1.4</b> The internal transcribed spacer regions.....                                                         | 19  |
| <br>                                                                                                                   |     |
| <b>Figure 2.1</b> An illustration of the barcoding “gap” .....                                                         | 27  |
| <b>Figure 2.2</b> Sequences for <i>matK</i> and <i>rbcl</i> are deposited in various publicly available databases..... | 34  |
| <br>                                                                                                                   |     |
| <b>Figure 3.1</b> The most parsimonious cladogram constructed from chromosomal banding patterns .....                  | 52  |
| <b>Figure 3.2</b> Molecular Phylogenetic analysis based on ITS sequences .....                                         | 56  |
| <b>Figure 3.3</b> Negative correlation between genome size and basic chromosome number .....                           | 57  |
| <b>Figure 3.4</b> Correlations within and between <i>Clivia</i> and <i>Cryptostephanus</i> .....                       | 66  |
| <b>Figure 3.5</b> Correlation between geographical distribution and genome sizes .....                                 | 67  |
| <br>                                                                                                                   |     |
| <b>Figure 4.1</b> The geographical distribution of the <i>Clivia</i> and <i>Gethyllis</i> samples in this study .....  | 86  |
| <b>Figure 4.2</b> Bayesian Inference phylograms.....                                                                   | 92  |
| <b>Figure 4.3</b> The cut-off distribution graphs.....                                                                 | 99  |
| <b>Figure 4.4</b> BI phylogram of the two-loci barcode.....                                                            | 107 |
| <b>Figure 4.5</b> The barcoding gap of the combined dataset .....                                                      | 109 |
| <b>Figure 4.6</b> Heatmap drawn for the combined datasets .....                                                        | 111 |
| <b>Figure 4.7</b> The virtual enzyme digestion of certain gene regions.....                                            | 112 |
| <br>                                                                                                                   |     |
| <b>Figure 5.1</b> Morphological variation in <i>Lachenalia</i> in the greenhouse .....                                 | 120 |
| <b>Figure 5.2</b> Morphological variation in different <i>Lachenalia</i> species.....                                  | 121 |
| <b>Figure 5.3</b> Different <i>Lachenalia</i> cultivars developed at ARC - Roodeplaat VOPI.....                        | 122 |
| <b>Figure 5.4</b> Histogram of the number of taxa per basic chromosome number in the genus <i>Lachenalia</i> .....     | 131 |
| <b>Figure 5.5</b> Evolutionary relationships based on the <i>ITS1-2</i> region .....                                   | 142 |
| <b>Figure 5.6</b> Evolutionary relationships based on the <i>trnL-F</i> region.....                                    | 144 |
| <b>Figure 5.7</b> Network of <i>Lachenalia</i> species based on <i>ITS</i> data .....                                  | 145 |
| <b>Figure 5.8</b> Network of <i>Lachenalia</i> species based on <i>trnL-F</i> data .....                               | 146 |
| <br>                                                                                                                   |     |
| <b>Figure 6.1</b> Bayesian Inference (BI) phylograms .....                                                             | 167 |
| <b>Figure 6.2</b> The Bayesian Inference cladogram from sequences from BOLD .....                                      | 169 |
| <b>Figure 6.3</b> The cut-off distribution for each gene region .....                                                  | 173 |
| <b>Figure 6.4</b> The geographical distribution of the <i>L. bifolia</i> samples.....                                  | 176 |
| <b>Figure 6.5</b> The distribution range of the focus species in the barcoding study of <i>Lachenalia</i> .....        | 179 |

|                                                                                                                       |     |
|-----------------------------------------------------------------------------------------------------------------------|-----|
| <b>Figure 6.6</b> The Bayesian Inference cladogram from sequences of the study of Hamatani <i>et al.</i> (2008) ..... | 183 |
| <b>Figure 6.7</b> Examples of the phenotypes represented in the cladogram .....                                       | 183 |
| <b>Figure 6.8</b> Flowers of some specimens of <i>L. unifolia</i> and <i>L. schlechterii</i> .....                    | 184 |
| <b>Figure 6.9</b> Flowers of <i>L. mediana</i> and the unknown sister species .....                                   | 184 |
| <br>                                                                                                                  |     |
| <b>Figure 7.1</b> Unrooted <i>Clivia</i> tree based on <i>matK</i> and <i>rbclA</i> .....                             | 192 |
| <b>Figure 7.2</b> Unrooted <i>Lachenalia</i> tree based on <i>matK</i> and <i>rbclA</i> .....                         | 193 |

## List of Tables

---

|                                                                                                                           |     |
|---------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Table 1.1:</b> A comparison between <i>Clivia</i> and <i>Lachenalia</i> .....                                          | 5   |
| <b>Table 1.2</b> A list of some of the barcoding regions.....                                                             | 11  |
| <b>Table 1.3</b> Comparison between the variability in some DNA regions .....                                             | 16  |
| <br>                                                                                                                      |     |
| <b>Table 2.1</b> The main DNA-barcoding tools available for analyses.....                                                 | 25  |
| <b>Table 2.2</b> A comparison between the published records found for the three families.....                             | 35  |
| <b>Table 2.3</b> Primers used for PCR in the present study.....                                                           | 37  |
| <b>Table 2.4</b> Recipes for the direct PCR and standard PCR methods. ....                                                | 38  |
| <b>Table 2.5</b> Cycling conditions for the gene regions amplified in this study. ....                                    | 39  |
| <br>                                                                                                                      |     |
| <b>Table 3.1</b> List of the described <i>Clivia</i> species .....                                                        | 49  |
| <b>Table 3.2</b> Summary of the Giemsa C-banding banding patterns.....                                                    | 51  |
| <b>Table 3.3</b> Species from the tribe Haemantheae used for the Maximum likelihood (ML) tree. ....                       | 55  |
| <b>Table 3.4</b> Summary of the DNA content and basic chromosome numbers.....                                             | 58  |
| <b>Table 3.5</b> Alkaloids isolated from <i>Clivia</i> .....                                                              | 70  |
| <b>Table 3.6</b> Summary of species that contain alkaloids .....                                                          | 73  |
| <br>                                                                                                                      |     |
| <b>Table 4.1</b> Availability of different barcoding markers of the genera <i>Clivia</i> and <i>Cryptostephanus</i> ..... | 82  |
| <b>Table 4.2</b> Samples used in this study.....                                                                          | 84  |
| <b>Table 4.3</b> Summary of PCR and sequence amplification success per DNA barcoding locus in <i>Clivia</i> .....         | 87  |
| <b>Table 4.4</b> A comparison between the sequencing successes vs. variable sites for the gene regions .....              | 89  |
| <b>Table 4.5</b> A summary of all five cladograms drawn for each gene regions.....                                        | 91  |
| <b>Table 4.6</b> The species delimitation in <i>Clivia</i> .....                                                          | 97  |
| <b>Table 4.7</b> A summary of the data for each gene region generated in jMOTU .....                                      | 100 |
| <b>Table 4.8</b> A summary of the polymorphisms in the sequencing datasets .....                                          | 102 |
| <b>Table 4.9</b> A comparison between the three methods used for DNA-barcoding of <i>Clivia</i> .....                     | 104 |
| <b>Table 4.10</b> A selection of the primers that can be used in future SNP studies to quickly identify species..         | 113 |
| <br>                                                                                                                      |     |
| <b>Table 5.1</b> List of <i>Lachenalia</i> species with the somatic- and gametic chromosome numbers .....                 | 124 |
| <b>Table 5.2</b> Number of inter-species crosses made over a 35 year period .....                                         | 137 |
| <br>                                                                                                                      |     |
| <b>Table 6.1</b> Availability of different barcoding markers of the genera <i>Lachenalia</i> and <i>Polyxena</i> .....    | 157 |
| <b>Table 6.2</b> A list of the <i>Lachenalia</i> samples used in the study .....                                          | 159 |
| <b>Table 6.3</b> Summary of success per DNA barcoding locus in <i>Lachenalia</i> .....                                    | 164 |
| <b>Table 6.4</b> A summary of the cladograms to indicate monophyletic regions.....                                        | 166 |
| <b>Table 6.5</b> The species delimitation in <i>Lachenalia</i> .....                                                      | 171 |

|                                                                                                           |         |
|-----------------------------------------------------------------------------------------------------------|---------|
| <b>Table 6.6</b> A summary of unique polymorphisms for the focus species .....                            | 175     |
| <b>Table 6.7</b> A comparison between the three methods used for DNA barcoding analysis.....              | 178     |
| <b>Table 6.8</b> A comparison of monophyletic species per gene region.....                                | 179     |
| <b>Table 6.9</b> A summary of the output from SequenceMatrix after combining the sequences.....           | 181     |
| <b>Table 6.10</b> The branch support expressed as PP in the Bayesian Inference cladograms .....           | 182     |
| <br><b>Table 7.1</b> Comparison between the PCR and sequencing success of the different gene regions..... | <br>191 |
| <b>Table 7.2</b> Comparing the gene regions of <i>Clivia</i> and <i>Lachenalia</i> .....                  | 192     |

## Acknowledgments

---

This journey would not have been possible without the help and support of the dear people in my life, to only some of whom it is possible to give particular mention here.

Thank you to my promoters, Proff JJ Spies and JP Grobler for your advice and guidance throughout this study. I cannot thank you enough for your support. Despite your hectic schedules, you always made time available to give your valuable inputs. Thank you for the opportunity to take this journey with you and to learn from your expertise.

My co-author of numerous papers, Mrs Riana Kleynhans: This has been a long road for us both. Thank you for sharing this journey with me; for your friendly advice and support during the past decade.

I would also like to thank Mrs Susan Reynecke for her valuable contribution to the chromosome studies. I appreciate the endless hours spent in front of the microscope searching for minute *Lachenalia* chromosomes. Thank you to Ms Hesmari van der Westhuizen for managing the automated sequencer and always being willing to 'run' the sequences. Thank you to my MSc students, Marli, Anrie, Bulelani and Ryno for your patience and support. A particular thank to Anri for setting ground research from which the *Lachenalia* study was built on, and for Marli, a *Clivia* team member who made valuable contributions to the research on *Clivia*.

I would like to thank Dr Ilia Leitch and Dr Jaume Pellicer from Kew Botanical Gardens for the help with the genome size analyses during my visit in 2010.

A special thank you to the following people/institutes for supplying the leaf material used in the study: Mrs Riana Kleynhans (ARC-Roodeplaat) and Mr Graham Duncan (Kirstenbosch Botanical Garden) for providing the *Lachenalia* samples. Mr and Mrs Able, Mr Fred van Niekerk, Mr Sean Chubb, Mr Brian Tarr, Mr Norman Weitz, Mr Francois van Rooyen, Mr Andy Forbes-Hardinge, Mr Mick Dower, Mrs Stella van Gas, Mr John Roderick and Owen, Mr Mias Volgraaff, Mr Kobus van Zyl for providing *Clivia* material. Also a word of thanks to Mr. Jaco Nel and Mr. Hans Joschko for providing additional *Clivia* and *Cryptostephanus* material. A

special thanks to a very kind collector from South Africa for donating *Cryptostephanus* plants for research.

The University of the Free State, the Clusters of the Faculty of Natural and Agricultural Sciences (UFS) and the Clivia society are sincerely thanked for financial assistance during this study. The Department of Genetics (UFS) is thanked for providing the equipment used during the study.

A final word of thank to the Canadian Centre of DNA Barcoding (CCDB) for providing the *matK* and *rbcLa* sequences.

## *Dedications*

---

The journey from the start to the completion of this thesis has been long and many times bumpy. Without the support, encouragement, patients and love of my family, husband, children, friends and colleagues, I may not have reached this final moment of completing this study.

I want to thank all the very dear and special people in my life. Each one of these people has made an impact in my life that contributed to the successful completion of this thesis. Thus, in no specific order, thank you to my dear husband (for his love, support, patience and encouragement), daughter and son (for their patience, love, respect and understanding when they were neglected at times), father (for his hard work and sacrifices to make study and an academic career possible and keeping the curiosity for genetics alive with interesting talks and articles), mother (who's support, love, sacrifices and encouragement made this journey possible), two sisters (for their encouragement, support and advice), grandparents (for teaching me the skills of patients, love, integrity and hard work) and in-laws for their love and support.

I dedicate this thesis to you.

Paula

# Part A: Introduction and Methods

## *Chapter 1*

### General introduction





## 1.1 INTRODUCTION TO THE GENUS *CLIVIA* LINDL.

The genus *Clivia* is considered a small genus, belonging to the family Amaryllidaceae, with only six species and one natural hybrid species being described: *C. caulescens* R.A.Dyer, *C. gardenii* Hook., *C. miniata* (Lindl.) Regel, *C. mirabilis* Rourke, *C. nobilis* Lindl. and *C. robusta* B.G.Murray *et al.*, of which *C. nobilis* is the type specimen of the genus. Five of the six species are distributed in the eastern parts of South Africa and one species, *C. mirabilis*, has a very small distribution area along the western escarpment on the border between the Northern and Western Cape Provinces (Rourke, 2002). Most of the species can be identified with certainty if enough morphological traits are available when identifying these species, i.e. root system morphology, flower and leaf morphology and length of the reproduction cycle. Unfortunately the distribution areas overlap for some species in some parts of the eastern distribution range, which implies that hybrids can easily be produced between different species. Speciation and hybridization are two events that are currently still impeding the identification and classification of many plant species.

Previous studies on *Clivia* include cytogenetic and molecular studies, such as hybrid identification and phylogenetic analysis using chromosome banding patterns and genomic *in situ* hybridization (Ran *et al.*, 2001a; b). Other studies on *Clivia* include RAPDs to infer phylogeny (Ran *et al.*, 2001c), growth studies (de Smedt *et al.*, 1996) and alkaloid isolations (Ieven *et al.*, 1982; Jeffs *et al.*, 1988). Most recent molecular research studies are a phylogeographic study based DNA-sequences of the *trnL-F* chloroplast region (Conrad, 2008) as well as a study establishing DNA-barcoding regions for two species (*C. mirabilis* and *C. nobilis*) (van der Westhuizen, 2010).

### 1.1.1 Statement of the taxonomic problem in *Clivia* and its cultivars

*Clivia* has a broad geographical range with some species overlapping in small distribution areas. There is a lack of absolute geographical barriers between some of the species, as well as a high degree of self-incompatibility in individual plants that results in high levels of cross pollination. Because of these two factors, ancient and/or recent hybridization events resulted in overlapping morphological characteristics between species as well as morphological variation within species. Putative new species that does not comply with any

of the taxa keys for any of the described species, hinders identification of individual plants and even classification of probable new species.

*Clivia* species and cultivars are very sought after in especially in Europe, Japan and the USA and are therefore of a very important economic resource to South Africa. Fraud in the trade does exist and a non-conventional system needs to be established to identify plants sold under false species and cultivar names.

There are three potential benefits from establishing a DNA-barcoding database for *Clivia*: **1)** To aid in the classification of possible new species; **2)** To serve as mechanism for identification of plants sold under false species names; and **3)** To identify plants confiscated from the traditional healer trade, identify the area from which the plants were taken by comparing it to the database and aid in the re-establishment of the plants into their natural habitat.

## 1.2 INTRODUCTION TO THE GENUS *LACHENALIA*

The genus *Lachenalia* (family Asparagaceae) is a numerically large genus of small bulbous geophytes consisting of 133 species (Duncan, 2012). The majority of species are distributed in the winter rainfall areas of southern Africa (thus in the Western Cape). A few species occur further inland and in the Eastern Cape Province (in summer rainfall areas). Most of the species are winter growers and remain dormant under the soil during the warm summer months (Duncan, 1988).

Research on *Lachenalia* is important since:

- Almost half of the species are listed in the IUCN Red Data List as being endangered, vulnerable, near threatened, critically rare, rare or declining (SANBI, 2012).
- *Lachenalia* is of horticultural importance in South Africa (Kleynhans *et al.*, 2009). Cultivars are being produced and are popular export products to several countries, of which the Netherlands is the most important.
- Molecular studies are needed to aid in the classification and identification of species.
- The phylogenetic relationships between the species need to be determined.

- Hybrid species need to be identified (this data will be applied in breeding studies).

No comprehensive molecular study have yet been undertaken on the genus, but the chromosome numbers, chromosome morphology and chromosome banding patterns have been studied on many of the species in several labs (Moffett, 1936; Therman, 1956; De Wet, 1957; Riley, 1962; Gouws, 1965; Mogford, 1978; Ornduff & Watters, 1978; Nordenstam, 1982; Crosby, 1986; Müller-Doblies *et al.*, 1987; Hancke & Liebenberg, 1990; Hancke, 1991; Duncan, 1996; Johnson & Brandham, 1997; Dold & Phillipson, 1998; Hamatani *et al.*, 1998; Hancke & Liebenberg, 1998; Kleynhans & Spies, 1999; Spies *et al.*, 2000; Duncan, 2001; Du Preez *et al.*, 2002; Spies *et al.*, 2002; Van Rooyen *et al.*, 2002; Hamatani *et al.*, 2004, 2007; Spies *et al.*, 2008; Hamatani *et al.*, 2009; Spies *et al.*, 2009; Hamatani *et al.*, 2010). Duncan (1988) suggested a complete revision of the genus, and since then, comprehensive morphological studies (Duncan, 1988, 2005, 2012) clarified many of the morphological uncertainties in the genus. The implementation of molecular DNA data to support the morphological classification should be investigated. Molecular data will be used to evaluate the use of a DNA-barcoding database for easy species identification, thus assisting the breeding programme at the Ornamental Plant Institute at ARC-Roodeplaat.

### 1.2.1 Statement of the taxonomic problem in *Lachenalia*

*Lachenalia* is one of the largest flowering genera in southern Africa (Langlois *et al.*, 2005). The size of the genus, together with diverse morphological variation, overlapping of certain morphological traits between species, natural hybridization and possible recent diversification of some species, all add to the problem of identification and even classification of the species. There have been several inconsistent attempts to subdivide the genus into subgroups (Baker, 1897; Crosby, 1986; Duncan, 1988, 2002; Spies, 2004). Closer related species are easier to cross and with greater success rates (Kleynhans *et al.*, 2009). Therefore, if breeders were to have a system based on the correct phylogeny of the species as basis for selecting parents in crosses, it would be more economical and less time consuming to breed exportable *Lachenalia* cultivars.

### 1.3 COMPARISON BETWEEN *CLIVIA* AND *LACHENALIA*

There are several differences (Table 1.1), as well as similarities between the two genera investigated in this study. Both these genera are members of the order Asparagales and individuals of both these genera complete their reproductive cycle in more or less the same time. Meiosis of both of these genera are instigated and completed in the bulb (or in the case of *Clivia* in the pseudo bulb). Theoretically, it could be expected that the mutation rates for the DNA regions in question would be approximately similar since their reproductive cycle is similar, making the barcoding regions chosen for this study comparable between the two genera. Furthermore, natural hybridization and incomplete speciation events in both these genera adds to the difficulty of identifying and classifying some closely related species with conventional methods, therefore both these genera need a DNA-barcoding system and species-specific database to simplify identification. Other similarities between the genera include the horticultural importance within South Africa as well as in numerous other countries because both are sought-after export products. Both genera are under threat of extinction in nature due to development that destroy their natural habitats, as well as illegal removal of the plant from nature.

**Table 1.1:** A comparison between the small genus *Clivia* and the large genus *Lachenalia*.

|                          | <i>Clivia</i>                 | <i>Lachenalia</i>               |
|--------------------------|-------------------------------|---------------------------------|
| Families                 | Amaryllidaceae                | Asparagaceae                    |
| Genus size               | 6                             | 133                             |
| Root system              | Thick root                    | Bulb                            |
| Main distribution        | Eastern parts of South Africa | Western part of southern Africa |
| Deciduous vs. evergreen  | Evergreen                     | Deciduous                       |
| Basic chromosome numbers | 11                            | 5, 6, 7, 8, 9, 10, 11, 13, 15   |

Previous molecular, FISH and karyotype studies in both genera (Moffett, 1936; De Wet, 1957; Gouws, 1965; Müller-Doblies & Müller-Doblies, 1997; Hamatani *et al.*, 1998, Hancke & Liebenberg, 1998; Pfosser & Speta, 1999; Ran *et al.*, 1999, 2001a, b, c; Kleynhans & Spies, 2000; Hancke *et al.*, 2001; Du Preez *et al.*, 2002; Conrad *et al.*, 2003; Pfosser *et al.*, 2003; Hamatani *et al.*, 2004; Manning *et al.*, 2004; Meerow & Clayton, 2004; Spies, 2004; Hamatani *et al.*, 2007, 2008, 2009; Swanevelder & Fisher, 2009; van der Westhuizen, 2010; Bay-Smidt *et al.*, 2011; Murray *et al.*, 2011) contributed to our current knowledge, but there has not been a comprehensive DNA-barcoding study on either of these genera. A comparison of the genera shows that *Clivia* has a basic chromosome number of  $x = 11$  with no variation in chromosome

numbers between the species, whereas *Lachenalia* has various basic chromosomes numbers and therefore a high degree of chromosomal variation. *Clivia* is a small genus consisting of 6 species, compared to the high number of species (133) and several subspecies and varieties in *Lachenalia*.

Samples of both *Lachenalia* and *Clivia* are easily obtainable from legal breeders and collectors, therefore, no new samples need to be collected and removed from their natural environment. For this, and all of the preceding reasons, *Lachenalia* and *Clivia* were chosen as subject material in this study.

## 1.4 PLANT DNA-BARCODING

Although there are controversy amongst some researchers regarding the effective use of DNA-barcodes (Hebert *et al.*, 2004; Moritz & Cicero, 2004; Will & Rubinoff, 2004; Ebach & Holdrege, 2005a, b; Will *et al.*, 2005; Ebach & de Carvalho, 2010), it has been proven in numerous studies to (See key concepts on p7):

- **recognize hidden diversity** in species leading to reclassification (Saunders & McDonald, 2010);
- **identify insect host-parasitic infections** (Hrcek *et al.*, 2011; Smith *et al.*, 2012);
- aid in **local control strategies** in East Africa by analysing the blood meals of tsetse flies (Muturi *et al.*, 2011);
- **identify the predator-prey interaction** of bats by barcoding the DNA found in their faeces (Bohmann *et al.*, 2011; Clare *et al.*, 2011) and identifying the plant-herbivore interaction in tropical forests (Navarro *et al.*, 2010);
- **monitor biodiversity** (Hajibabaei *et al.*, 2007a; Hausmann *et al.*, 2011) and detection of biodiversity i.e. in bryophytes which even experts have difficulty in identifying (von Cräutlein *et al.*, 2011);
- **identify species** misclassified or unclassified (Stern *et al.*, 2010), species identification in taxonomically difficult groups such as Eurasian yews (Liu *et al.*, 2011), identify venom sam-

## KEY CONCEPTS

**DNA-barcodes**

A DNA-barcode is “a short DNA-sequence that identifies a species” (Stoeckle *et al.*, 2003), by comparing the sequence of an unknown specimen to barcodes in a sequence database of known species (Kress & Erickson, 2007). The main use of these sequences is for identification and not for phylogenetic reconstruction (Kress & Erickson, 2007) or as only criterion in describing new species (Stoeckle *et al.*, 2003).

**Benefits of DNA-barcoding:**

- 1) facilitate species identification
- 2) enable identification where traditional methods are unrevealing
- 3) provide new technology that can be applied in the field to identify specimens
- 4) provide evolutionary insights (Stoeckle *et al.*, 2003). Although DNA-barcoding is not recommended for phylogenetic reconstruction, it has successfully been used in phylogenetic studies. For example, Kress *et al.* (2009, 2010) used super-matrices of barcoding regions to construct community- and species-level phylogenies.

**Speciation**

Speciation through polyploidization, hybridization and isolation barriers (i.e. geographical barriers, fertilization barriers) play such an integral role in the speciation of Angiosperms (Soltis & Soltis, 2009), that it is inevitable to include in this short key concepts.

Polyploids can develop by hybridization of two distant related species and the combination of their genomes (allopolyploidy), by doubling of the same genome (autopolyploidy) or by hybridization of related taxa (segmental allopolyploidy). With the development of genomic (Soltis & Soltis, 2009) and fossil studies (Masterson, 1994), it was determined that many (70% - 80%) of the angiosperms had an ancient polyploid origin, contributing to the species diversity found at present. Speciation is an ongoing process, and within the past 150 years new species has surfaced via polyploidization, such as *Spartina anglica* C.E. Hubbard (Nehring & Adersen, 2006). In this study, the hypothesis is that there exist on-going speciation events in *Clivia* Lindl.

The main problem with hybridization as speciation event (in contrast to divergence), is the difficulty in analysing phylogenetic data when this process is involved. Genera where hybridization events are suspected will show a reticulate evolution compared to a well resolved divergent cladogram. With the problem of hybridization in plants, comes the challenge of choosing a proper species concept to classify species. Species concepts can be divided into

- The morphology-based taxonomic species concept, which has for centuries been used and is still used widely in plants (Grant, 1981).
- The biological species concept, following the concept that groups are reproductively isolated from similar groups, and that two species can thus not hybridize (Mayr, 1942).
- The evolutionary species concept, where a species is described as: “A single lineage of ancestor-descendent populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate” (Simpson, 1961; Wiley, 1978).
- The phylogenetic species concept, which is used to “reveal the smallest units that are analyzable by cladistic methods and interpretable as the result of phylogenetic history” (Nixon & Wheeler, 1990; Judd *et al.*, 2002).

Identifying, naming and classification of organism is mainly based the morphological system (Linnaeus, 1758, 1759), but because of the limitations of relying solely on morphology, modern taxonomy includes molecular data such as gene sequences, polymorphisms in non-coding DNA regions, iso-enzymes, as well as physiology, behaviour, population biology and geography (Stoeckle *et al.*, 2003). Despite this modern technology, a large number of species can be correctly identified by only one or two experts in the world (Stoeckle *et al.*, 2003).

The high degree of hybridization in plants, renders it problematic to apply the morphological-, biological- and phylogenetic species concepts. The evolutionary species concept can tolerate hybrids but only if two hybrids have not hybridized (Soltis & Soltis, 2009).

The hypothesis that both ancient and recent hybridization events resulted in speciation in *Clivia* and *Lachenalia* Jacq. f. ex Murray will be tested in this study.

ples of unidentified or misidentified snake species and delimitation of species (Dong *et al.*, 2011; Vanhaecke *et al.*, 2012);

- **identify fossil seeds** excavated from ancient caves and ruins (Gismondi *et al.*, 2012);
- **control quality and trade** in the food and timber industries by monitoring the ingredients in, for example dietary supplements where harmful species can accidentally be misidentified and used in the supplements (Baker *et al.*, 2012), monitoring the ingredients in ‘cooling’ beverages (consisting of wild plants) in China and Asia (Li, M. *et al.*, 2012), distinguish between wrong and correctly identified plant species used in medicine (Xue & Li, 2011) and in cuisine and phytotherapy (Horn *et al.*, 2012), have a vital role in the trade control of important timber species (Muellner *et al.*, 2011) and be used to help correctly identify plants in the international trade (Pryer *et al.*, 2010) ;
- **identify fraud** in the food industry where, for example, locally caught fish are mislabelled and sold as imported (Yancy *et al.*, 2008; Lowenstein *et al.*, 2009; Hanner *et al.*, 2011);
- **protect threatened species** by, i.e. identifying shark body parts in the trade (Holmes *et al.*, 2009; Barbuto *et al.*, 2010), monitoring illegal trade in plants (SAPA, 2010; Liu *et al.*, 2011), identifying threatened species in natural health products (Wallace *et al.*, 2012) and identification of endangered snake species in illegal trade of snake skin (Dubey *et al.*, 2011);
- **be useful in forensic studies** including identifying poached wildlife (Dalton & Kotze, 2011), identifying forensic relevant fly species in forensic cases (Desmyter & Gosselin, 2009), identifying species in illegal egg (Coghlan *et al.*, 2012) and timber smuggle as well as in ecological forensic studies (Kress *et al.*, 2009);
- **monitor biological invasions** in soil (Porco *et al.*, 2012) and water (Geoffroy *et al.*, 2012);
- **pinpoint the need for taxonomic revision** (Puillandre *et al.*, 2011);
- **investigate special patterns of root diversity** to give insight in the below-ground ‘structure associated with depth, root morphology, soil chemistry and soil texture (Kesanakurti *et al.*, 2011).

The potential to apply barcoding in plant taxonomy were first explored during an exploratory workshop in 2003 (held at the “Cold Spring Harbor Banbury Conference Center”

from 9 – 12 March; accessed on <http://www.barcodeoflife.org/content/about/what-cbol>) and it was predicted that barcoding will in future be utilized in species identification, conservation biology and mapping the extent of species by linking maps to barcodes. It was also predicted that the cost of barcoding a sample would decrease to such a degree that it would be affordable to be used by science teachers and “backyard naturalists” (Stoeckle, 2003).

#### 1.4.1 History of DNA-barcoding

The use of the *CO1* gene region (also known as *cox1*) as a DNA-barcode system to identify animal life has been suggested by Hebert *et al.* (2003). The *CO1* is a 600 bp segment (Kress & Erickson, 2007) consisting of the mitochondrial *cytochrome c oxidase subunit 1* (*cox1*). This region has successfully been implemented in DNA-barcoding studies discriminating between species in 95% of the cases (Hebert *et al.*, 2003b; Hajibabaei *et al.*, 2007b). The ribosomal RNA (rRNA) may be a good candidate to use in prokaryotic barcoding (Barns *et al.*, 1996).

In 2004, the successful use of DNA-barcodes on animals has led to the establishment of an international initiative, the Consortium for the Barcode of Life (CBOL) to develop and promote DNA-barcoding (CBOL, 2010). CBOL established working groups and the main objectives of the Plant Working Group (PWG) was to establish a suitable gene region for barcoding as well as establish and complete a pilot project on one group of plants (Stoeckle *et al.*, 2004).

The ideal qualities of a DNA-barcoding region is that one or two DNA regions should provide more intraspecific than interspecific variation so that genera and even species can be identified based on their unique DNA-barcodes (Hebert *et al.*, 2003b; Stoeckle, 2003; Kress & Erickson, 2007). These DNA-barcodes should be short (~750 bp), universally and easily amplifiable across all taxa and have low intraspecific and ample interspecific variation to identify species (Hebert *et al.*, 2003b, 2004a; Savolainen *et al.*, 2005; Chase *et al.*, 2007; Hajibabaei *et al.*, 2007b). Other criteria for a suitable barcoding region are that the sequences should align readily and contain a limited number of INDELS (Cowan & Fay, 2012).

Even though the mitochondrial genome has a rapidly changing gene structure (Adams



& Palmer, 2003), plant mitochondria has very little variation in most genera (Kress *et al.*, 2005; Chase & Fay, 2009; CBOL Plant Working Group, 2009). It transfer genes between the nuclear, plastid and mitochondrial genomes (Palmer *et al.*, 2000) and in the angiosperms (Cho *et al.*, 1998) estimated over 1 000 previous horizontal transfer events of the *cox1* gene. For these reasons the mitochondria and specifically the *cox1* gene is unsuitable as a source for DNA-barcoding in plants.

The focus for choosing a universal plant DNA-barcode has thus been on chloroplast and nuclear regions (CBOL Plant Working Group, 2009), but finding universal barcoding regions is complicated by the controversy that the barcode should be universal and simultaneously have enough discrepancy between species (Kress & Erickson, 2007). Due to low mutation rates in plants, it has been agreed upon that more than one gene region should be used as universal plant DNA-barcoding regions (Stoeckle *et al.*, 2004; Kress *et al.*, 2005; Rubinoff *et al.*, 2006; Chase *et al.*, 2007).

The first proposed DNA-barcoding regions for universal plant DNA-barcoding suggested by the PWG, were the multicopy nuclear Internal Transcribed Spacer (*ITS*), the *rbcL* subunit and *matK* (Stoeckle *et al.*, 2004). Since then, several barcoding regions have been investigated, tested and proposed for different groups (Table 1.2).

Gene regions that are popular in phylogenetic studies have been investigated for possible candidate regions to be used in barcoding. Loci that are popular in plant systematics are *rbcL*, the *trnL-F* intergenic spacer, *matK*, *ndhF* and *atpB*. Two of these regions, *rbcL* and *atpB*, are used in phylogenetic studies to distinguish at genus level and above. Even though the characteristic of a suitable barcoding region is that there should be distinction at the species level, *rbcL* and *atpB* have been considered as barcoding regions (Blaxter, 2004). The regions *matK* and *ndhF* have enough variation to be used in phylogeny on interspecific level, but unfortunately only when the sequenced length is more than 1000bp does it provide enough variation for discrimination (Kress *et al.*, 2005).

The most common regions tested for its suitability as universal barcoding region includes:

- **plastid non-coding regions** *trnH-psbA* intergenic spacer, *trnL* intron, *trnL-F*, the *rbcL* subunit, *atpF-atpH* spacer, *psbK-psbI*, *rps4* regions;
- **plastid coding regions** *accD*, *ndhJ*, *rpoB*, *rpoC1*, and *ycf5*, ribulose-bisphosphate carboxylase (*rbcL*), maturase K (*matK*), *ndhF*, 23S rDNA and *atpB*;
- **nuclear non-coding regions** Internal Transcribed Spacer (*ITS* consisting of *ITS1* and *ITS2*).

The CBOL plant working group proposed the use of *rbcL* and *matK* as universal plant barcoding regions (CBOL Plant Working Group, 2009). The SciVerse Scopus bibliography database (accessed on October 2012) have 283 citations to this article and the common conclusion of many of these studies is that a universal barcode system can still not be agreed upon due to lack of universality, sequence quality and lack of discriminatory power (CBOL Plant Working Group, 2009).

**Table 1.2** A list of some of the suggested barcoding regions as either universal plant DNA-barcoding regions or suggestions made for specific plant families/genera

| Reference                                              | Non-coding plastid | Coding plastid                           | Nuclear  | Barcoding for                                |
|--------------------------------------------------------|--------------------|------------------------------------------|----------|----------------------------------------------|
| Chase <i>et al.</i> (2005)                             | * <i>trnH-psbA</i> | <i>rbcL</i>                              | ITS      | *                                            |
| Armenise <i>et al.</i> (2012)                          | <i>trnH-psbA</i>   | <i>rbcL</i>                              |          | Conifers (Italy)                             |
| Sun <i>et al.</i> (2012)                               |                    | <i>matK</i>                              |          | Dioscorea (China)                            |
| Liu <i>et al.</i> (2011)                               | <i>trnL-F</i>      |                                          | ITS      | Eurasian yews ( <i>Taxus L.</i> , Taxaceae)  |
| Li, Y. <i>et al.</i> (2011)                            | * <i>trnL-F</i>    | <i>rbcL</i> , <i>matK</i>                |          | Ferns                                        |
| de Groot <i>et al.</i> (2011)                          | <i>trnL-F</i>      | <i>rbcL</i>                              |          | Ferns (NW-Europe)                            |
| Li, M. <i>et al.</i> (2012)                            |                    | <i>ITS</i>                               |          | <i>Ficus</i> (Moraceae) (China)              |
| Ferri <i>et al.</i> (2009)                             |                    |                                          |          | Forensic botany                              |
| Fu <i>et al.</i> (2011)                                |                    | <i>rbcL</i> , <i>matK</i>                | ITS      | Genus <i>Tetrastigma</i> (Miq.) Planch.      |
| Guo <i>et al.</i> (2011)                               | * <i>petD</i>      |                                          | ITS      | <i>Hedyotis L.</i> (Spermacoceae, Rubiaceae) |
| Xiang <i>et al.</i> (2011)                             |                    | <i>matK</i>                              | ITS      | Holcoglossum (Orchidaceae: Ageridinae)       |
| Theodoridis <i>et al.</i> (2012)                       | <i>trnH-psbA</i>   | <i>matK</i>                              |          | Labiatae (Lamiaceae)                         |
| De Mattia <i>et al.</i> (2011)                         | <i>trnH-psbA</i>   | <i>matK</i>                              |          | Lamiaceae                                    |
| Han <i>et al.</i> (2012)                               |                    |                                          | ITS/ITS2 | Medicinal plants of Lamiaceae                |
| Liu <i>et al.</i> (2010)                               |                    |                                          |          | Mosses                                       |
| de Vere <i>et al.</i> (2012)                           |                    | <i>rbcL</i> , <i>matK</i>                |          | Native Flowering Plants and Conifers (Wales) |
| Jeanson <i>et al.</i> (2011)                           |                    | <i>rbcL</i> , <i>matK</i>                | ITS2     | Palms                                        |
| Luo <i>et al.</i> (2010)                               |                    |                                          | ITS2     | Plant                                        |
| Hollingsworth (2011)                                   | * <i>trnH-psbA</i> | <i>rbcL</i> , <i>matK</i>                | ITS      | Universal plant                              |
| CBOL Plant Working Group (2009)                        |                    | <i>rbcL</i> , <i>matK</i>                |          | Universal plant                              |
| Kress & Erickson (2007)                                | <i>trnH-psbA</i>   | <i>rbcL</i>                              |          | Universal plant                              |
| Kress <i>et al.</i> (2005)                             | <i>trnH-psbA</i>   |                                          | ITS      | Universal plant                              |
| Li, D.-Z. <i>et al.</i> (2011) (China plant BOL group) |                    | <i>rbcL</i> , <i>matK</i>                | ITS/ITS2 | Universal plant                              |
| Starr <i>et al.</i> (2009)                             |                    | <i>matK</i>                              |          | Universal plant                              |
| Wang <i>et al.</i> (2011)                              | <i>trnH-psbA</i>   | <i>rbcL</i> , <i>matK</i>                | ITS      | Universal plant                              |
| Yao <i>et al.</i> (2010)                               |                    |                                          | ITS2     | Universal plant                              |
| Chen <i>et al.</i> (2010)                              |                    |                                          | ITS2     | Universal plant & Medicinal plants           |
| Chase <i>et al.</i> (2007)                             |                    | <i>rpoC1</i> , <i>rpoB</i> , <i>matK</i> |          | Universal plant 1                            |
| Chase <i>et al.</i> (2007)                             | <i>trnH-psbA</i>   | <i>rpoC1</i> , <i>matK</i>               |          | Universal plant 2                            |
| Shi <i>et al.</i> (2011)                               |                    |                                          | ITS2     | Zingiberaceae                                |

\*Assessment in the possibility to be used as barcoding regions

# Additional barcoding regions that can be used in conjunction with the others

The CBOL plant working group recommended the 2-locus combination of *rbcLa* and *matK* (CBOL Plant Working Group, 2009), whereas other studies suggested that these two loci will not work as a universal barcode in all families (Zhang *et al.*, 2009; Nicolalde-Morejón *et al.*, 2010; Roy *et al.*, 2010; Nicolalde-Morejón *et al.*, 2011; Arca *et al.*, 2012; Maia *et al.*, 2012). When re-evaluating the core barcoding regions, Hollingsworth (2011) suggested that the nuclear *ITS* region should routinely be added to the barcoding core regions (*rbcLa* and *matK*) since the discriminatory power may increase by up to 20%. The use of *ITS2* as alternative can increase discrimination by 10-15%. Other studies also supported the importance of using combined analyses, thus more than one region (Kress *et al.*, 2005; Chase *et al.*, 2007; Kress & Erickson, 2007; Fazekas *et al.*, 2008; CBOL Plant Working Group, 2009; Li, D.-Z. *et al.*, 2011; Wang *et al.*, 2011).

#### 1.4.2 Challenge of recently diverged organisms and DNA-barcoding in general

Recently diverged species will have few characters to discriminate them from close relatives since the limited time would have an effect on the number of nucleotide changes. These species can have unclear barcode matches or, when the tree-based analysis is implemented, barcode clusters may be absent. It is recommended that tree-based methods should not be implemented when investigating recently diverged species (van Velzen *et al.*, 2012).

Three factors influence the degree of inter- and intraspecific variation and will indirectly influence the effective use of DNA-barcoding. The first is the time of divergence of the species, the more recent the speciation, the smaller the barcoding “gap” will be (Nichols, 2001, Wallman & Donnellan, 2001; Meyer & Paulay, 2005; Kaila & Stahls, 2006; Lou & Brian Golding, 2010; Yassin *et al.*, 2010). Second, the intraspecific variation are influenced by the population size, thus a larger population will have species with larger intraspecific variation (Nichols, 2001). The third factor will be the mutation rate, which, if slow, can result in two morphological distinct species sharing identical haplotypes (Lou & Brian Golding, 2010).

Van Velzen *et al.* (2012) regards the time (measured in generations), as well as the population size, to be the most influential factors contributing to lineage sorting and overall to the problems using DNA-barcoding for identification purposes in some taxa. The success rate

for DNA-barcoding in plants due to these factors is estimated to be only 70%. Another problem that can influence the effective use of DNA-barcoding analyses is hybridization, which may be a common phenomenon in many plant taxa. *Clivia* is known as a genus that hybridizes readily in cultivation. Some individuals have very poor seed offset when self-pollinated, and seems to yield higher seed offset when cross-pollinated with individuals from the same species. Cross-pollination occurs between different species in distribution areas where two species co-occur (e.g. *C. × nimbicola* resulting from a cross between *C. miniata* and *C. caulescens*). *Lachenalia* on the other hand seems to have cross-pollination barriers in many species (Kleynhans *et al.*, 2009). Hybridization will result in similar or shared sequences in different species. Since the chloroplast is maternally inherited, there may be incongruence's between chloroplast and species trees (Hebert *et al.*, 2004b). This incongruence's may be expected in the genus *Clivia* and will further be investigated in this study.

#### 1.4.3 Plastid and nuclear genomes

Chloroplast genes have several advantages such as the uniparental mode of inheritance, the fact that it is nonrecombining and are structurally stable (Kress *et al.*, 2005), and these genes are therefore more readily exploited in phylogenetic studies compared to the nuclear genome.

The core plant DNA-barcoding regions are *matK*, *rbcL*, *trnH-psbA* and the nuclear region *ITS*. In this study, the following regions were investigated as possible DNA-barcoding regions in *Lachenalia* and *Clivia*: *ITS2*, *matK*, *trnH-psbA*, *atpH-atpI*, *trnL* intron, *trnL-F*, *rbcLa*, *rpoC1*, *rpoB*, *trnT-L* and *rpL16*.

#### 1.4.4 Evaluation of some of the core chloroplast coding regions

***matK*:** The chloroplast maturase K gene (*matK*) is, with the exception of some ferns, situated within an intron of the *trnK* gene (Neuhaus & Link, 1987) (Figure 1.1). The gene is approximately 1535 bp long in monocots (Yu *et al.* 2011) and is the only chloroplast-encoded group II intron maturase (Barthet & Hilu, 2007). Universal primers situated in the *trnK* gene are used to amplify the entire gene region for phylogenetic studies (Wang *et al.*, 2006; Li &

Zhou, 2007) in orders or families, but are sometimes effectively used on genus or species level, i.e. in the genus *Paeonia* (Paeoniaceae) (Sun & Hong, 2012).

Only a 600-800 bp region of the *matK* gene are utilized for DNA-barcoding purposes (Yu *et al.*, 2011). The *matK* gene evolves fast (three times faster than *rbcL* and *atpB*) (Hilu *et al.*, 2003) and some studies suggest it can effectively discriminate between species in the angiosperms. A problem with the *matK* gene region as universal barcoding region is it has a low amplification success rate and the universal primers need to be improved (CBOL Plant Working Group, 2009).



**Figure 1.1** The *matK* chloroplast coding region based on the schematic drawing of Wakasugi *et al.* (1998), Matsumoto *et al.* (1998), Shaw *et al.* (2005) and Barthet & Hilu (2007) (not drawn to scale). The areas in the boxes represent the coding exon regions, and the connecting lines represent intergenic spacer- and intron regions and the numbers centred on the lines are the lengths (bp) of the intergenic spacer and intron regions based on the study of Shaw *et al.* (2005).

***rbcL*:** The large subunit ribulose-bisphosphate carboxylase (*rbcL*) (Figure 1.2) (Yoshinaga *et al.*, 1996) is part of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) protein in land plants. This protein consists of eight small subunits (Rutner & Lane, 1967; Nishimuran *et al.*, 1973; Baker *et al.*, 1977) (encoded in the nucleus) and eight large subunits which are encoded by a single gene in the chloroplast (Kellogg & Juliano, 1997). RuBisCO is involved in photosynthesis and interacts with its substrates CO<sub>2</sub>, O<sub>2</sub> and ribulose 1,5 bisphosphate (RuBP) (Kellogg & Juliano, 1997).

Because the *rbcL* gene codes for a protein, many regions in the gene need (to a large degree) to be conserved to ensure the correct three dimensional folding of the protein. This implies that the gene cannot resolve a systematic study on a large dataset (Kellogg & Juliano, 1997). The *rbcL* secondary structure has been used in a systematic study (Kellogg & Linder, 1995) and results suggested that the *rbcL* sequences should be translated and that amino acid changes should be plotted onto phylogenetic trees (Kellogg & Juliano, 1997).

The *rbcl* region is comparatively easy to amplify and sequenced over a broad spectrum of taxa (CBOL Plant Working Group, 2009) and it has been suggested as a core barcoding region (CBOL Plant Working Group, 2009), but it has been proven in some studies to have a low divergence rate, such as in the Solanaceae (Kress *et al.*, 2005). In many taxa it cannot be used to discriminate on species level (Renner, 1999).



**Figure 1.2** The chloroplast coding region *rbcl* is situated between the *atpB* and *trnR* coding regions (Yoshinaga *et al.*, 1996) (not drawn to scale). The areas in the boxes represent the coding exon regions, and the connecting lines represent intron regions.

***rpoB* and *rpoC1*:** These two plastid coding genes are part of a group of genes encoding for subunits of the plastid RNA polymerase (PEP), which is responsible in photosynthesis in higher plants (Serino & Maliga, 1998). The *rpoB* gene is responsible for coding the RNA polymerase beta subunit and *rpoC1* codes for the RNA polymerase beta' subunit. The latter has an intron of 738 bp in tobacco (Wakasugi *et al.*, 1998). Functional copies only occur in the plastid, and without functional genes *rpoA*, *rpoB*, *rpoC1* and *rpoC2*, a plant will be photosynthetically defective (Serino & Maliga, 1998). Although good quality sequences are routinely obtained for *rpoB* and *rpoC1* (CBOL Plant Working Group, 2009; Ford *et al.*, 2009), there has been controversies regarding the use of these two regions as barcodes, where Chase *et al.* (2007) and Ford *et al.*, (2009) recommend them as members of a three-region barcode, and Lahaye *et al.* (2008) and Seberg & Petersen (2009) opposed that and suggested that these regions are too conserved in Angiosperms.

#### 1.4.5 Evaluating some chloroplast non-coding regions

***atpH-atpI intergenic spacer:*** This region is located between the *atpH* coding gene (that codes for the ATP synthase III subunit) and *atpI* (coding for the ATP synthase IV subunit) (Wakasugi *et al.*, 1998) in the Large Single Copy region (Shaw *et al.*, 2007). Although this region has not been extensively studied for its potential as barcode, Shaw *et al.* (2007) identified it as one of the top nine gene regions to use in sequence-based studies in

Angiosperms. Poly-A/T regions occur in this spacer region and depending of the length of the A/T run, this may cause problems during sequencing. The frequency of having this problem is low, and Shaw *et al.* (2007) observed only a single lineage in their study with a 24 bp repeat interfering with the sequencing.

***rpL16 intron*:** The *rpL16* intron is situated in the *rpL16* gene, a chloroplast gene encoding for the ribosomal protein L16 (Wakasugi *et al.*, 1998). This chloroplast DNA region occurs in the Large Single Copy (LSC) region located in the chloroplast genome (Shaw *et al.*, 2005) and is regularly used in plant molecular studies (Shaw *et al.*, 2007).

***trnH-psbA*:** This orientation is based on the *Nicotiana* chloroplast map of Wakasugi *et al.* (1998), starting at the Inverted Repeat A gene and is in several publications referred to as *psbA-trnH*. The *trnH<sup>GUG</sup>-psbA* is a chloroplast intergenic spacer region between tRNA-His(GUG) (*trnH*) and the 5' adjacent *psbA* (coding for PSII 32kD protein) (Figure 1.1; Aldrich *et al.* (1988). This region has a high degree of INDELS even between closely related species, which are often flanked by directly repeated sequences. The high variability in this region, which varies more than *matK*, *trnL-F*, ITS, *rbcL*, and *matK* in some taxa (Sang *et al.*, 1997; Kress *et al.*, 2005; Kress & Erickson, 2007), make it an ideal region to be used in phylogenetic studies between closely related genera and species (Shaw *et al.*, 2005). The *trnH-psbA* region is relative short with an average length of 465 bp (range between 198 – 1077 bp) (Shaw *et al.*, 2005) and in most flowering plants it ranges between 340 – 660 bp (Li, D.-Z. *et al.*, 2011). The longest length that has been recorded i.e. in *Trillium-Pseudotrillium* (Table 1.3), is atypical (Shaw *et al.*, 2005).

**Table 1.3** Comparison between the aligned length, number of INDELS, the average INDEL length and the percentage variability in some chloroplast regions in the monocotyledons based on a study of Shaw *et al.* (2005).

| MONOCOTS            | <i>trnH-psbA</i> | <i>trnT-L</i> | <i>trnL</i> | <i>trnL-F</i> | <i>rpL16</i> |
|---------------------|------------------|---------------|-------------|---------------|--------------|
| Aligned length (bp) | 1077             | 777           | 566         | 384           | 1055         |
| INDELS              | 8                | 2             | 6           | 3             | 9            |
| Avg. INDEL length   | 6.1              | 27.6          | 4.8         | 2.6           | 8.9          |
| % variability       | 3.81             | 2.32          | 2.30        | 4.17          | 3.51         |

The *trnH-psbA* region has been used in DNA-barcoding studies due to the high interspecific variation, the ease of amplification amongst different taxa (Kress *et al.*, 2005), and because the region can be sequenced with only one primer in many taxa (Shaw *et al.*, 2005), due to the ease of obtaining full length unidirectional sequences.

Due to some problems such as the presence of poly-A/T structures in the region (Aldrich *et al.*, 1988) influencing successful sequencing (Zhu *et al.*, 2010), difficulties in amplification and difficulty in aligning some taxa due to palindrome inversions and gene insertions within the region (Shaw *et al.*, 2005; Chase *et al.*, 2007; CBOL Plant Working Group, 2009; Whitlock *et al.*, 2010), this region has been rejected as core DNA-barcoding region in land plants, but it has been suggested to be used as additional barcoding region (Newmaster *et al.*, 2006; Kress & Erickson, 2007; Seberg & Petersen, 2009).

***trnT-trnL-trnF***: This region consists of the *trnT* gene [tRNA-Thr(UGU)], the *trnL* gene [coding for tRNA-Leu(UAA)] and *trnF* [coding for tRNA-Phe(GAA)] (Wakasugi *et al.*, 1998). The *trnT-trnL-trnF* cistron is in the large single-copy region of the chloroplast genome and consists of the group I *trnL* intron, as well as the *trnT-trnL* and *trnL-F* intergenic spacer regions (Figure 1.3). The *trnL* intron has a conserved secondary structure and the spacer regions are variable but can contain hairpin structures in the *trnL-F* spacer region (Won & Renner, 2005).

This conserved gene order in the cistron is unique in land plants (Quandt *et al.*, 2004) and has three characteristics which made it popular in various phylogenetic studies on genus and species level (Alejandro *et al.*, 2011; Razafimandimbison *et al.*, 2011; Voshell *et al.*, 2011; Barrabé *et al.*, 2012): **1)** It has a conserved gene order, **2)** The non-coding regions are variable and **3)** The intergenic spacer region (IGS) and intron are long enough for phylogenetic studies (Taberlet *et al.*, 1991; Won & Renner, 2005).

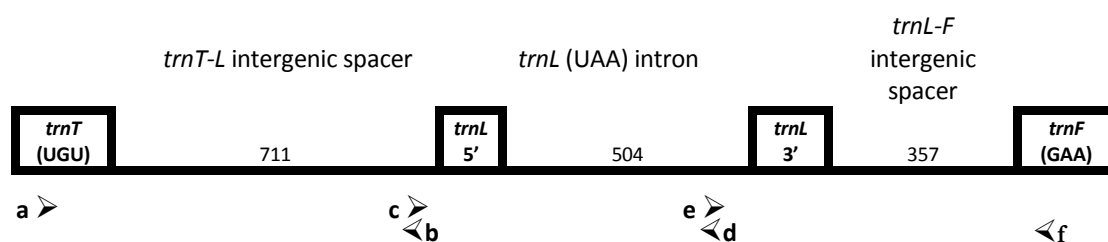


**Figure 1.3** The *trnT-L-F* cistron consisting of the *trnT-L* intergenic spacer, the *trnL* intron and the *trnL-F* intergenic spacer of the chloroplast genome based on the representation of Taberlet *et al.* (1991), Won & Renner (2005), and Shaw *et al.* (2005) (not drawn to scale). The areas in the boxes represent the coding exon regions, and the connecting lines represent intergenic spacer- and intron regions. Numbers on the lines are the lengths of these region (in bp) based on that of *Nicotiana* (Shaw *et al.*, 2005). The relative positions of the primers and their amplification directions are indicated with arrows.



The *trnL-F* region has been recommended as one of the barcoding regions in ferns and yews ( de Groot *et al.*, 2011; Li, D.-Z. *et al.*, 2011; Liu *et al.*, 2011), and the *trnL* intron has also been considered as a barcoding region for degraded samples (Taberlet *et al.*, 2007). Both these regions have however been rejected as universal barcoding regions because of low interspecific variation (Kress & Erickson, 2007).

#### 1.4.6 The nuclear *ITS* region

The internal transcribed spacer (*ITS*) region of the nuclear ribosomal cistron (18S-5.8S-26S) (Figure 1.4) has been used broadly across eukaryotes in phylogeny since the region is much more variable (3 – 4x) than chloroplast genes (Chase *et al.*, 2007). Nuclear DNA (nrDNA) is transmitted through the pollen and seeds of plants, compared to the mainly maternal inherited plastid DNA that is transmitted only through the seed. Seeds are usually dispersed poorly compared to pollen, and this could explain why the *ITS* region has a higher resolving power in DNA-barcoding than plastid DNA markers (CBOL Plant Working Group, 2009). It has been suggested (Stoeckle *et al.*, 2003; Chase *et al.*, 2005; Kress *et al.*, 2005;) that *ITS* should be included as barcoding region based on its successful amplification and discrimination in flowering plants.

In spite of the positive characteristics that the barcoding regions pose, there might be limitations in some taxa: **1) Fungal amplification** instead of plant sample amplification is not uncommon. This can result in the fungal sequences being interpreted under the false impression that it is sequences of the plant (Hollingsworth, 2011); **2) Multiple copies** of the *ITS* region is present in each cell, and it usually undergoes concerted evolution. Paralogous gene copies can be found in some plant taxa (Álvarez & Wendel, 2003). Sequencing of these paralogous copies can result in unreadable sequences due to the simultaneous amplification of the variants (Hollingsworth, 2011). It has been found in hybrid species that *ITS* can ‘behave’ in three manners. First, the *ITS* from both parental species can be maintained in the hybrid species. Second, the two parental *ITS* gene regions can cross over to form chimeric *ITS* sequences. Lastly, only one of the parental *ITS* gene regions will be maintained (Álvarez & Wendel, 2003); **3) Problems with amplification** is another drawback of the *ITS*, where it is difficult to amplify and sequence in some taxa (Hollingsworth, 2011); **4) Reduced variability** is

possible between recently diverged taxa; **5) Secondary structures** in the *ITS* spacer regions can result in lower amplification success (Baldwin *et al.*, 1995; Álvarez & Wendel, 2003).

Some of these problems can be overcome by cloning the multiple copies of divergent paralogues (Baldwin *et al.*, 1995; Álvarez & Wendel, 2003) and eliminating amplification of fungi with the use of plant-specific primers (Cullings & Vogler, 1998) (though the latter approach would negate the usefulness of *ITS* as a barcoding gene). Addition of DMSO to the amplification reactions should overcome the problem with secondary structure formation (Choi *et al.*, 1999). Considering all aspects, even though the use of the *ITS* nrDNA has limitations, it is strongly suggested to include it in barcoding studies of plants (Hollingsworth 2011).

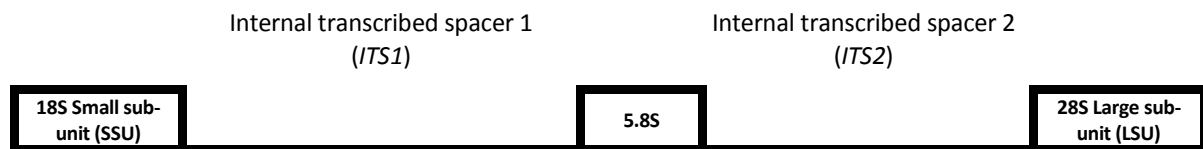


**Figure 1.4** The internal transcribed spacer regions (*ITS1* and *ITS2*) are situated between the coding genes for the 18S, 5.8S and 28S ribosomal subunits (not drawn to scale). The areas in the boxes represent the coding regions, and the connecting lines represent the internal transcribed spacer regions.

#### 1.4.7 Why implement DNA-barcoding?

The shortage of ‘conventional’ (non-molecular) taxonomists in South Africa (Smith *et al.*, 2008), calls for an urgent additional or alternative method to identify species (Hebert *et al.*, 2003a). Conventional taxonomy has several limitations in general, and these limitations are also to a large degree applicable in both the genera *Clivia* and *Lachenalia*: **1)** Species can be incorrectly identified due to variability in the characters used in species recognition (Hebert *et al.*, 2003a); **2)** Morphological keys can often only be used effectively during certain developmental stages of the plants, i.e. when flowering. Seedlings and young plants are mostly difficult to identify; **3)** Keys are often difficult to use, and an inexperienced person may incorrectly identify a species (Hebert *et al.*, 2003a).

DNA-barcoding is a relatively rapid, inexpensive and reliable method to identify species. In theory, a good barcoding region can be used in conjunction with a taxonomic

investigation to identify species (Köhler, 2007), or alternatively the potential exists for it to be used alone for identifying plant species (Kress *et al.*, 2005).

Different species in the genus *Clivia* hybridize easily, and many plant specimens have greater seed sets from cross-hybridizing than from self-pollination (personal observation). This implies that, unless a species is isolated by a distinct geographical barrier, it will readily hybridize with other species in nature. There are overlaps in some areas between species, such as between *C. miniata* and *C. gardenii* (in KwaZulu-Natal), *C. miniata* and *C. caulescens* (on the mountain Bearded man bordering Swaziland and Mpumalanga) and *C. nobilis* with *C. robusta* (Eastern Cape Province). Morphological variation is very common in *Clivia*, and many researchers have suggested that there should be more species described. This study will explore the use of DNA-barcodes to aid in the identification of species and possible new species.

Based on previous chromosome studies in *Lachenalia* (Moffett, 1936; Therman, 1956; De Wet, 1957; Riley, 1962; Gouws, 1965; Mogford, 1978; Ornduff & Watters, 1978; Nordenstam, 1982; Crosby, 1986; Müller-Doblies *et al.*, 1987; Hancke & Liebenberg, 1990; Hancke, 1991; Duncan, 1996; Johnson & Brandham, 1997; Dold & Phillipson, 1998; Hamatani *et al.*, 1998; Hancke & Liebenberg, 1998; Kleynhans & Spies, 1999; Spies *et al.*, 2000; Duncan, 2001; Du Preez *et al.*, 2002; Spies *et al.*, 2002; Van Rooyen *et al.*, 2002; Hamatani *et al.*, 2004, 2007; Spies *et al.*, 2008; Hamatani *et al.*, 2009; Spies *et al.*, 2009; Hamatani *et al.*, 2010; Hamatani *et al.*, 2012), some of the species in the genus have evolved from ancient hybridization events. Most of the *Lachenalia* species do not hybridize freely in a greenhouse environment (Graham Duncan [SANBI, Kirstenbosch] and Riana Kleynhans [VOPI, ARC-Roodeplaat], personal comm.) and only one natural hybrid have been identified (Graham Duncan, personal comm.). Unfortunately, data proving the occurrence of cross pollination in nature do not exist, but at this stage it can be assumed that cross pollination in nature is relative uncommon. Despite of this, some *Lachenalia* species are difficult to identify correctly due to interspecific variation and morphological overlap between species. A barcoding system for the genus will improve the proper identification of species, as with the genus *Clivia*. The aim of the study is thus to investigate the possibility of supplementing morphological data with a DNA-barcode to aid in identification of species.

## 1.5 AIMS AND OBJECTIVES

The *rbcL* + *matK* and additionally the *trnH-psbA* + *ITS* regions have been identified as the universal plant barcoding regions. However, several authors have come to the conclusion that these regions are not the best candidate regions for all plant groups (Table 1.2). For this reason it has been decided to evaluate the suggested DNA regions as barcoding regions, as well as additional putative barcoding regions that can be most effectively used for species identification. The additional regions chosen for this study have been used in previous studies in either DNA-barcoding or phylogenetic contexts and consist of the chloroplast regions *matK*, *atpH-atpI*, *trnH-psbA*, *trnL-F*, *trnT-L*, *rpL16 intron* and the nuclear region *ITS2*. The specific objectives of this study are

1. to assess the efficacy of the universal plant DNA-barcoding regions in *Clivia* and *Lachenalia* and compare these regions to putative new regions;
2. to reconstruct the phylogenies of *Clivia* and *Lachenalia* based on sequence data;
3. to investigate the influence of polyploidy on DNA-sequences in these genera;
4. to investigate phylogeographical and cytogeographical evolution in *Clivia* and *Lachenalia*;
5. to analyse levels of inter- and intraspecific variation in *Clivia* and *Lachenalia*.

## 1.6 STRUCTURE OF THESIS

The thesis is written as a series of papers submitted to scientific journals. Each chapter represents a separate paper. The thesis is subdivided into four parts, covering an Introduction and Methods (Part A), *Clivia* (Part B), *Lachenalia* (Part C) and a comparison between these genera (Part D). Part A comprises two chapters: this chapter and *Chapter 2* – a comprehensive description of barcoding techniques, as well as the methods used during this study which will not be published separately, but is included to avoid unnecessary duplication.

Part B, “*Clivia*”, consists of two chapters: *Chapter 3* presents a review of all previous cytogenetic and phylogenetic studies on the genus *Clivia* and includes a review on general studies (such as alkaloids) on *Clivia* species. This paper has already been published but has

been updated for the chapter. *Chapter 4* explores the identification and establishment of DNA-barcodes in the genus *Clivia* using tree-based, distance-based and character-based analyses. The inter and intraspecific variation in the barcoding regions is tested statistically. A manuscript based on this chapter will soon be submitted for publication.

Part C, "*Lachenalia*", starts with *Chapter 5* which provides a complete revision of the genus *Lachenalia*. All aspects of cytogenetics, phylogenetics, cross-ability and a comparison of these genera are discussed in this chapter. This paper has already been published but in this thesis it was adapted to accommodate recent name changes suggested by Duncan (2012). *Chapter 6* covers the same aspects as Chapter 4, but the focus is on the genus *Lachenalia*. A manuscript of this chapter will also be submitted for publication.

The thesis ends with Part D where *Chapter 7* summarizes the whole thesis and compares the efficacy of barcoding a small genus compared to a large genus. A manuscript of this chapter will also be submitted for publication.

# Part A: Introduction and Methods

## *Chapter 2*

### Barcoding Techniques



## 2.1 DNA-BARCODING DATA ANALYSES

Choosing the best barcoding region should be based on the following criteria: the region should be able to discriminate between closely related species by having a high interspecific variability, and simultaneously specimens should readily be assigned to species because of a lower intraspecific variation; it should be short ( $\leq 750$  bp); and (depending on the type of analysis used), sequences should align readily, therefore it should contain a limited number of INDELS.

The type of data analysis system can be crucial for the analyses of DNA-barcoding data, especially to use the data effectively on species level. Many tools or statistical frameworks (Munch *et al.*, 2008a; Sandionigi *et al.*, 2012) are available to analyse DNA-barcoding data, and these tools use different analysis methods or combinations thereof for analysing the data (Table 2.1). These methods can be divided into three categories: 1) Similarity methods/pairwise distance; 2) Hierarchical clustering methods/tree-based; 3) Character-based and diagnostic methods (Sandionigi *et al.*, 2012; van Velzen *et al.*, 2012).

### 2.1.1 Similarity methods/pairwise distance methods

The distance methods compare the query sequences as a whole unit (Bergmann *et al.*, 2009) to a reference dataset and trees are drawn from which similarities can be determined (Bergmann *et al.*, 2009; Sandionigi *et al.*, 2012). The programs BLAST (Altschul *et al.*, 1990), JMOTU (Jones *et al.*, 2011) and TAXON DNA (Meier *et al.*, 2006) make use of similarity methods/pairwise distances to uncover patterns of clustering (Sandionigi *et al.*, 2012). The advantage of distance methods is that the exact distance is used in the calculations, rather than a distance estimation from aligned sequences (Jones *et al.*, 2011).

The disadvantages are that INDELS are not recognised as characters and this can influence the number of clusters that are estimated (Jones *et al.*, 2011). Clustering can also be superficial due to the assumption that individuals from the same species are more similar to each other than to individuals of another species and because this method groups individuals based on a specific “threshold value” (a certain level of variability). Hebert *et al.* (2004) proposed that this “standard sequence threshold” should be ten times the mean intraspecific

variation for the study group. The standardised threshold of 2.7% proposed for birds (Hebert *et al.*, 2004), proved to be unrealistic and implementing this threshold as a benchmark will lump many species as being similar despite real biological differences (Moritz & Cicero, 2004). When intraspecific variation is large, false positives can be obtained, resulting in an over-splitting of species (Meyer & Paulay, 2005). Hebert *et al.* (2004) predicted that this threshold value may not be foolproof, especially for species with short evolutionary histories and species resulting from recent hybridization. The threshold can however serve as a screening tool for species that has been taxonomically neglected (Hebert *et al.*, 2004).

**Table 2.1** The main DNA-barcoding tools available for data analyses. This table was adapted from Sandionigi *et al.* (2012) and Bhargava & Sharma (2013)

| Class           | Method                                                           | Software                                |
|-----------------|------------------------------------------------------------------|-----------------------------------------|
| Similarity      | Similarity                                                       | BLAST, CLOTU                            |
|                 | Pairwise distance                                                | Taxon DNA, jMOTU, TaxI                  |
| Tree-based      | Neighbor-Joining                                                 | MEGA5, R package APE, Mesquite tool     |
|                 |                                                                  | NINJA, PAUP                             |
|                 | Parsimony                                                        | MEGA5, TNT, Phylip                      |
|                 | UPGMA                                                            | PAUP, MEGA5                             |
|                 | Maximum likelihood                                               | MEGA5, RAxML                            |
| Character-based | Bayesian inference                                               | SAP                                     |
|                 | Diagnostic                                                       | CAOS, BRONX, BLOG, BLOG2, DNA-BAR, OFBG |
| Other           | Portal of data analysis                                          | Web browser <sup>1</sup>                |
|                 | Complete R-package analysis                                      | SPIDER                                  |
|                 | PTIGS based DNA-barcoding analyses                               | PTIGS-Idit                              |
|                 | Identifying new barcode markers and their associated PCR primers | ecoPrimers                              |
|                 | R package for Operational Taxonomic Unit data                    | OTUbase                                 |
|                 | Measures sequence success                                        | B                                       |
|                 | Visualise inter- and interspecific variation                     | TaxonGap                                |

**APE** = Analyses of Phylogenetics and Evolution (Paradis *et al.*, 2004); **B** = Barcode quality index (Little, 2010); **BLAST** = Basic Local Alignment Search Tool; **BLOG** = Barcoding with LOGic formulas (Bertolazzi *et al.*, 2009); **BLOG2** = Barcoding with LOGic formulas (Weitschek *et al.*, 2013); **BRONX** = Barcode Recognition Obtained with Nucleotide eXpose's (Little, 2011); **CAOS** = Characteristic Attribute Organization System (Sarkar *et al.*, 2008); **CLOTU** = Processing of amplicon reads followed by taxonomic annotation (Kumar *et al.*, 2011); **DNA-BAR** (DasGupta *et al.*, 2005); **ecpPrimers** (Riaz *et al.*, 2011); **jMOTU** = Java program to identify Molecular Operational Taxonomic Units (Jones *et al.*, 2011); **MEGA** = Molecular Evolutionary Genetics Analysis (Tamura *et al.*, 2011); **NINJA** (Wheeler, 2009); **OFBG** = Oligonucleotide Frequency Barcode Generator (Tyagi *et al.*, 2010); **PAUP** = Phylogenetic Analysis Using Parsimony (Swofford, 2003); **PTIGS** = psbA-trnH Intergenic Spacer (Liu *et al.*, 2011); **RAxML** = Randomized Axelerated Maximum Likelihood (Stamatakis *et al.*, 2012); **SAP** = Statistical Assignment Package (Munch *et al.*, 2008a; b); **SPIDER** = SPecies IDentity and Evolution in R (Brown *et al.*, 2012); **TaxI** (Steinke *et al.*, 2005) **TaxonGap** (Slabbinck *et al.*, 2008); **TNT** = Tree analysis using New Technology (Goloboff *et al.*, 2008).

<sup>1</sup> <http://www.ibarcode.org> (Singer & Hajibabaei, 2009)



Two solutions to the threshold problem of false positives, are (i) to adapt the threshold value according to the choice of marker and the taxonomic group, and (ii) to determine the barcoding “gap” (Figure 2.1). The barcoding “gap” refers to the gap between intra- and interspecific variation. This gap should be ample to distinguish between species (Hebert *et al.*, 2004; Meyer & Paulay, 2005). The barcoding “gap” relies on the bin size of distribution and sometimes uninformed decisions can differ between scientists. Automatic Barcode Gap Discovery (ABGD) is a reliable method to successfully determine the barcoding “gap” in many taxonomic groups. The only input required is the approximate maximum prior intraspecific distance,  $P$ . The ABGD algorithm uses a sequence alignment or pairwise distances matrix as input file, and the computation time is extremely fast compared to other methods, such as reconstructing a phylogeny. Another advantage of ABGD is that differences in intraspecific diversity between species does not influence analyses (Puillandre *et al.*, 2011). To effectively determine the gap, it is suggested that enough samples per species (more than 1-2) (Meyer & Paulay, 2005; Puillandre *et al.*, 2011), or even 3-5 when using ABGD (Meyer & Paulay, 2005; Puillandre *et al.*, 2011) should be included in the analysis (Meyer & Paulay, 2005). When the number of sequences per species is less than 3-5, it may be impossible to determine the barcoding “gap” and the ABGD software will not be able to delimit species. It is suggested that ABGD should not be used for delimitation purposes, but only as a tool as a “first species partition hypothesis” (Meyer & Paulay, 2005; Puillandre *et al.*, 2011).

The barcoding “gap” is determined by calculating the mean intra- and interspecific gaps (Figure 2.1). Meier *et al.* (2008) suggested that the smallest interspecific distance should rather be used, since using the mean intra- and interspecific gaps in identification can give false-positives and negatives. Lou & Brian Golding (2010) even suggested that identification can be successful in the absence of a barcoding gap when using the Bayesian method.

The Basic Local Alignment Search Tool (BLAST; Altschul *et al.*, 1990) is a method to quickly search DNA and protein sequence databases, to search for motifs and perform gene identification searches. Problems that can be encountered using BLAST are:

- 1) It uses a local alignment heuristic search method to find homologous sequences rather than a global alignment. Due to this there might be a loss of data (matches).

- 2) There is no measure of confidence when a search is made. The method relies on the E-value, which is only an indication of the probability of finding a match by chance (Munch *et al.*, 2008b) and it is therefore ineffective for choosing among multiple close matches (Munch *et al.*, 2008a). The scores are influenced by both the sequence length and sequence similarity, which can cause interpretation difficulties for some researchers (Ratnasingham & Hebert, 2007).
- 3) Issues of population and phylogenetic relationships are not considered when searching for an identification during a BLAST search (Munch *et al.*, 2008a).

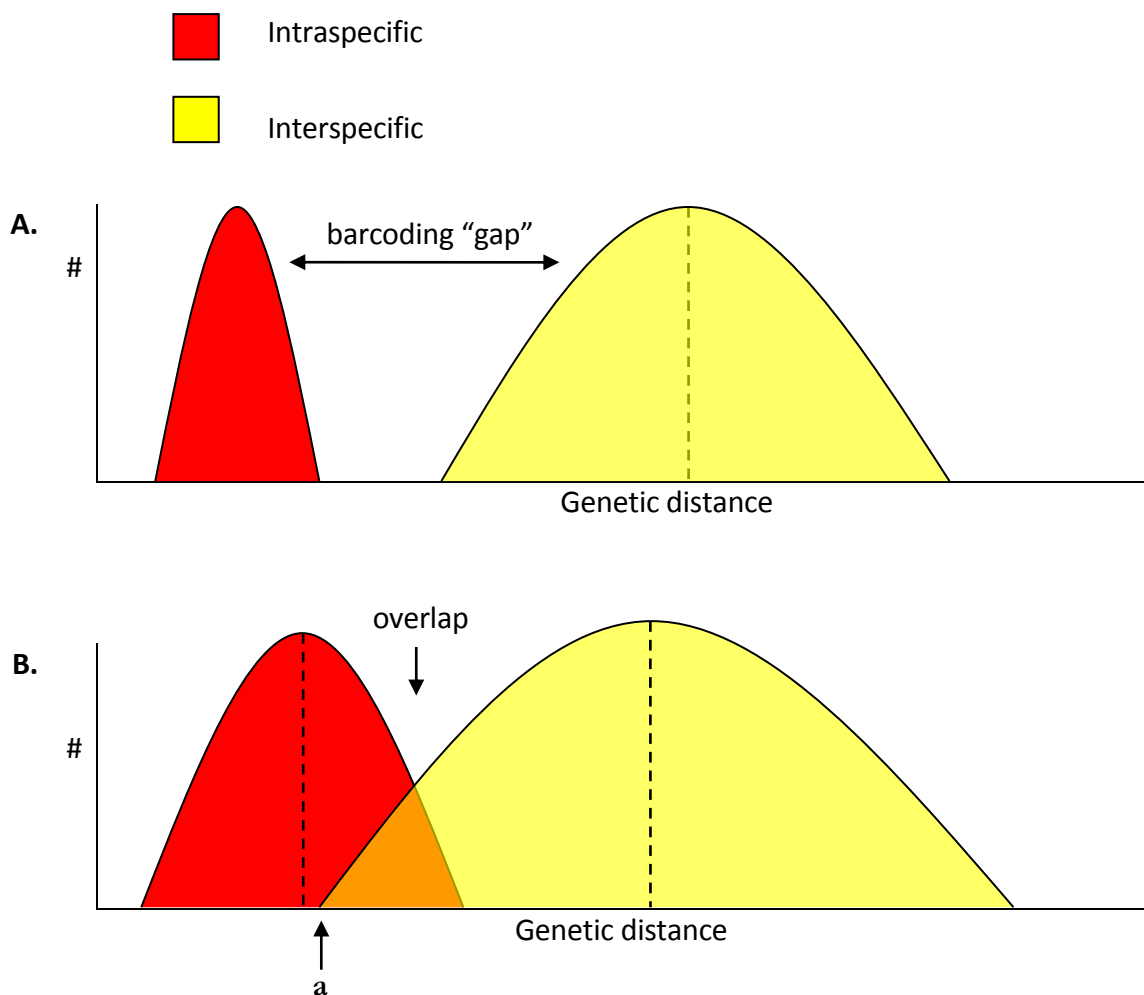


**Figure 2.1** An illustration of the barcoding “gap”, adapted from (Meyer & Paulay, 2005). **A.** The ideal barcode will have little intraspecific variation (red) and should have a proper barcoding gap to distinguish between species. **B.** When the barcoding “gap” is small, there may be an overlap between the inter- and intraspecific variation and identification of species may become problematic. When calculations are based on the mean taxonomic distances (indicated by the dashed lines), an organism (**a**) from taxon 2 (yellow) with a distance <1% may mistakenly be identified as a member of taxon 1 (red) (Meier *et al.*, 2008).

Some critics (Will & Rubinoff, 2004; Meier *et al.*, 2008) suggest that distance matrices may not correctly represent the true relationships of species. Munch *et al.* (2008b) support this hypothesis after comparing BLAST identifications with a statistical assignment approach.

### 2.1.2 Tree-based methods

These methods make use of the clades in a cladogram to identify an unknown species. The species is thus identified according to the cluster it groups with. This is considered a better method to implement since the need for a threshold value is eliminated. The principle is similar to that of a phylogenetic reconstruction, where the sequences need to be aligned and a tree needs to be constructed. The aim is however not to draw phylogenetic conclusions, but to identify a species based on the clustering compared to reference samples. There are four methods used for tree-based clustering: Parsimony, Maximum Likelihood (Edwards & Cavalli-Sforza, 1964), Bayesian Inference (Huelsenbeck & Ronquist, 2001) and Neighbor-Joining (Saitou & Nei, 1987), combined with bootstrapping (Felsenstein, 1989).

As the name suggests, the parsimony method (also known as PAR) make use of algorithms to determine the most parsimonious cladogram. The PAR method has several disadvantages. Nevertheless, this character-state method uses a heuristic approach, has a low calculation time and is overall a better tool than NJ (Sandionigi *et al.*, 2012) and likelihood (DeSalle, 2006). The performance of PAR is well suited for unambiguous alignments (Little, 2011), such as the *trnH-psbA* region used in this study, as will be discussed in Chapter 4.

Population genetic assumptions should be considered in any DNA-barcoding analysis. The identification of unknown organisms by assignment of query sequences to specific species can fail if the demographics have not been properly modelled (Nielsen & Matz, 2006). The Bayesian Interference (BI) method (Huelsenbeck & Ronquist, 2001) uses a tree-based principle combined with a population genetics statistical approach (Nielsen & Matz, 2006). The Statistical Assignment Package (SAP) uses a Bayesian method with a Markov chain Monte Carlo (MCMC) approach (Munch *et al.*, 2008a). Similar to the other tree-based methods, the BI method estimates the probability of the query sequence falling into a monophyletic group (Munch *et al.*, 2008a), thus the identity of the query sequence is determined by the clade it clusters with. Although this method solves problems related to limited sampling coverage and

single gene reconstruction (Munch *et al.*, 2008a; Sandionigi *et al.*, 2012), it can make incorrect phylogenetic assumptions (Nielsen & Matz, 2006) and have difficulty in coping with the computational requirements of very large datasets generated from metagenomic applications (Munch *et al.*, 2008b).

The Neighbor-joining (NJ) method with Kimura 2 parameter (K2P) (Kimura, 1980) uses a heuristic approach instead and is the most widely used tree drawing method for DNA-barcoding analysis (Munch *et al.*, 2008b; Sandionigi *et al.*, 2012). Analysis of large datasets is fast and has a good performance if the sequence divergence is low (Hebert *et al.*, 2004). Despite its simpler approach compared to complex MCMC analysis, the NJ method has the same (or even better) performance compared to the full Bayesian approach (Munch *et al.*, 2008b).

There are controversies in the opinions as to which method is the best for barcoding analyses. Both MP and BI are tools for reconstructing phylogenies, and in spite of the fact that BI requires high calculation power (Little, 2011), it seems that both these tools are superior to NJ (Sandionigi *et al.* 2012).

### 2.1.3 Character-based and diagnostic methods

Character-based analyses focus on the similarities and differences (or presence/absence) of characters or a set of characters (DeSalle *et al.*, 2005; DeSalle, 2006; Sarkar *et al.*, 2008; Rach *et al.*, 2008; Sandionigi *et al.*, 2012). Bergmann *et al.* (2009) describes these characters as unique SNPs (single nucleotide polymorphisms). These authors state that a character-based barcode for the pre-described taxon is created by combining the single SNPs using a complex algorithm.

An advantage of the character-based method is that the data is not dependent on molecular (SNP) data alone, but other type of characters can be included in the data matrix. Additional data such as amino acids (Sarkar *et al.*, 2002a), expression patterns (Sarkar *et al.*, 2002b), morphological data and even geographical distribution (Rach *et al.*, 2008) can thus be incorporated to increase the number of diagnostic characters. In this way, the character-based barcoding system can have the same degree of effectiveness as a traditional

morphological identification system and be effective for identifying species, subspecies and populations (Bergmann *et al.*, 2009).

The sequences to be analysed can be aligned, or unaligned, depending on the analyses and type of software that is used. The main programs to use for these analyses are BLOG (Bertolazzi *et al.*, 2009), BRONX (Little, 2011), CAOS (Sarkar *et al.*, 2008) and DNA-BAR (DasGupta *et al.*, 2005). Both the programs CAOS and BLOG uses and analyses aligned sequences, compared to BRONX and DNA-BAR which are not dependent on alignments (Sandionigi *et al.*, 2012). Using the P-GNOME analyses with CAOS speeds up and simplifies the data analysis (Rach *et al.*, 2008).

Some gene regions are extremely variable, especially between distantly related taxa and are difficult to align. Improper alignments can have an influence on the analysis of the data. Sandionigi *et al.* (2012) suggests the use of alignment-free diagnosis methods, and in a study of van Velzen *et al.* (2012), it was determined that BLOG has the best performance of all methods. Each of these tools have advantages and shortcomings for various types of datasets and different stages of analyses (Sandionigi *et al.*, 2012).

## 2.2 REFERENCE DATABASES

To properly implement the use of DNA-barcoding analyses in species identification, a good referencing dataset is essential. Although GenBank<sup>2</sup> contains sequences of many DNA regions used in plant DNA-barcoding, it is not a good tool to use in DNA-barcode identification. GenBank contains many taxonomical errors, thus sequences stored as reference may have been incorrectly assigned to species. Furthermore, many species are represented by only a single sequence for a specific region on GenBank, implicating that there can be an underestimation of the variation in a species due to introgression, incomplete lineage sorting and geographical coverage (Sandionigi *et al.*, 2012).

Sandionigi *et al.* (2012) suggests three steps to consider when identification needs to be made based on a barcode. Even though the data in GenBank is not always very reliable,

---

<sup>2</sup> <http://www.ncbi.nlm.nih.gov/genbank/>

due to wrong species identification, it does contain a large depository of sequences and is a good tool to identify species where morphological data is lacking, especially for samples obtained from forensic cases. The first step will therefore be to do a BLAST search on GenBank. The BLAST (Basic Local Alignment Search Tool) algorithm is used to compare a sequence to the available reference sequences in GenBank.

Step two will be to search in BOLD, using a combination of similarity methods and distance tree reconstruction. The data in BOLD is more reliable, but finding a match can be more difficult.

The last step will be used in case that of an unsuccessful identification in BOLD due to the lack of samples for that specific organism. Identification of that organism will have to rely on the comparison of the unknown organism to a local database (Sandionigi *et al.*, 2012). The database of BOLD will have to expand before this system can be used with a high success rate (Sandionigi *et al.*, 2012).

### 2.2.1 GenBank

The National Centre for Biotechnology Information (NCBI) is a subdivision of the National Library of Medicine (NLM) situated in Bethesda, MD, USA and is the builder and distributor of the publicly available nucleotide sequence database GenBank (Benson *et al.*, 2008). GenBank, together with the European Molecular Biology Laboratory Nucleotide Sequence Database (EMBL) and the DNA Databank of Japan (DDBJ), form part of the International Nucleotide Sequence Database Collaboration (INSDC).

GenBank support the submission of DNA-barcoding sequences and the NCBI, together with CBOL<sup>3</sup>, has created a tool for bulk DNA-barcode submissions to GenBank<sup>4</sup> (Benson *et al.*, 2008). All the DNA-barcode sequences in GenBank are tagged as “barcodes”.

Some scientists have criticised the quality of data in GenBank, e.g. Kwong *et al.* (2012) compared the DNA-barcoding data available in GenBank with that in BOLD. Some of the shortcomings are that a large amount of the sequences are not assigned to species (Kwong *et*

---

<sup>3</sup> [www.barcoding.si.edu/index.htm](http://www.barcoding.si.edu/index.htm)

<sup>4</sup> [www.ncbi.nlm.nih.gov/BankIt/websub/?tool=barcode](http://www.ncbi.nlm.nih.gov/BankIt/websub/?tool=barcode)

*al.*, 2012), and that there is no direct link between sequences and data of the source specimen, such as an image or GPS coordinates. Also, no data standard is enforced (Ratnasingham & Hebert, 2007). The data in GenBank are in many instances incomplete and unreliable.

### **2.2.2 The Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007)**

BOLD is the most relevant barcoding database available (Sandionigi *et al.*, 2012). The main aim of developing BOLD was to create software that can accommodate large scale, as well as novel aspects of DNA-barcoding data. BOLD has formal guidelines for the submission of DNA-barcodes. The main criteria are that the specimen must be taxonomically reviewable through linkage to a specimen in a collection, the sequences must be of good quality and lastly, the sequences must derive from designated gene regions. BOLD provides a repository of DNA-barcoding sequences (including the trace files) with the specimen data (such as species name, voucher data, collection record, identifier, primer information and an image of the specimen). All these data are organized, stored and open to access after the data have been published (Ratnasingham & Hebert, 2007).

The management and analysis system (MAS) in BOLD provide an analysis platform for various types of data analyses. Firstly a taxon ID tree is constructed by using distance metrics, followed by a NJ tree to match specimens. Second, nearest-neighbour analysis is used to detect incongruities in the sequence data, i.e. cases when two identical sequences are assigned to two different species. When the data are ready for publication, a copy of the sequences are transferred to NCBI or to the other major genomic repositories [DNA Data Bank of Japan (DDBJ) and European Molecular Biology Laboratory (EMBL)] (Ratnasingham & Hebert, 2007).

BOLD uses an identification system (IDS) to identify unknown specimens. A match is made when the query sequence has less than 1% divergence compared to a reference sequence. To identify a specimen, the query sequence is pasted in the ID page. A Hidden Markov Model (HMM) is used to align this sequence to a global alignment of the reference sequences. A linear search is performed to find the nearest neighbours. The IDS searches all

the sequences in the database, even where reference sequences have not been validated or made public (Ratnasingham & Hebert, 2007).

When sequences that are deposited in the database are validated by the IDS, they are tested for any incongruence. An example is when two species with identical sequences are listed as different species. All the invalidated sequences remain in a separate database, but can also be used in search queries. The validated sequences are used as the default search library. These sequences have a minimum of three representatives in the database and a maximum intraspecific divergence of 2% (Ratnasingham & Hebert, 2007). By analysing multiple sequences per species, the population structure can also be investigated and considered in assigning specimens to species (Nielsen & Matz, 2006).

Unfortunately, the BOLD database is still incomplete (Munch *et al.*, 2008b; Sandionigi *et al.*, 2012). Based on a search through BOLD (April 2013), most publicly available records for the *matK* and *rbcl*a regions are from data deposited in GenBank, confirming the need for a major expansion of the more appropriate BOLD database (Figure 2.2). The number of published records based on sequences of *matK* and *rbcl*a for the family Asparagaceae far exceeds that of the families Amaryllidaceae and Hyacinthaceae (Table 2.2). More species from the family Asparagaceae are also represented in these publications than for the other two families. The number of records for the Hyacinthaceae is included in the comparison due to the fact that the genus *Lachenalia* previously was a member of the family Hyacinthaceae.

BOLD is also criticised for using the mean interspecific gap instead of the smallest interspecific distance (Meier *et al.*, 2008). It is therefore recommended that BOLD should be used in conjunction with an online tool, The Barcode of Life Data Portal (BDP)<sup>5</sup>. Most DNA-barcoding analytical tools are only available as separate software. The “Aggregator” tool of BDP can retrieve DNA-barcoding records from BOLD, INSDC databases (GenBank, ENA and DDBJ) and the user’s own collection of sequences and then do analyses that comply with the prerequisites needed for barcoding. Five of the analytical tools developed by the DAWG (BLOG, DNA-Barcode Linker, BLAST and the Barcode Data Release Report) are included in BDP and the future goal is to incorporate other tools such as geo-location or “morphology based

---

<sup>5</sup> <http://bol.uvm.edu/>



analyses” (Sarkar & Trizna, 2011). In order to eliminate possible DNA-barcoding errors, the molecular data should be combined with traditional morphological approaches (Dexter *et al.*, 2010).

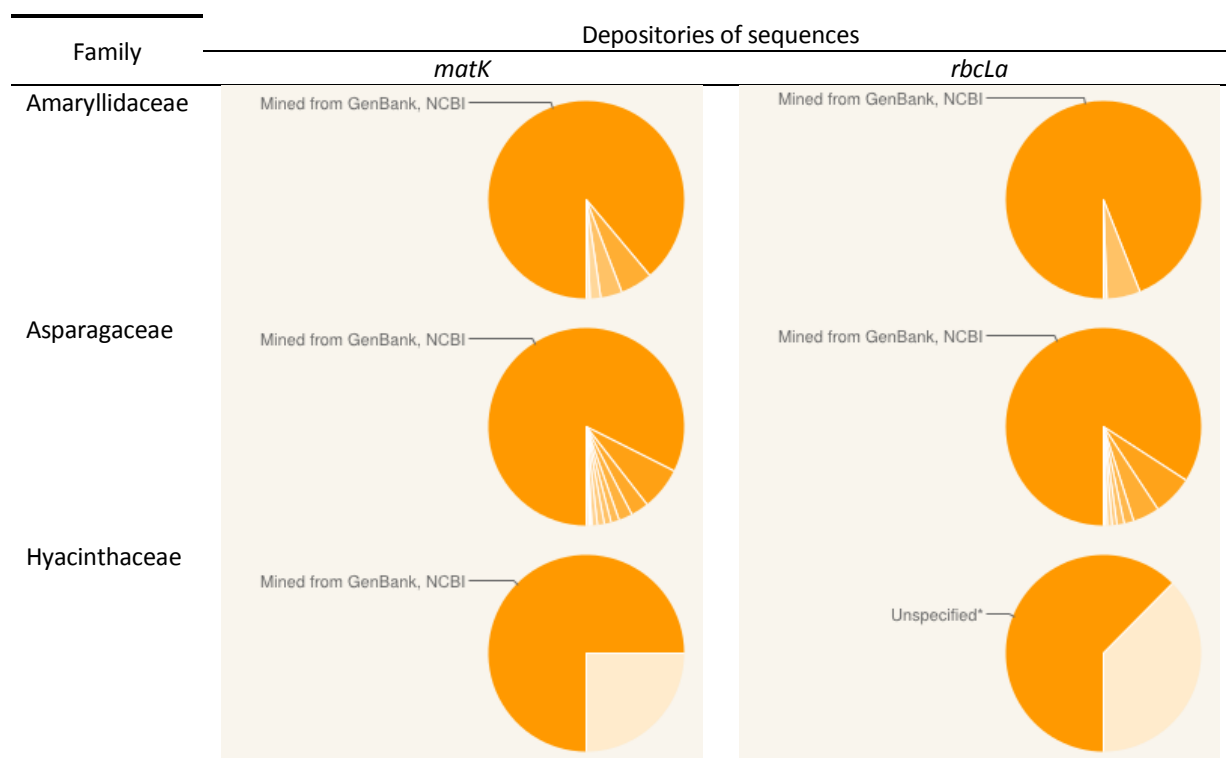


**Figure 2.2** Sequences for *matK* and *rbcLa* are deposited in various publicly available databases. The majority sequences for these two regions are deposited in GenBank, NCBI (dark shade in the pie charts). The rest of the sequences are deposited in institutes such as museums, universities and herbaria represented by the different shades in the pie chart (see Appendix A for detail for each pie chart). Charts were obtained through the search function of the BOLD website ([www.boldsystems.org](http://www.boldsystems.org)).

In addition to BOLD and BDP, other search systems have been developed, such as the Google based web server<sup>6</sup> (Hajibabaei & Singer, 2009) and the DNA-Barcode Linker (Albu *et al.*, 2011).

## 2.3 DNA-BARCODE DATA STANDARDS

The Database Working Group (DBWG) was established in 2004 and proposed a five-component standard that all reference DNA-barcoding sequences should comply with

<sup>6</sup> <http://www.ibarcode.org/gg>

(Hanner, 2009). The most important of these components are the guidelines for the quality and coverage of the sequences.

**Table 2.2** A comparison between the published records found for the three families Amaryllidaceae, Asparagaceae and Hyacinthaceae. These data were obtained in April 2013 through the search function of the BOLD website ([www.boldsystems.org](http://www.boldsystems.org)).

|                | Number of published records<br>( <i>matK/rbcLa</i> ) | Number of names with records<br>( <i>matK/rbcLa</i> ) | Number of species represented<br>( <i>matK/rbcLa</i> ) |
|----------------|------------------------------------------------------|-------------------------------------------------------|--------------------------------------------------------|
| Amaryllidaceae | 170/182                                              | 166/181                                               | 104/140                                                |
| Asparagaceae   | 443/503                                              | 438/498                                               | 273/314                                                |
| Hyacinthaceae  | 4/8                                                  | 4/8                                                   | 4/8                                                    |

The DBWG recommends that, prior to creating the contig sequences, the ends of the sequences should be trimmed to remove bases with low Phred scores. A Phred score > 20 has a base call accuracy of 99% and is an indication of high quality base calls. Bases which are considered to be of very high quality have a Phred score of > 30, thus 99.9% accuracy. It is thus important to record all bases with a Phred < 20 as “N” (unknown nucleotide). When manually editing the bidirectional sequences, the bases with the high or very high Phred scores should be retained. To be considered as a barcode, the contig of the reference sequence should have bi-directional sequence coverage and the “non-N base calls” should not be less than 40% of the sequence (Hanner, 2009).

A PERL script have been developed to compute a barcode quality index (*B*) (Little, 2010) based on the quality and contig overlap of the DNA-barcoding sequences and this is freely available for download<sup>7</sup>.

## 2.4 METHODS USED DURING THE CURRENT STUDY

All the chemicals for DNA extraction, PCR, sequencing and flow cytometry were of analytical grade. The following ready mix PCR kits were used: KapaTaq Readymix (KapaBiosystems), Fermentas PCR Master Mix (Thermo Scientific Fermentas), EmeraldAmp GT PCR Master Mix (Takara) and Phire<sup>®</sup> Plant Direct PCR Kit (Thermo Scientific Finnzymes). Proteinase K (Promega) and RNase (Sigma) were used in some of the DNA extractions and for the flow cytometry. The BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems<sup>®</sup>) was used for the sequencing PCR.

<sup>7</sup> <http://www.nybg.org/files/scientists/dlittle/B.html>

### 2.4.1 DNA extraction

The leaves collected from *Clivia* enthusiasts were mostly the old, outer leaves of the plants. These leaves contain more secondary compounds compared to young leaves and it was therefore more difficult to obtain good quality DNA. To overcome this problem, several different CTAB extraction methods were tested, combined and/or modified (Saghai-Marooft *et al.*, 1984; Doyle & Doyle, 1987; Doyle & Dickson, 1987; Pyle & Adams, 1989). To remove phenols, combinations of the anti-oxidants ascorbic acid, PVP and  $\beta$ -mercapto ethanol were added to the extraction buffer. To remove polysaccharides, different NaCl and sodium sulphite ( $\text{Na}_2\text{SO}_3$ ) concentrations were added to the extraction buffer. DIECA was added to reduce polyphenol oxidase activity and to inhibit nuclease activities. Proteinase K and RNase was used to remove proteins and RNA respectively (Henry, 2008). When young *Clivia* leaves were available, DNA was extracted using a modified protocol described by (Rogstad, 1992).

Prior to DNA extraction from both *Clivia* and *Lachenalia* leaves, CTAB (3% m/v) and 0.2% (m/v) 2-Beta-mercapto-ethyl-alcohol was added to the extraction buffer (pH 8) (100 mM Tris-HCl; 25 mM EDTA; 1.4 M NaCl) and preheated to 65°C. Approximately 1 g of plant material was cut into a mortar. A smaller amount of silica-preserved leaves were used since these leaves are dehydrated and this will alter the ratio between leaf material and extraction buffer.

Purified sand (0.1-0.2 g), as well as 1 ml of the preheated extraction buffer, were added to the leaf material. The mixture was ground with a pestle until it formed a paste and transferred to a 15 ml test tube. This was followed by  $\pm$  60 minutes incubation in a preheated water bath at 65°C. The test tubes were vortexed briefly every 10-15 minutes. Thereafter, one volume of chloroform:iso-amylalcohol (24:1 v/v) was added and the tube contents thoroughly mixed. The test tubes were centrifuged for 5 minutes at 3 000 g and the supernatants were transferred to clean test tubes. The DNA was precipitated with cold (-20°C) absolute ethyl-alcohol with 3 M sodium acetate (25:1) for at least 60 minutes at -20°C or overnight at 4°C. The test tubes were centrifuged at 10 000 g for 10 minutes, the supernatant discarded and the pellet washed with 70% (v/v) ethyl-alcohol, containing 10 mM ammonium acetate. Another centrifugation step for 5 minutes at 10 000 g followed, and the supernatants were discarded. The DNA pellets were allowed to dry at room temperature. DNA was

dissolved (overnight at 4°C) in 20-100 µl sterile water. This volume depended on the size of the pellet. The DNA was treated with RNase for 30 minutes at 37°C and transferred to micro-tubes and stored at -20°C.

## 2.4.2 PCR

Two different PCR techniques were used for amplifying all the gene regions in this study (Table 2.3). These techniques were direct PCR (Phire® Plant Direct PCR Kit, Finnzymes) and standard PCR protocols.

**Table 2.3** Primers used for PCR in the present study. T<sub>m</sub>, CG and length were calculated with Thermo Scientific online primer calculator<sup>8</sup>

| DNA region       | Primer                         | Primer sequence 5'-3'      | T <sub>m</sub> (°C) | CG%  | Length |
|------------------|--------------------------------|----------------------------|---------------------|------|--------|
| <i>ITS2</i>      | ITS2-S2F                       | ATGCGATACTTGGTGTGAAT       | 51.9                | 40.0 | 20     |
|                  | ITS4                           | TCCTCCGCTTATTGATATGC       | 52.1                | 45.0 | 20     |
| <i>matK</i>      | <sup>1</sup> 472F              | CCCRTYCATCTGGAAATCTTGTTTC  | 71.0                | 48.0 | 25     |
|                  | <sup>1</sup> 1248R             | GCTRTRATAATGAGAAAGATTCTGC  | 62.3                | 34.6 | 26     |
|                  | <sup>2</sup> MatK2.1           | CCTATCCATCTGGAAATCTTAG     | 58.7                | 40.9 | 22     |
|                  | <sup>2</sup> MatK5             | GTTCTAGCACAAAGAAAGTCG      | 57.3                | 45   | 20     |
|                  |                                |                            |                     |      |        |
| <i>psbA-trnH</i> | psbA3_f                        | GTTATGCATGAACGTAATGCTC     | 61.4                | 40.9 | 22     |
|                  | trnHf_05                       | CGCGCATGGTGGATTACAATCC     | 76.1                | 56.5 | 23     |
| <i>atpH-atpI</i> | atpH-P                         | CCAGCAGCAATAACGGAAGC       | 67.1                | 55   | 20     |
|                  | atpI-M                         | ATAGGTGAATCCATGGAGGG       | 62.9                | 50   | 20     |
| <i>trnL-F</i>    | c                              | CGAAATCGGTAGACGCTACG       | 64.8                | 55   | 20     |
|                  | f                              | ATTTGAACTGGTGACACGAG       | 60.7                | 45   | 20     |
|                  | <sup>3</sup> Nested primer PS1 | CTACGGACTTAATTGGATTGAGC    | 62.9                | 43.5 | 23     |
|                  | <sup>3</sup> Nested primer PS4 | AGGATTTTCAGTCCTCTGCTC      | 61.8                | 47.6 | 21     |
|                  |                                |                            |                     |      |        |
| <i>rbclLa</i>    | rbclLa-F                       | ATGTCACCACAAACAGAGACTAAAGC | 65.6                | 42.3 | 26     |
|                  | rbclLa-R                       | GTAATAATCAAGTCCACCRGC      | 62.3                | 47.5 | 20     |
| <i>rpoC1</i>     | rpoC1 2                        | GGCAAAGAGGGGAAGATTTTCG     | 65.1                | 50   | 20     |
|                  | rpoC1 4                        | CCATAAGCATATCTTGAGTTGG     | 60.5                | 40.9 | 22     |
| <i>rpoB</i>      | rpoB 2                         | ATGCAACGTCAAGCAGTTCC       | 65.6                | 50   | 20     |
|                  | rpoB 4                         | GATCCCAGCATCACAAATTC       | 64.7                | 50   | 20     |
| <i>trnT-trnL</i> | a                              | CATTACAAATGCGATGCTCT       | 60.8                | 40   | 20     |
|                  | b                              | TCTACCGATTTCCGCATATC       | 61.9                | 45   | 20     |
| <i>rpl16</i>     | F71                            | GCTATGCTTAGTGTGACTCGTTG    | 66.1                | 48   | 25     |
|                  | R1661                          | CGTACCCATATTTTCCACCECGAC   | 69.7                | 50   | 24     |

<sup>1</sup>MatK primers used for *Lachenalia* samples

<sup>2</sup>MatK primers used for *Clivia* samples

<sup>3</sup>Nested primers were used for the sequencing PCR reactions and were named in this study as PS1 and PS4 (based on the first letters of the authors).

Primer references: ITS2-S2F (Chen *et al.*, 2010); ITS4 (White *et al.*, 1990); psbA3\_f (Sang *et al.*, 1997); trnHf\_05 (Tate & Simpson, 2003); atpH-P & atpI-M (Heinze, 2007); primers a, b, c & f (Taberlet *et al.*, 1991); nested PS1 & PS2 primers; rbclLa-F (Levin *et al.*, 2003); rbclLa-R (Kress & Erickson, 2007); matK2.1, matK5, rpoC1 2, rpoC1 4, rpoB 2, rpoB 4 (Ford *et al.*, 2009); F71, R1661 (Jordan *et al.*, 1996); 472F & 1248R (Yu *et al.*, 2011).

<sup>8</sup> [http://www.finnzymes.com/java\\_applets/multiple\\_primer\\_analyzer.html](http://www.finnzymes.com/java_applets/multiple_primer_analyzer.html)

**Direct PCR** The DNA regions *trnL-F*, *psbA-trnH*, *atpH-I*, *matK* and *ITS2* in some *Clivia* and *Lachenalia* species were amplified from fresh or silica dried leaves with a direct PCR kit (Finnzymes) using the suggested PCR reaction mix (Table 2.4). The PCR conditions were used according to the protocol (Table 2.5) with the following annealing temperatures: *ITS2* (53°C), *psbA-trnH* (53.7°C), *trnL-F* (52.8°C), *atpH-atpI* (53.7°C) and *matK* (primers 472F and 1248R) (53.7°C). Because of time constraints the direct PCR approach was chosen for most of the *Lachenalia* samples.

**PCR** All the *Clivia* samples were amplified using either a standard reaction mix (Table 2.4) or different ready mixes. These mixes (KapaTaq Ready Mix DNA Polymerase, EmeraldAmp GT PCR Master Mix and Fermentas PCR Master Mix) were used according to the protocols provided. The MgCl<sub>2</sub> concentration for amplification of *atpH-atpI* was 2 mM as suggested by Heinze (2007). The conditions for the different regions were optimised and the *Clivia* samples in the study were amplified using different PCR conditions (Table 2.5).

**Table 2.4** Recipes for the direct PCR and standard PCR methods.

| Component                                  | Final concentration |                    |
|--------------------------------------------|---------------------|--------------------|
|                                            | Direct PCR          | PCR                |
| Water                                      | up to total volume  | up to total volume |
| Buffer                                     | 1 X                 | 1 X                |
| F primer                                   | 0.5 µM              | 1 µM               |
| R primer                                   | 0.5 µM              | 1 µM               |
| DMSO*                                      | 5%                  | 5%                 |
| Phire® Hot Start II DNA Polymerase mixture | 0.4 µl in 20 µl     |                    |
| TaqPol                                     | 0 <sup>#</sup>      | 1.5U               |
| MgCl <sub>2</sub>                          | 0 <sup>#</sup>      | 2.5 mM             |
| dNTPs                                      | 0 <sup>#</sup>      | 0.4 mM             |
| DNA/DNA punch                              | 10-100 ng           | 50-200 ng          |

\*In some cases omitted from PCR reactions

<sup>#</sup>Already included in the Phire Hot Start mixture

### 2.4.3 Qualification and quantification of DNA and PCR products

The quality of the DNA and PCR products was determined by visualising on 1% (m/v) agarose gels prepared with TAE buffer {1% (m/v) agarose; 1x TAE buffer [50x TAE (48.44 g Tris; 11.42 ml acetic acid; 2.92 g EDTA)]}. The DNA and PCR products were stained with GelRed<sup>TM</sup> that was added to the loading buffer according to the specifications of the supplier. Nucleic acids were visualised and photographed with the G:Box Syngene system using the software GeneSnap V7.09 (Synoptics).

Nucleic acids were further quantified and qualified by measuring the concentrations and the A260/A280 ratio's with a Nanodrop Lite spectrophotometer (Thermo Scientific).

#### 2.4.4 Sequencing

All the DNA regions were sequenced by using the BigDye® Terminator v3.1 Cycle Sequencing Kit according to the protocol provided. Quarter volume reactions were prepared with 0.5 µl sequencing premix and a 3.2 µM final concentration for the primers. The other components were 5x sequencing buffer and 3-20 ng PCR template. Standard cycling conditions were used [25 cycles of denaturation (10 sec @ 96°C); primer annealing (5 sec @ 50°C); extension (4 min @ 60°C)].

**Table 2.5** Cycling conditions for the gene regions amplified in this study.

|                        | atpH-atpI, trnL-F     | <sup>1</sup> MatK, rpoB, rpoC1 | trnT-L, rpl16 | ITS2          |
|------------------------|-----------------------|--------------------------------|---------------|---------------|
| <b>Initial step 1</b>  | 30 min @ 4°C          |                                |               |               |
| <b>Initial step 2</b>  | 3 min @ 94°C          |                                |               |               |
| <b>Cycle 1</b>         | Touchdown:<br>x2 each | x30                            | x28           | x35           |
|                        | 45 sec @ 94°C         | 30 sec @ 94°C                  | 1 min @ 94°C  | 30 sec @ 95°C |
|                        | 45 sec @ 58°C;        | 40 sec @ 53°C                  | 1 min @ 50°C  | 30 sec @ 54°C |
|                        | 57°C; 56°C; 55°C;     |                                |               |               |
|                        | 54°C; 53°C            |                                |               |               |
|                        | 90 sec @ 72°C         | 40 sec @ 72°C                  | 3 min @ 72°C  | 1 min @ 72°C  |
| <b>Cycle 2</b>         | x25                   |                                |               |               |
|                        | 45 sec @ 94°C         |                                |               |               |
|                        | 45 sec @ 51°C         |                                |               |               |
|                        | 90 sec @ 72°C         |                                |               |               |
| <b>*Cycle 3</b>        | x10                   |                                |               |               |
|                        | 45 sec @ 94°C         |                                |               |               |
|                        | 45 sec @ 49°C         |                                |               |               |
|                        | 90 sec @ 72°C         |                                |               |               |
| <b>Final extension</b> | 7 min @ 72°C          |                                |               |               |
| <b>Store</b>           | 30 min @ 4°C          |                                |               |               |

<sup>1</sup> Primers MatK2.1 & MatK5

\* Denaturing temperature for the KapaTaq readymix

The products were purified with EDTA/EtOH precipitation in a 96 well plate, where 2.5 µl 125 mM EDTA was first added to the PCR products to remove metals in the reaction, and 25 µl 100% ethanol was added to precipitate the extension product. The reactions were protected with an aluminium tape covering and inverted four times. The extension products were precipitated at room temperature for 15 minutes. The centrifuge was cooled to 4°C and the plate was centrifuged for 45 minutes at 1 650 *g*. The supernatant was subsequently aspirated by inverting the plate and centrifuging it up to 185 *g*. A volume of 30 µl ethyl-

alcohol (70% v/v) was added to each well and the plate was centrifuged at 4°C for 15 minutes at 1 650 *g*. The supernatant was again aspirated by inverted centrifugation for 1 minute at 185 *g* and the samples were dried in the Perkin Elmer GeneAmp PCR system 9600 at 60°C for 5 minutes (the samples were protected from light during this step).

The dried sequencing products were resuspended in buffer and analysed with an automated ABI 3130 sequencer.

Some specimens were processed by the Canadian Centre for DNA-Barcoding (CCDB) for DNA-barcoding according to a draft protocol<sup>9</sup> submitted to the DNA Working Group by Ivanova *et al.* (date unknown).

#### 2.4.5 2C measurements

The genome sizes (2C-values) of some *Clivia* and *Lachenalia* samples were measured to compare the genome sizes of different samples with their chromosome numbers. The genome sizes were measured using the two-step procedure with Otto I+II buffers (Otto, 1990) and by the chopping method of Galbraith *et al.* (1983). A small amount of fresh leaf sample (1 g) was finely chopped with a razor blade in a petri dish with 1 ml of ice-cold Otto I buffer [0.1 M citric acid monohydrate; 0.5 % (v/v) Tween 20]. The suspension was filtered through a CellTric 30 µm nylon mesh (Partec) into an eppendorf tube and incubated for 10 minutes on a shaker. The samples were centrifuged (1 200 rpm for 5 min), the supernatant removed and 100 µl of fresh ice-cold Otto I buffer was added. The samples were incubated at room temperature for 5-60 minutes with occasional shaking. Otto II buffer (0.4M Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O) was supplemented with propidium iodide (diluted 1 mg/ml with a final concentration of 50 µg/ml), β-mercaptoethanol (2 µl/ml) and RNase stock solution (1 mg/ml) with a final RNase concentration of 0.15 mg/ml. After incubation, 1 ml of Otto II buffer were added, the tubes were stored on ice in a dark cupboard for 5-15 minutes, and the isolated nuclei were analysed with the BD Accuri C6 flow cytometer. Three leaf samples per species was measured and three replicates per sample were measured. For each run, 5000 particles were analysed.

---

<sup>9</sup> [http://barcoding.si.edu/PDF/Protocols\\_for\\_High\\_Volume\\_DNA\\_Barcode\\_Analysis.pdf](http://barcoding.si.edu/PDF/Protocols_for_High_Volume_DNA_Barcode_Analysis.pdf) (accessed 23 May 2013)

*Pisum sativum* (2C = 9.09 pg) was used as a reference standard to estimate the genome sizes (Doležal *et al.*, 1998).

## 2.4.6 Data analyses

### 2.4.6.1 Editing and alignment of sequences

The software programme Geneious Pro 6.1.2<sup>10</sup> was used to view, assemble and edit the sequence trace files. Consensus sequences were aligned with the MUSCLE (Edgar 2004a, b) plugin in Geneious R6. Other statistical values for each gene region such as the GC content, number of parsimonious informative sites, number of variable sites, the minimum, maximum and mean lengths for the sequences as well as the pairwise % identity were also obtained in Geneious R6.

To combine loci, the datasets were imported into SequenceMatrix 1.7.8. (Vaidya *et al.*, 2011). The number of gene-sets per sample were determined with this software, and the combined matrixes were constructed and exported as NEXUS (Maddison *et al.*, 1997) format files to be used in other software programs.

### 2.4.6.2 Tree-based analysis

Tree-drawing/searching methods can be divided into two categories, i.e. the character *versus* distance-based methods. Character-based methods include Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian (BI) methods, whereas the distance-based methods include Neighbor-Joining (NJ) and UPGMA methods to create trees (Hall, 2011).

**Character-based tree drawing method:** The MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001) plugin to Geneious R6 was used to construct Bayesian Interference (BI) cladograms. The HKY85 substitution model was used with an equal rate of variation. The MCMC settings were kept on default values: the rate variation was equal with a chain length of 1 100 000 with four heated chains and a heated chain temperature of 0.2. The sub-sampling frequency was 200 and the burn-in length was set to 100 000. Under the priors setting, the molecular

---

<sup>10</sup> [www.geneious.com](http://www.geneious.com)



clock with uniform branch lengths was chosen with an exponential tree age of one.

The ML and MP phylograms were constructed in MEGA 5.05 (Tamura *et al.*, 2011), after determining the best substitution model for each analysis. To determine the best model, the “find best DNA/Protein Models (ML)” function was chosen using default settings. Thus a NJ tree was used in the analysis and ML for statistical purposes. All gaps/missing data were included.

The best fit model differed for the gene regions, and was determined by the “find best DNA/Protein Models” in MEGA. The reliabilities of the ML trees were tested with 500 bootstrap replicates including all missing data such as gaps. For inferring the tree, the nearest-neighbor-interchange (NNI) option was chosen. All the trees were rooted with the relevant outgroup genus.

For the MP trees, the number of bootstrap replications was decreased to 100 replicates. It would be more ideal to use bootstrap replicates of 1 000, but extremely slow calculation times for the MP cladograms compelled the use of these lower number of replicates. All gaps and missing data were included in the analysis and the Close-Neighbor-Interchange (CNI) was used in random trees in the tree inference options. The number of initial trees was 10.

**Distance-based tree-drawing method:** The best DNA substitution model for each dataset was determined, applied in the substitution analysis and the distance-based trees (NJ, UPGMA) was constructed in MEGA version 5.05 (Tamura *et al.*, 2011). All trees were tested using 500 bootstrap replications.

**Tree editing:** All the cladograms were saved in Enhanced Metafile (.emf) format in MEGA 5.05. The Maximum Likelihood (ML) cladogram from each region was selected as representative of all the cladograms from the different tree drawing methods. The bootstrap values of each tree were added to the braches in Microsoft Word.

#### **2.4.6.3 Character-based barcoding analysis**

Variation and SNP's were located in Geneious R6 and the results were exported as a

comma separated values (CSV) file. The SNP's and INDELS were compared to different aspects that may be involved in the evolution or speciation processes, e.g. geographical distribution, chromosome numbers and ploidy levels.

#### **2.4.6.4 Evolutionary distances and the barcoding gap**

Pairwise differences were used to cluster the samples in Molecular Operational Taxonomic Units using the program jMOTU<sup>11</sup> (Jones *et al.*, 2011). The FASTA sequences for each data set were separately loaded in jMOTU. A multiple range (1-20) was used as parameters for the MOTU definition and the low BLAST identity filter was set on 97. The graphs created for the cut off distribution as well as detailed Microsoft Excel documents for each cut off value were exported for further analyses.

The distances within and between species were determined in MEGA 5.05 using the Tamura 3-parameter model (Tamura, 1992). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). All positions containing gaps and missing data were eliminated. The standard deviations for the intraspecific variation were determined using MEGA 5.05. Interspecific standard deviations were calculated in Microsoft Excel 2010.

The barcoding gap of the combined two-loci barcodes of *matK* and *rbcLa* was determined with the online tool Automatic Barcode Gap Discovery (ABGD)<sup>12</sup> using the default settings (Puillandre *et al.*, 2011). The barcoding gaps for *matK* and *rbcLa* was separately determined with the online tools of BOLD.

#### **2.4.6.5 Data analyses**

Geneious R6 and MEGA 5.05 was used for determining the sequence statistics for each separate gene region, e.g. the sequences length, GC content, percentage of gaps, number of parsimonious informative sites and the number of variable sites.

---

<sup>11</sup> <http://www.nematodes.org/bioinformatics/jMOTU/index.shtml>

<sup>12</sup> <http://www.abi.snv.jussieu.fr/public/abgd/>

BOLD software was used to analyse the *matK* and *rbcLa* barcoding sequences that was submitted by the CCDB. The Kimura 2 Parameter (K2P) is a good metric to use when distances are low (Nei & Kumar, 2000) and was used as a distance model to construct distance summaries and to determine the sequence composition

#### **2.4.6.6 Phylogeography and specimen mapping**

The geographical distribution of the specimens were visualised in QuantumGIS version 1.8.0-Lisboa<sup>13</sup> on a Köppen-Geiger map. The approximate original collection site provided by each collector for each sample, was notated as GPS coordinates with the header of the latitude and longitude abbreviated as *Lat* and *Lon* respectively. The document was saved as a comma delimited (.csv) file. This file was imported in QuantumGIS by selecting the option “create a layer from a delimited text file” and consequently each coordinate was plotted and visualised on a map.

The free software GenGIS 2.2.1 was used for constructing a visual phylogeographical comparison between the Bayesian Inference cladogram and geographical distribution according to the user manual.

#### **2.4.6.7 SpeciesIdentifier analyses**

SpeciesIdentifier 1.7.8 was used for the combined *matK* and *rbcLa* matrices to determine aspects discussed by (Meier *et al.*, 2006), such as the intraspecific and interspecific as well as the congeneric pairwise (uncorrected) distances. In addition, sequences with unusual genetic distances were determined. The ‘Best match and best close match’ (Meier *et al.*, 2006) was used to determine the degree under which a species are identifiable. The ‘consensus barcode generator’ option in SpeciesIdentifier was used to construct consensus barcodes for species that has intraspecific variation.

---

<sup>13</sup> <http://www.qgis.org/>

#### 2.4.6.8 Networks

Splitstree (Huson & Bryant, 2006), NETWORK 4.6.1.1<sup>14</sup> and Geneious were used to construct networks using the NeighborNet and median-joining options (Bandelt *et al.*, 1999) respectively. The data for NETWORK was prepared by removing uniform sites in Geneious R6. The polymorphism file was exported in a strict Phylip (\*.phy) format and opened in NETWORK for further analyses.

Networks were enhanced by the public software Inkscape 0.48<sup>15</sup>. The unrooted trees were saved in the Scalable Vector Graphic (.svg) data format in Geneious, edited in Inkscape and exported as an Enhanced Metafile (.emf). These pictures were imported into Microsoft Word to present the final edited format.

The sequences of all the gene regions were exported using the online tool Format Converter 2.2.5<sup>16</sup> with the 'pretty print' option and was sequentially added to the appendixes.

---

<sup>14</sup> <http://www.fluxus-engineering.com/sharenet.htm>

<sup>15</sup> <http://www.inkscape.org>

<sup>16</sup> [http://www.hiv.lanl.gov/content/sequence/FORMAT\\_CONVERSION/form.html](http://www.hiv.lanl.gov/content/sequence/FORMAT_CONVERSION/form.html)

## Part B: *Clivia*

### *Chapter 3*

A review of phylogenetic relationships in the  
genus *Clivia*



modified from Spies, P., Grobler, J.P. and Spies, J.J. 2011 *Philosophical Transactions in Genetics* 1: 168-207

## ABSTRACT

The results of several published phylogenetic studies, based on RAPDs, karyotyping, chromosome banding techniques, GISH, FISH and sequencing of the nuclear *ITS1-2* and chloroplast *trnL-F* regions, were combined for the six species of the genus *Clivia*. The cladogram topologies between these studies are inconsistent and none of these studies provided all the answers regarding the evolution and relationships in the genus. These studies suggest that development of *Clivia* is from western South Africa to the east and that the divergence of *C. mirabilis* in the west from the eastern species occurred during the Late Miocene and Pliocene. Most studies confirm that *C. mirabilis* and *C. nobilis* are the most primitive species and basal to the rest of the species. The relationship between *C. miniata*, *C. gardenii* and *C. robusta* are not yet established. RAPD studies revealed inter- and intraspecific variation, especially in *C. miniata* and interspecific variation in *C. caulescens* based on chromosome banding. Genome sizes seem to correlate with the evolution patterns in *Clivia*. An expanded DNA phylogenetic study, based on sequencing of various chloroplast and nuclear DNA regions and aimed at elucidating current uncertainties, is currently in progress.

## PREFACE

This chapter was published in *Philosophical Transactions in Genetics* (PTG), a conference proceeding for the Inaugural Genetics Symposium held in Bloemfontein, 9 September 2011. The paper was completely written by me but I acknowledge the intellectual input from my promoters. The article is a complete review on the genus *Clivia*, with special focus on the cytogenetic and molecular studies in the genus. It is a combination of published data (by several other authors) and my own unpublished data, represented here in a new perspective.

Other relevant aspects in *Clivia* that was previously studied, are also included in this chapter. This is not only to highlight the importance of this genus as research organism but also to highlight the economical value of *Clivia* due to its export, as well as its medicinal value.

For the purpose of the thesis, the chapter ends with a clear statement of the research questions and the sub-questions that was addressed during the study.

### 3.1 INTRODUCTION

The family Amaryllidaceae consists of 59 genera, most having perennial bulbs. Although the distribution is worldwide, many of the genera are concentrated in South America and South Africa (Simpson, 2010).

*Clivia* Lindl is a member of the family Amaryllidaceae, which have been subdivided into three tribes: Amaryllideae, Cyrtantheae and Haemantheae Salisb. (Meerow & Snijman, 1998, 2001; Meerow *et al.*, 1999; Meerow & Clayton, 2004; Meerow & Snijman, 2006). The correct placement of the genus into tribes and subtribes have been debated several times (Traub, 1963; Melchior, 1964; Dahlgren *et al.*, 1985; Meerow & Snijman, 1998; Ito *et al.*, 1999; Meerow *et al.*, 1999; Meerow & Clayton, 2004).

*Cryptostephanus* Welw. ex Baker is the closest relative of *Clivia* and these are the only two genera with rhizomes instead of true bulbs. Some *Scadoxus* Raf. species do, however, also have rhizomes (Meerow & Clayton, 2004).

Six *Clivia* species have been described: *C. caulescens* R.A.Dyer (Dyer, 1943), *C. gardenii* Hook. (Hooker, 1856), *C. miniata* (Lindl.) Regel (Regel, 1864), *C. mirabilis* Rourke (Lindley, 1828), *C. nobilis* Lindl (Lindley, 1828) and *C. robusta* B.G.Murray *et al.* (Murray *et al.*, 2004) (Table 3.1), with *C. nobilis* as the type species of the genus. A rare yellow flowering form has been observed in nature in most *Clivia* species. Watson (1899) recognised this yellow form in *C. miniata* as a new variety (*C. miniata* var. *citrina* Watson) and since then new yellow varieties has been described for *C. gardenii* (*C. gardenii* var. *citrina* Swanevelder *et al.*) as well as *C. robusta* (*C. robusta* var. *citrina* Swanevelder *et al.*). Although natural hybridization can occur freely between *Clivia* species when growing sympatrically, only one natural hybrid, *C. ×nimbicola* Z.H.Swanevelder *et al.* between *C. caulescens* and *C. miniata*, is recognised (Swanevelder *et al.*, 2006).

Many species of the family are economically important due to large scale ornamental propagation of cultivars, as well as several taxa being used in traditional medicine (Simpson, 2010). Several publications have elaborated on and compared, amongst others, the morphology, habitats and distribution of different species (Koopowitz, 2002; Duncan, 2008;

Swanevelder & Fisher, 2009). This review paper will focus mainly on the evolution of *Clivia* based on chromosomal and phylogenetic studies. *Clivia* is threatened by extinction in nature, and other aspects and studies that have an impact on the conservation of *Clivia* will therefore also be discussed in this paper.

**Table 3.1.** A list of the described species and varieties in the genus *Clivia*, with a comparison between plant height, altitude and distribution (Koopowitz, 2002; Snijman & Archer, 2003; Duncan, 2008; own observations).

| Species, varieties & hybrids                                | Height (m) | Altitude (m) | Distribution                                       |
|-------------------------------------------------------------|------------|--------------|----------------------------------------------------|
| <i>C. caulescens</i>                                        | 0.5-1.5    | ± 1500       | Limpopo, Mpumalanga, Swaziland                     |
| <i>C. ×nimbicola</i>                                        | 0.5-1.5    | ± 1500       | Mpumalanga                                         |
| <i>C. gardenii</i> & <i>C. gardenii</i> var. <i>citrina</i> | 0.8-1.3    | 0-1200       | KwaZulu-Natal, Eastern Cape, possibly Swaziland    |
| <i>C. miniata</i> var. <i>citrina</i>                       | 0.4-0.6    | Unknown      | Swaziland, KwaZulu-Natal, Eastern Cape             |
| <i>C. miniata</i> var. <i>miniata</i>                       | 0.5-1      | 30-1200      | Mpumalanga, Swaziland, KwaZulu-Natal, Eastern Cape |
| <i>C. mirabilis</i>                                         | 0.6-1.2    | ± 600        | Northern Cape, Western Cape                        |
| <i>C. nobilis</i>                                           | 0.5-1.1    | 15-600       | Eastern Cape                                       |
| <i>C. robusta</i> & <i>C. robusta</i> var. <i>citrina</i>   | 0.5-2.1    | 25-1200      | KwaZulu-Natal, Eastern Cape                        |

## 3.2 CYTOGENETIC STUDIES

### 3.2.1 Chromosome numbers

All six *Clivia* species have been cytogenetically studied and a basic chromosome number of  $x = 11$  was reported for the genus. A somatic chromosome number of  $2n = 2x = 22$  has been reported for *C. miniata* (Inariyama, 1937; Gouws, 1949; Kato, 1960; Nandi, 1973; Niu *et al.*, 1986; Sveshnikova & Zemskova, 1988; Ran *et al.*, 1999; Chen *et al.*, 2003), *C. caulescens* (Gouws, 1949; Ran *et al.*, 1999), *C. gardenii* (Ran *et al.*, 1999), *C. robusta* (Murray *et al.*, 2004), *C. nobilis* (Inariyama, 1937; Sveshnikova & Zemskova, 1988; Ran *et al.*, 1999; Chen *et al.*, 2003) and *C. mirabilis* (Murray *et al.*, 2011). The only polyploid number ( $2n = 4x = 44$ ) was reported for *C. nobilis* (Satô, 1938, 1942). No chromosome numbers have been reported for the natural hybrid *C. ×nimbicola*, but several studies reported a basic chromosome number of  $x = 11$  for interspecific hybrids (He & Deng, 1989) and cultivars (Sveshnikova & Zemskova, 1988; He & Deng, 1989). The only deviation from the chromosome number ( $2n = 18$ ) in the genus has been reported for a hybrid between *C. miniata* and *C. nobilis* (Wittlake, 1940), but this is most probably a miscount due to the techniques used at the time or mistaken identity of the specimen studied.



### 3.2.2 Karyotype analysis

Karyotype analysis in two studies (Gouws, 1949; He & Deng, 1989), had conflicting karyotypes. The arm ratio criteria were not clearly defined in these studies, and the variability in the contraction of the large chromosomes (Ran *et al.*, 1999) could have resulted in the identification of two large, five medium and five small pairs in *C. caulescens* compared to two large, three small and six medium sized pairs in *C. miniata* (Gouws, 1949). Another study (He & Deng, 1989) described the karyotype of *C. nobilis* and reported one large metacentric chromosome pair. In addition to these karyotypic studies, He & Deng (1989), Ran *et al.* (1999, 2001) and Murray *et al.* (2011), employed more modern techniques and analysed the karyotypes of *Clivia* and *Clivia* hybrids with Giemsa C-banding, fluorochrome staining, silver impregnation and in situ hybridization (GISH and FISH).

These modern studies revealed that all the *Clivia* species have similar karyotypes with three large and three small chromosome pairs, with the rest being intermediate. With a combination of chromosome lengths and arm ratio's with banding patterns or ISH, it is possible to identify chromosome pairs 1, 2, 3, 8, and 10 in *C. miniata* and *C. gardenii*, as well as 1, 2, 3, 8, 9, 10 and 11 in *C. caulescens* and *C. nobilis*. *Clivia miniata* and *C. gardenii* can be differentiated from *C. nobilis* and *C. caulescens* based on the presence of a centromeric C-band in the first two species and the absence of the bands in the latter. The four species, *C. miniata*, *C. nobilis*, *C. gardenii* and *C. caulescens* could, despite their similar karyotypes, be distinguished without difficulty from the data of the combined techniques (Ran *et al.*, 1999). *Clivia mirabilis* has a unique karyotype, but has been proven to be related to the other species by sharing some banding patterns (Murray *et al.*, 2011).

Both *C. caulescens* and *C. nobilis* have band polymorphisms which are an indication of intraspecific variation in these species, and an interesting observation is that the taxa of *C. caulescens* in the study of Ran *et al.* (1999) were all from the same population at God's Window (Mpumalanga Province, South Africa). Knowledge on the uniqueness of the species karyotypes was followed by a similar study where chromosome banding and *in situ* hybridization was successfully utilised for hybrid identification (He & Deng, 1989).

To determine the karyotype evolution in *Clivia*, Ran *et al.* (2001) compared

phylogenetic studies (discussed later in more detail) with the chromosomal banding patterns based on a previous study (Ran, 2000) and concluded that *C. miniata*, *C. gardenii* and *C. robusta* are the most derived species. This is also supported by the overlapping distribution area in eastern South Africa. Centromeric C-bands are present in these three species and absent in the rest, therefore the conclusion from this study was that centromeric C-bands are a derived state. In a follow up study of Murray *et al.* (2011) including the primitive *C. mirabilis*, this statement was however questioned, since this primitive species also has centromeric C-bands present. The conclusion was that karyotypic evolution in *Clivia* is a complex process and that there might be gain and loss of chromosome bands in the genus. These authors postulated two different scenarios for the gain and loss of these bands based on a comparison of the *ITS* cladogram and the banding patterns.

C-banding techniques were the most consistent and provided most markers of all the staining techniques (Ran *et al.*, 1999). For the purpose of this review, a data matrix was constructed (Table 3.2) based on the C-banding patterns from Ran *et al.* (1999, 2001) and Murray *et al.*, (2011) and a parsimonious cladogram with a tree length of seven, was constructed (Figure 3.1). Of the six characters, four were parsimoniously informative, and one character (45s site on chromosome 8), according to one of the scenarios of Ran *et al.* (2001), might have disappeared from *C. gardenii* (as well as *C. mirabilis* as can be seen from Figure 3.1). This homoplasy is reflected by the consistency index (CI = 0.86).

**Table 3.2.** Summary of the Giemsa C-banding banding patterns, where “1” = band present, “0” = band absent and “?” = missing information. Species are represented by (A) *C. miniata*; (B) *C. gardenii*; (C) *C. nobilis*; (D) *C. caulescens*; (E) *C. robusta* and (F) *C. mirabilis*.

| Giemsa C-banding staining:                                        | A    | B    | C    | D    | E | F |
|-------------------------------------------------------------------|------|------|------|------|---|---|
| Centromeric bands                                                 | 1    | 1    | 0    | 0    | 1 | 1 |
| Interstitial band on chr. 8                                       | 1    | 1    | 1    | 1    | 1 | 1 |
| Terminal band on chr. 8                                           | 1    | 0    | 1    | 1    | 1 | 0 |
| Terminal band on chr. 2                                           | 1    | 1    | 0    | 0    | 1 | 1 |
| Terminal band on chr. 9                                           | 0    | 0    | 1    | 1    | 0 | 0 |
| Terminal band on chr. 10                                          | 0    | 0    | 1    | 0    | 0 | 0 |
| % Heterochromatin:                                                | 9.77 | 7.86 | 4.87 | 1.55 | ? | ? |
| Silver bands at centromeres:                                      | 0    | 0    | 0    | 1    | ? | ? |
| Number of hybridization sites (pairs) from ISH of 45S rRNA genes: | 2    | 1    | 3    | 2    | ? | 1 |

Based on the cladogram of the centromeric C-bands, *C. mirabilis*, *C. gardenii*, *C. robusta* and *C. miniata* share two characters and have a closer phylogenetic relationship. This phylogeny can, however only be used with caution since very few samples were included in

the study and very few characters have been used to construct the phylogeny. Differences in banding patterns between *C. gardenii* and the robust form of *C. gardenii* (Ran *et al.*, 2001) resulted in a subsequent morphological investigation, which lead to the splitting of these two forms into the separate species, *C. gardenii* and *C. robusta* (Murray *et al.*, 2004). Ran *et al.* (2001) studied two samples of 'Robust Gardenii' (now classified as *C. robusta*) and stated that insufficient material was available for the analysis of active NORs in this putative new species. For this reason an ongoing investigation is in progress to determine if *C. robusta* should indeed be regarded as a separate species.

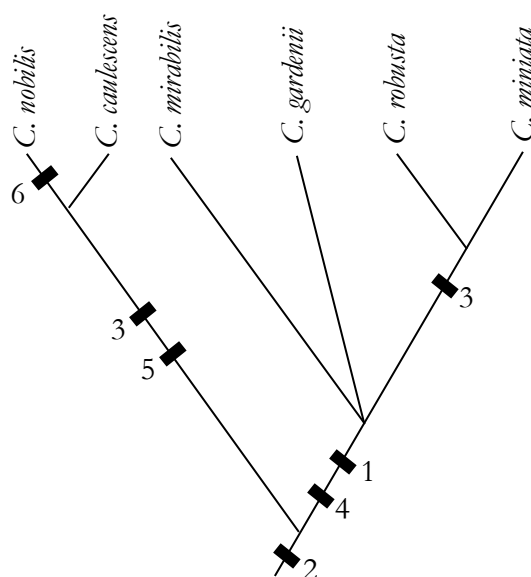


**Figure 3.1.** The most parsimonious cladogram constructed manually from the matrix of the chromosomal banding patterns (Ran *et al.*, 1999, 2001; Murray *et al.*, 2011). Numbers on the branches represents the following chromosomal characters from the C-banding patterns. 1 – centromeric bands present; 2 – interstitial band on chromosome 8; 3 – terminal band on chromosome 8; 4 – terminal band on chromosome 2; 5 – terminal band on chromosome 9; 6 – terminal band on chromosome 10.

### 3.3 PHYLOGENETIC STUDIES

#### 3.3.1 Position of *Clivia* in Amaryllidaceae

*Clivia* is a member of the tribe Haemantheae Salisb., which is subdivided into three subtribes: Cliviinae D.Müll.-Doblies & U.Müll.-Doblies (consisting of *Clivia* and *Cryptostephanus*.), Haemanthineae Pax (consisting of *Haemanthus* L. and *Scadoxus* Raf.) and Gethyllidinae Dumort (consisting of *Apodolirion* Baker and *Gethyllis* L.) (Meerow & Clayton, 2004).

Meerow & Clayton (2004) summarized the different taxonomic positions of *Clivia* in the different tribes and subtribes over time, so this will not be discussed in detail in this review. They used sequences of the *trnL-F* (no bootstrap support) and *ITS* (99 bootstrap support) to indicate that *Clivia* is a monophyletic group in the tribe Haemantheae (which consists of *Apodolirion*, *Clivia*, *Cryptostephanus*, *Gethyllis*, *Haemanthus* and *Scadoxus*). Their phylogeny supports previous reports based on taxonomical and molecular investigations (Traub, 1963; Melchior, 1964; Dahlgren *et al.*, 1985; Müller-Doblies & Müller-Doblies, 1996; Meerow & Snijman, 1998; Meerow *et al.*, 1999) of the close relatedness of *Clivia* and *Cryptostephanus*.

*Clivia* and *Cryptostephanus* share a bulbless state and are the only two genera in the tribe exclusively with rhizomes (Meerow & Clayton, 2004). Similarly, the evolution of berry fruits in conjunction with forest understory habitat in these genera is considered plesiomorphic. Even though *Cryptostephanus* is not endemic to South Africa, it forms a monophyletic clade with *Clivia*, the other rhizomatic genus. These two genera are phylogenetically separated from the sisterclade with true bulbs (Meerow & Clayton, 2004).

*Scadoxus* groups with the bulbous genera but have polymorphic underground storing systems, meaning that some species have bulbs and some share the rhizomatic trait with *Clivia* and *Cryptostephanus*. It could not be confirmed if the bulb vs. rhizome formation is limited to certain species or to specific environmental conditions (Meerow & Clayton, 2004) in South Africa.

Bay-Smidt *et al.* (2011) expanded the matrix of Meerow & Clayton (2004) by including additional *Clivia* taxa, and constructed a combined cladogram from *trnL-F* and *ITS* sequences. Their Maximum Parsimony (MP) cladogram had a similar topology to that of Meerow & Clayton (2004), supporting the basal placement of *Clivia* and *Cryptostephanus* to the Haemantheae with a bootstrap support of 66. As with the study of Meerow & Clayton (2004), only four of the six *Clivia* species were included in the study, but the monophyletic nature of these four *Clivia* species is supported with a 100 bootstrap value.

### 3.3.2 Chromosome and genome evolution in the tribe Haemantheae

Meerow & Clayton (2004) constructed a phylogeny of the tribe Haemantheae and speculated on the chromosomal evolution in the tribe. *Clivia*, as previously stated, has a somatic chromosome number of  $2n = 22$ . This is the plesiomorphic number for the family Amaryllidaceae (Meerow, 1995) and it seems that evolution favours the reduction of chromosomes from the ancestral  $2n = 22$  number to lower numbers (Meerow & Clayton, 2004). Reduction in chromosome numbers occurs in both the plant and animal kingdoms and seems to be a key evolutionary process (Vosa & Marchi, 1980). Translocations are frequent in the genus (Meerow & Clayton, 2004) and dysploid reduction has been described in the genera *Scadoxus* ( $2n = 18$ ) and *Haemanthus* ( $2n = 16$ ). Chromosomes in these two genera are very similar and Vosa & Marchi (1980) concluded that *Haemanthus* evolved from a “*Scadoxus*-type ancestor” by the occurrence of a translocation event. This dysploid event explains the decrease in chromosome number (Vosa & Marchi, 1980). Meerow & Clayton (2004) compared the chromosome numbers with the phylogeny of tribe Haemantheae. According to the basal position in their cladogram, the oldest genera are *Clivia* and *Cryptostephanus* and these two genera also have the highest chromosome numbers ( $2n = 22$  and  $2n = 24$  respectively). There is a need for similar studies to that of Vosa & Marchi (1980), to determine if dysploidy events were involved in the evolution of the other genera in the tribe. Because of the ancient nature of *Clivia*, it has been used as outgroup in phylogenetic studies of other genera and families, such as the phylogenies of *Cyrtanthus* (Meerow & Snijman, 2001; Snijman & Meerow, 2010) and the Orchidaceae (Neyland & Urbatsch, 1996).

For the purpose of this review paper, published *ITS1-2* sequences were obtained from GenBank (Table 3.3) and a Maximum Likelihood (ML) phylogram (Figure 3.2) was constructed using MEGA5 (Tamura *et al.*, 2011). The ML phylogeny was compared with the mean 2C genome sizes (measured in pg) from other studies (Olszewska & Osiecka, 1982; Vosa, 1987; Zonneveld *et al.*, 2005; Zonneveld, 2010).

Since there already seems to be an evolutionary relationship based on chromosome morphology between *Haemanthus* and *Scadoxus*, a possible relationship based on the genome sizes of *Clivia*, *Scadoxus* and *Haemanthus* were investigated. There seems to be a negative correlation ( $R^2 = 0.9707$ ) between the mean 2C values and the chromosome

numbers in *Clivia*, *Scadoxus* and *Haemanthus* (Figure 3.3).

**Table 3.3.** Species from the tribe Haemantheae used for the construction of the Maximum likelihood (ML) tree for this paper (with GenBank accession numbers for the *ITS* sequences). *Crinum* was used as outgroup.

| Species reference                                              | <i>ITS</i> accession number                      |
|----------------------------------------------------------------|--------------------------------------------------|
| <i>Apodolirion</i>                                             |                                                  |
| <i>A. cedarbergense</i> D.Müll-Doblies                         | AY280344                                         |
| <i>A. lanceolatum</i> (Thunb.) Baker.                          | AY280345                                         |
| <i>Clivia</i>                                                  |                                                  |
| <i>C. caulescens</i>                                           | AF324022                                         |
| <i>C. gardenii</i>                                             | AF324023, AF324024, AF324021, AY280346, AY280347 |
| <i>C. robusta</i>                                              | AF324025, AF324026                               |
| <i>C. miniata</i>                                              | AF324027, AF324028, HM140798, AY280348           |
| <i>C. nobilis</i>                                              | AF324029, AF324030, HM140799, AY280349           |
| <i>C. mirabilis</i>                                            | This study                                       |
| <i>Crinum</i> L.                                               |                                                  |
| <i>C. buphanoides</i> Welw. ex Baker                           | AY139124                                         |
| <i>C. macowanii</i> Baker                                      | EF111017, AF373094, DQ386442                     |
| <i>C. minimum</i> Milne-Redh.                                  | EF111014, DQ386435                               |
| <i>Cryptostephanus</i>                                         |                                                  |
| <i>C. vansonii</i> Verd.                                       | AF324032, HM140800, AY280351                     |
| <i>Gethyllis</i>                                               |                                                  |
| <i>G. afra</i> L.                                              | HM140801                                         |
| <i>G. britteniana</i> Baker                                    | AY280352                                         |
| <i>G. ciliaris</i> (Thunb.) Thunb.                             | AY280353                                         |
| <i>G. grandiflora</i> L.Bolus                                  | HM140802                                         |
| <i>G. lanuginosa</i> Marloth                                   | AY280354                                         |
| <i>G. verticillata</i> R.Br. ex Herb.                          | AY280355                                         |
| <i>Haemanthus</i>                                              |                                                  |
| <i>H. albiflos</i> Jacq.                                       | HM140803, AY280356                               |
| <i>H. amarylloides</i> Jacq.                                   | HM140804                                         |
| <i>H. coccineus</i> L.                                         | AF324031, HM140805                               |
| <i>H. deformis</i> Hook.f.                                     | HM140806, HM140807                               |
| <i>H. graniticus</i> Snijman                                   | AY280357                                         |
| <i>H. humilis</i> Jacq. subsp. <i>hirsutus</i> (Baker) Snijman | HM140808                                         |
| <i>H. montanus</i> Baker                                       | HM140809                                         |
| <i>H. pumilio</i> Jacq.                                        | AY280358                                         |
| <i>H. sanguineus</i> Jacq.                                     | HM140810, HM140811                               |
| <i>Scadoxus</i>                                                |                                                  |
| <i>S. cinnabarinus</i> (Decne.) Friss & Nordal                 | AY280359                                         |
| <i>S. membranaceus</i> (Baker) Friis & Nordal                  | AY280360                                         |
| <i>S. multiflorus</i> (Martyn) Raf.                            | HM140812                                         |
| <i>S. puniceus</i> (L.) Friis & Nordal                         | HM140813, HM140814, AY280361                     |

Within *Haemanthus*, *Scadoxus*, *Crinum* and *Clivia*, high chromosome numbers correlate with low genome content and based on the topology of the phylogram, evolution favours fewer chromosomes but larger genomes. The evolutionary direction of these three genera can be indicated as *Clivia* ( $2n = 22$ ;  $2C = 37.04$  pg), *Scadoxus* ( $2n = 18$ ;  $2C = 88.4$  pg) and *Haemanthus* ( $2n = 16$ ;  $2C = 96.85$  pg) (Table 3.4).

The correlation pattern between genome size and chromosome number is not applicable to *Cryptostephanus*, *Apodolirion* and *Gethyllis*. These three genera have the lowest

basic chromosome numbers in the tribe Haemantheae, but not the highest genome size as would be expected from the negative correlation observed in the other genera.

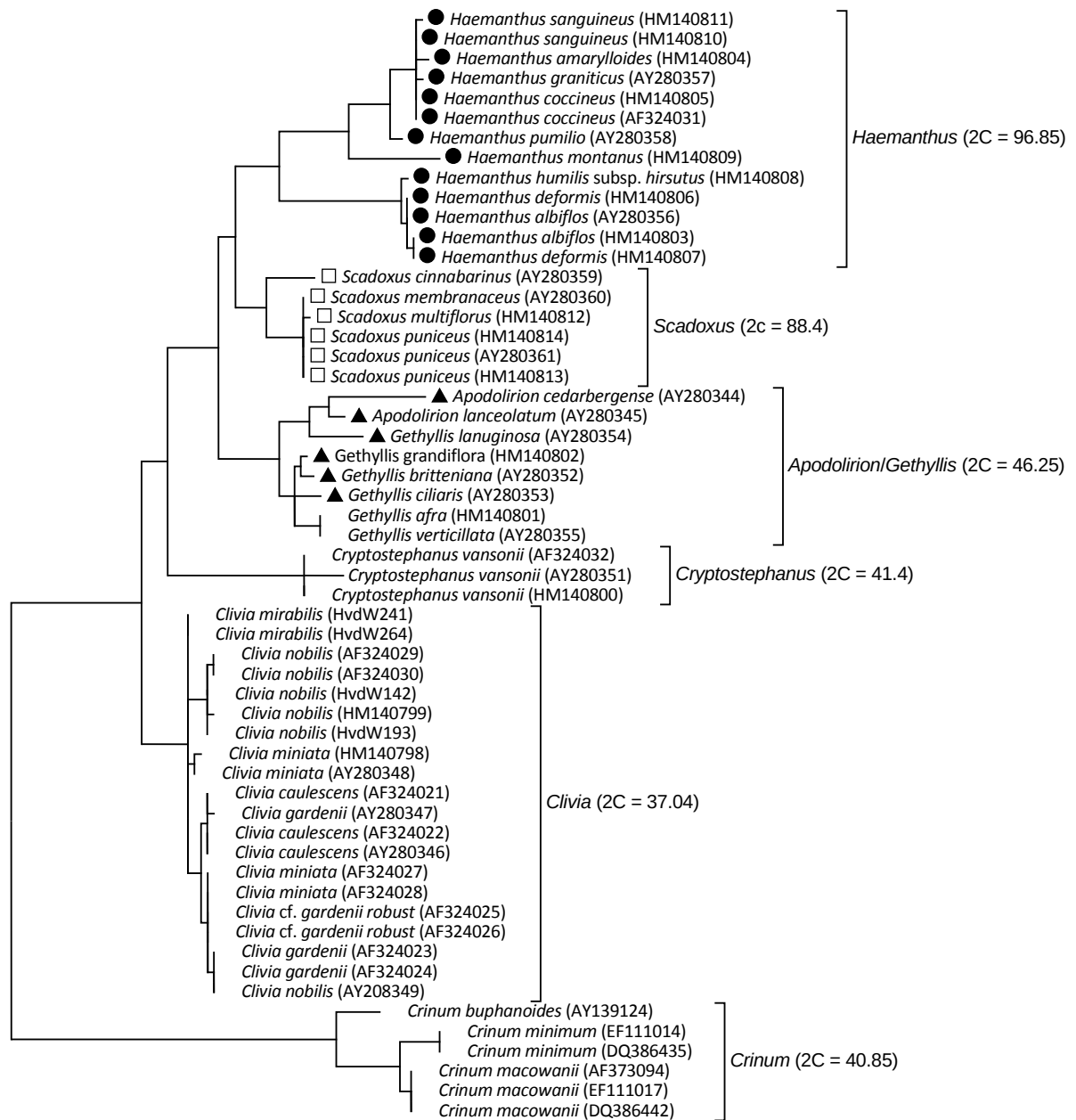


**Figure 3.2:** Molecular Phylogenetic analysis based on the ITS sequences of 56 taxa from GenBank (Tamura & Nei, 1993). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 60% variable and 51 parsimonious informative sites. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011). The taxon name is followed by the GenBank accession number. Bootstrap values are represented next to the branches. A mean genome size (2C) per taxonomic group is indicated in brackets (in pg). Basic chromosome numbers (x) are presented by the following symbols: ● = 8; □ = 9; ▲ = 6; ◇ = 11.

The genus *Crinum* with  $x = 11$  was used as outgroup for the Haemantheae. The ITS1-2 phylogeny favours an increase in genome size as a derived state and it seems as if lower genome content is ancestral in the tribe. Both *Crinum* and *Clivia* have the highest basic chromosome number  $x = 11$ , and also have the lowest DNA content (mean 2C = 40.85 and

37.04 respectively). The more evolved genera in the cladogram had higher 2C content. Both the ML and ME cladograms lack strong branch support, so this hypothesis needs further testing.

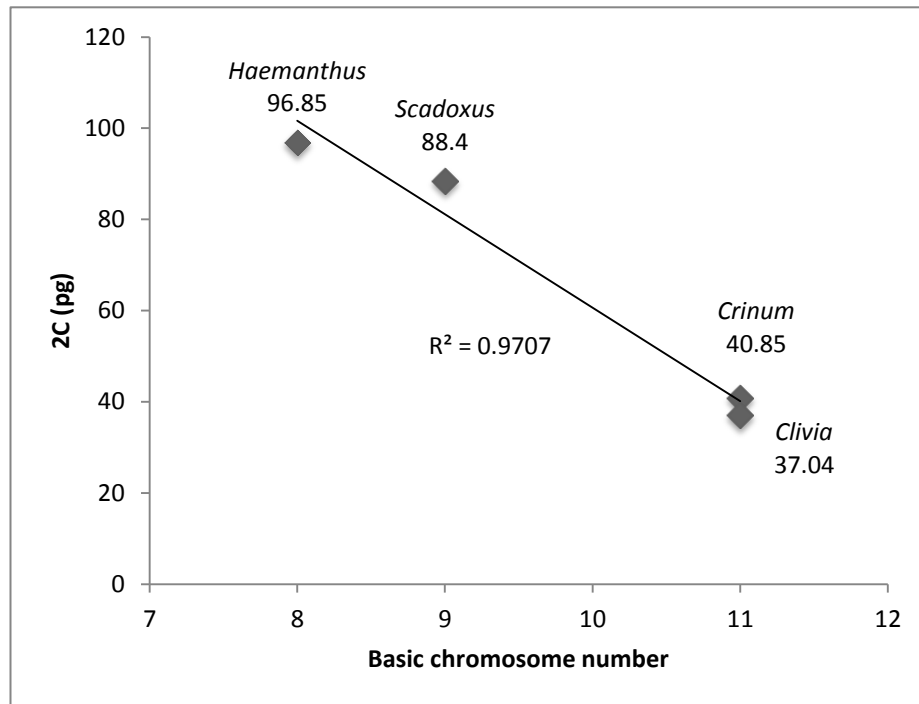


**Figure 3.3.** Negative correlation between the genome size (2C) and basic chromosome number (x) in three genera of the tribe Haemantheae and the outgroup genus *Crinum*.

Meerow & Clayton (2004) recognised that the subtribe Gethyllidinae (*Apodolirion*/*Gethyllis*) has distinct morphological characteristics compared to the rest of the genera in the tribe. The subtribe Gethyllidinae has a sister relationship with the subtribe Haemanthineae (*Haemanthus*/*Scadoxus*). The Gethyllidinae (and probably the genus *Cryptostephanus* as well), did not evolve from the subtribe Haemanthineae, through chromosome reduction in the same manner as *Haemanthus* has evolved from *Scadoxus*. The subtribe Gethyllidinae (and *Cryptostephanus*) must have evolved by other means, but based on the *ITS1-2* topology and the genome content of the x = 6 genera which have a mean size of about 46 pg, it seems as if there is some type of relationship between these genera.

Gouws (1949) suggested a tetraploid ( $2n = 24$ ) derivation of *Cryptostephanus* from a  $2n = 12$ , which would imply that *Cryptostephanus* evolved from one of the genera in the subtribe Gethyllidinae. Combined matrixes of the *ITS* and *trnL-F* genomic and chloroplast regions of



Meerow & Clayton (2004) lead these authors to the conclusion that  $x = 6$  in the Gethyllidinae originated after the divergence of *Clivia* and *Cryptostephanus*, thus rejecting the relationship that Gouws (1949) suggested. *Clivia* and *Cryptostephanus* both have a basic chromosome number of  $x = 6$ , and their genome sizes are very similar ( $2C = 36.47$  &  $46.25$  respectively). These findings suggest a close relationship between the genera, as suggested by Gouws (1949), and this will be studied in the near future.

**Table 3.4.** Summary of the DNA content (2C measured in pg), mean 2C, standard deviation and basic chromosome numbers ( $x =$ ) of members of the tribe Haemantheae and the outgroup genus (*Crinum*).

| Taxa                                  | 2n | 2C (pg)             | Mean 2C      | Std. dev.    | ( $x =$ ) |
|---------------------------------------|----|---------------------|--------------|--------------|-----------|
| <i>Crinum</i>                         |    |                     | <b>40.85</b> | <b>0.6</b>   | <b>11</b> |
| <i>C. bulbispermum</i>                | 22 | 40.20 <sup>d</sup>  |              |              |           |
| <i>C. moorei</i>                      | 22 | 41.50 <sup>d</sup>  |              |              |           |
| <i>Clivia</i>                         |    |                     | <b>37.04</b> | <b>2.12</b>  | <b>11</b> |
| <i>C. caulescens</i>                  | 22 | 38.13 <sup>e</sup>  |              |              |           |
| <i>C. gardenii</i>                    | 22 | 37.40 <sup>e</sup>  |              |              |           |
| <i>C. miniata</i>                     | 22 | 36.91 <sup>e</sup>  |              |              |           |
| <i>C. miniata</i> <sup>†</sup>        | 22 | 46.90 <sup>b</sup>  |              |              |           |
| <i>C. miniata</i>                     | 22 | 39.00 <sup>d</sup>  |              |              |           |
| <i>C. miniata</i> <sup>†</sup>        | 22 | 35.25 <sup>a</sup>  |              |              |           |
| <i>C. miniata</i> 'Chubbs peach'      | 22 | 37.17 <sup>e</sup>  |              |              |           |
| <i>C. miniata</i> var. <i>citrina</i> | 22 | 38.05 <sup>e</sup>  |              |              |           |
| <i>C. nobilis</i>                     | 22 | 34.48 <sup>e</sup>  |              |              |           |
| <i>C. robusta</i>                     | 22 | 36.16 <sup>e</sup>  |              |              |           |
| <i>C. robusta</i> cross***†           | 22 | 38.01 <sup>e</sup>  |              |              |           |
| <i>C. robusta</i> hybrid**†           | 22 | 36.17 <sup>e</sup>  |              |              |           |
| <i>C. robusta</i> *†                  | 22 | 34.65 <sup>e</sup>  |              |              |           |
| <i>Cryptostephanus</i>                |    |                     | <b>46.47</b> | <b>5.07</b>  | <b>6</b>  |
| <i>C. vansonii</i>                    | 24 | 41.4 <sup>e</sup>   |              |              |           |
| <i>C. haemanthoides</i>               | 24 | 51.53 <sup>e</sup>  |              |              |           |
| <i>Gethyllis</i>                      |    |                     | <b>46.25</b> | <b>0.6</b>   | <b>6</b>  |
| <i>G. villosa</i>                     | 24 | 45.65 <sup>c</sup>  |              |              |           |
| <i>G. britteniana</i>                 | 24 | 46.85 <sup>c</sup>  |              |              |           |
| <i>Haemanthus</i>                     |    |                     | <b>96.85</b> | <b>20.85</b> | <b>8</b>  |
| <i>H. katherinae</i>                  | 16 | 117.70 <sup>b</sup> |              |              |           |
| <i>H. albiflos</i>                    | 32 | 76.00 <sup>f</sup>  |              |              |           |
| <i>Scadoxus</i>                       |    |                     | <b>88.4</b>  | -            | <b>9</b>  |
| <i>S. multiflorus</i>                 | 18 | 88.40 <sup>d</sup>  |              |              |           |
| <i>Apodolirion</i>                    |    |                     |              |              | <b>6</b>  |
| No data                               |    |                     |              |              |           |

\* from KZN seed list

\*\* Hybrid between *C. robusta* (pink) x 'Harburg blush'

\*\*\* Unknown cross

† species excluded from the mean 2C and standard deviation calculations

References: <sup>a</sup> Bennett & Smith (1976); <sup>b</sup> Olszewska & Osiecka (1982); <sup>c</sup> Vosa (1987); <sup>d</sup> Zonneveld *et al.* (2005); <sup>e</sup> Leitch & Moscardo (Personal Communication); <sup>f</sup> Zonneveld (2010)

### 3.3.3 Phylogeography

Diversity and distribution patterns can not necessarily be linked to modern environments, but the evolutionary history and palaeoenvironment should also be investigated to understand these patterns (Tolley *et al.*, 2008). Geographical and climate

changes have influenced the evolution and distribution of the family Amaryllidaceae. The warm moist tropical climate that prevailed during the Cretaceous period resulted in the settlement of angiosperms worldwide. Major climatic changes during the Cenozoic period, such as progressive cooling and atmospheric CO<sub>2</sub> reductions, resulted in the ecological changes such as rainfall seasonality and an increase in aridity leading to declining forestation (Tolley *et al.*, 2008). These changes had a major influence on the distribution and evolution of the family Amaryllidaceae.

From the biogeographical study of Meerow & Clayton (2004) it was suggested that at least four genera from the Haemantheae (*Apodolirion*, *Gethyllis*, *Haemanthus* and *Clivia*) originated in Eastern South Africa and dispersed through three vicariance events to the Western Cape. This observation is based on DIVA analysis and the ancestral node of Haemantheae in their phylogeny that roots in Eastern South Africa. Meerow & Clayton (2004) considered climatic changes and suggested that the divergence of the three clades in the Haemantheae, and therefore the divergence of *Clivia* and *Cryptostephanus* from the rest of the genera, occurred during the Pleiocene. South-western Africa experienced a subtropical environment prior to the Pleiocene, and only in the late Pleiocene is there evidence of the modern semi-arid, winter-rainfall patterns.

Conrad *et al.* (2003) postulated that climatic changes resulted in the isolation of *C. mirabilis* in the Western Cape from the rest of the species in eastern South Africa, but did not elaborate on the geographical origin of the genus. The hypothesis from this study is that *Clivia* habituated a wide range from the east to the west, and that vicariance started during the late Miocene when the subtropical forests from the Pleiocene era retracted, due to the increase in aridity in the Northern and Western Cape. However, these authors raised concern if such a theory could be accepted, since there are no traces of the existence of clivias in the central area between east and west, such as in the Knysna forest. The absence of species in this area is contradictory to the theory of a previously wide distribution of the genus. Meerow & Clayton (2004) suggested that further speciation might be due to more recent paleoclimatic events.

Since *Clivia* prefer a natural forest-type habitat, another opinion is that *Clivia* either developed with these forests, or migrated with the Afromontane forest (Swanevelder &

Fisher, 2009). The Western Cape is a winter rainfall area with a high degree of plant endemism. Most of the plant diversity in the Western Cape probably evolved during the Late Miocene and Pliocene when the ice sheet expanded and the sea-ice was initiated (Goldblatt & Manning, 2002; Goldblatt *et al.*, 2002; Meerow & Clayton, 2004; Stuit *et al.*, 2004). The modern Afromontane forest cannot tolerate the long, dry summers of the winter rainfall areas of the Western Cape. These climatic changes therefore probably led to the extinction of the previous tropical flora (Linder *et al.*, 1992). Two types of phylogeographical evolution are postulated. The first hypothesis is that *Clivia* escaped the increasing arid areas in the west and migrated to more suitable climates, and implies that the origin of an ancestral species is in south-southwest Africa and that *Clivia* migrated from the west up to the eastern borders of South Africa (Swanevelder & Fisher, 2009). This hypothesis contradicts that of Meerow & Clayton (2004) who, as previously mentioned, suggested an easterly origin of the genus. The second hypothesis supports that of Conrad *et al.* (2003), suggesting a widespread ancestral species (Swanevelder & Fisher, 2009). Support for this hypothesis comes from a study on fauna in the region: a study on chameleon species (Tolley *et al.*, 2008) suggest a similar pattern of diversification from west-to-east, and propose that fragmentation of the closed habitats were due to dramatic changes in global and regional climates.

*Clivia* grow in very specific habitats in the Afromontane forests of eastern South Africa. The natural habitat of *Clivia* covers or partially covers ecoregion 10 (Northern escarpment mountains), ecoregion 14 (north eastern uplands), ecoregion 16 (south eastern uplands), ecoregion 17 (north eastern coastal belt), ecoregion 23 (western folded mountains) and ecoregion 31 (eastern coastal belt), as described by Kleynhans *et al.* (2005). The majority of *Clivia* species are distributed in areas with very high stream frequency and a mean annual precipitation of 700-1000 mm. Most of the species are distributed in areas with rainfall occurring in January, February or December, with the exception of *C. mirabilis* distributed in the winter rainfall area. *Clivia* cannot tolerate extreme temperatures and grow in areas with maximum temperatures of between 24-28°C in February and minimum temperature of between 6-10°C in July. All *Clivia* species except *C. mirabilis* prefer the subtropical forest, with some species occurring on slopes in the sub-escarpment Savannah, Highveld grassland and sub-escarpment grassland. The exception is *Clivia mirabilis* that adapted to a climate with less annual precipitation (100-200 mm) and more extreme temperatures.

*Clivia* favours warmer temperatures and the warmer ecoregion of the eastern coast corresponds to the warm Agulhas Sea current that stretches down to ecoregion 31. The most southern distribution of *Clivia* ends almost exactly where the colder Benguela sea current from the west meets with the warm Agulhas current from the east. From this point westwards the climate and vegetation changes. The mean annual precipitation decreases and rainfall seasonality changes from early/late summer to all year. From this point westward the stream frequency per square kilometre decreases and the vegetation changes into Albany thickets and Fynbos.

The preceding observations support the theory of recent paleoclimatic events having an influence on the geographic distribution of *Clivia*. The logical explanation for the distribution of *Clivia* will be that there was an ancestral species covering a vast majority of South Africa. Ancient (the development of the winter rainfall area in the Western Cape) as well as recent paleoclimatic events (the warm sea currents and relative warmer temperatures combined with high annual summer rainfall) resulted in the survival and speciation of the *Clivia* species as we know it today. The modern environmental conditions in the area between *C. nobilis* and *C. mirabilis* is not suitable for any *Clivia* species to grow, and this explains why there is no *Clivia* species found in any of the southern forests, including the Knysna forest. The combination of *Clivia mirabilis* adapting morphologically to the warmer environment and drier summers as well as the gorges that serve as protection to the plants, resulted in the survival of this species in the Western Cape.

### 3.3.4 Phylogenetic relationships within the genus *Clivia*

Ran *et al.* (2001) compared the karyological data of six *Clivia* species (excluding *C. mirabilis*), with the sequences of two ribosomal DNA regions, *ITS* and *5S* to construct the phylogeny in the genus. This study was extended with RAPD analysis of 46 plants representing five of the six species (excluding *C. mirabilis*). The results from all these studies corresponded closely and suggested that *C. miniata*, *C. gardenii* and *C. robusta* are closely related and diverged recently. *Clivia nobilis* is basal to all the species, with *C. caulescens* occurring intermediate between these groups. Both the molecular and karyological data support these groupings.

Based on the phylogeny and the gain and loss of certain regions on chromosomes, it is suggested that *C. nobilis* is ancestral. From the ancestral state, a region on chromosome 10 was lost resulting in an ancestral *C. caulescens* karyotype. The remaining three species (*C. miniata*, *C. gardenii* and *C. robusta*) are the most derived and share the translocation from a region on chromosome 9 to the long arm of chromosome 2. The final suggestion is that *C. gardenii* developed from the other two species in this group by losing the 45S site from chromosome 8, and is therefore the youngest species in the genus (Ran *et al.*, 2001).

The RAPD analysis yielded further informative results, showing 67.6% variation between the different *Clivia* species, which was greater than the within-species component (32.4%). *Clivia miniata* has 72.7% variation, compared to the 52.1% variation in *C. nobilis*. In addition *C. miniata* has 77.4% variation between populations and 22.6% variation within populations. These differences resulted in the division of the *C. miniata* species into five different groups, namely the Howick population; the second group consisted of the populations in Ixopo, Poynton and yellow cultivars from Japan. Group three consisted of the “German hybrids”, a New Zealand and Japanese cultivar. The fourth group was “Belgian hybrids”, and the last group had a mixture of accessions. RAPDs would have been a significant technique to use in the geographic identification of the origin from a plant sample, but it is unfortunate that RAPD analysis has limited repeatability (Ran *et al.*, 2001).

In a later study, Meerow & Clayton (2004) compared their combined analysis of *trnL-F* and *ITS* to those of Ran *et al.* (2001). These two studies yielded contradictory results regarding the phylogeny and evolution within *Clivia*, where the one study [as well as a study of Bay-Smidt *et al.* (2011)] suggest a sister species relationship of the group *C. caulescens* – *C. gardenii* to the group *C. miniata* – *C. nobilis* (Meerow & Clayton, 2004). As discussed previously, Ran *et al.* (2001) proposed that *C. nobilis* is ancestral to the other three species, *C. caulescens* is intermediate and that *C. miniata*, *C. gardenii* and *C. robusta* are monophyletic and the youngest group. The major explanation of these inconsistent results is that the sequences for some species (*C. gardenii* and *C. nobilis*) is incongruent between these studies and the sequence of *C. nobilis* from the study of Meerow & Clayton (2004) is closer to the sequence of *C. gardenii* from Ran *et al.* (2001). There is also a divergence in sequences between these studies in the *C. miniata* as well as *C. gardenii* sequences (Meerow & Clayton,

2004). Booysen (2003) compared the *Clivia* sequences available on GenBank with her own sequences, and experienced the same problem as Meerow & Clayton (2004). A *trnL-F* sequence of *C. nobilis* from GenBank do not group with *C. nobilis* from her study, but rather with *C. gardenii* and this may be due to misidentification or a hybrid specimen being used instead of a “pure species” (Booyesen, 2003).

A phylogeny using three non-coding chloroplast regions Conrad *et al.* (2003) suggested a sister relationship between *C. miniata* and *C. caulescens* with *C. gardenii* being sister to this clade. *Clivia mirabilis* is basal to the *Clivia* species and *C. nobilis* is intermediate between the ancestral species and the rest of the species.

The topologies of the cladograms between different studies are inconsistent, especially the relationships between *C. miniata*, *C. gardenii*, *C. caulescens* with *C. nobilis*. From a recent study on the phylogenetic position of *C. mirabilis* (including nine *C. mirabilis* and eight *C. nobilis* specimens as well as taxa from the other species), Van der Westhuizen (2010) discovered through combining five chloroplast regions that *C. mirabilis* and *C. nobilis* have a very well supported sister relationship with the rest of the species. This implies that *C. mirabilis* and *C. nobilis* are not ancestral to the rest of the species, but that the species rather share a common ancestor as discussed earlier.

The exact phylogenetic relationship between the other four *Clivia* species is unclear and it might be influenced by some degree of historical introgression of genes of one species into another (Meerow & Clayton, 2004). Since mixed populations are still being observed from time to time (Snijman & Archer, 2003), it is very likely that ancient gene flow might have been prevalent.

### 3.3.5 Phylogeny vs. DNA content (2C) of *Clivia*

Zonneveld (2002) measured a range between 31.2 and 39.2 pg for the somatic nuclear DNA contents (2C) of all six *Clivia* species. Our unpublished data confirm these measures. It can be concluded from the 8 pg difference between the largest and the smallest genome, that there is a 30% size difference between the chromosomes. *Clivia mirabilis* has the lowest DNA content (2C = 31.2 pg). The DNA content (2C in pg) for *C. miniata* varies between studies with

a standard deviation of 4.86 pg.

There seems to be a possibility that the 2C value gives additional phylogenetic support for the evolution of the tribe Haemantheae as well as the evolution within *Clivia*. Based on genome size, *C. mirabilis* and *C. nobilis* are the oldest (*C. nobilis* [AY280349] from GenBank does not group with the rest of the *C. nobilis* taxa, as have been observed in other studies). *Clivia caulescens* has the largest genome and might be the youngest species. Considering the *ITS1-2* cladogram constructed for this review paper (Figure 3.2), *C. caulescens* has long branch lengths. The length of the branches represents the number of substitutions per site. More substitutions thus either mean a faster evolutionary rate or that *C. caulescens* has the same evolutionary rate as the rest of the species but that it diverged early from *C. miniata*, *C. gardenii* and *C. robusta*.

*Clivia mirabilis*, *C. nobilis* and *C. caulescens* have been geographically isolated from the other species, leading to clear lineage formation in these three species. These species are each monophyletic and seems to have unique genome sizes (31.33 pg, 34.48 pg and 38.13 pg respectively). The *C. miniata* - *C. gardenii* - *C. robusta* - *C. caulescens* species has a sister relationship with each other and it seems as if these four species share a common ancestor.

Some of the *C. miniata* taxa have a very close relationship with *C. robusta* that are well supported with high bootstrap values. In addition to the close relationship between these species, *C. miniata* also has variable genome sizes overlapping with the genome sizes of *C. robusta* and *C. gardenii*. *Clivia miniata* is not monophyletic, probably due to hybridization and introgression events. An extended molecular study is underway to investigate the true relationship between *C. miniata*, *C. gardenii* and *C. robusta* and a definite correlation between genome size and evolution within *Clivia* will be further investigated based on a much greater sample size.

The *ITS1-2* phylogram, although weakly supported, indicate a possible evolutionary development between *Clivia* and *Cryptostephanus*, either by sharing an ancestor, or that the one genus evolved from the other. This relationship between the genera correlates positively ( $R^2 = 0.71$ ) with genome sizes (Figure 3.4a), and the same correlation ( $R^2 = 0.71$ ) is observed

with the geographical distribution between the species of *Clivia* and *Cryptostephanus* (Figure 3.4b).

Many studies, as already mentioned, suggest that the evolutionary trend in species is from the Western South Africa to eastern South Africa. This hypothesis is supported with the genome sizes in *Clivia* and *Cryptostephanus*, where the smallest genome of *C. mirabilis* is found in the Western Cape, with genome size increasing the further the species are distributed along the eastern escarpment up north into Africa (Figure 3.5). *Cryptostephanus haemanthoides* is distributed in Tanzania to Kenya, and has the largest genome ( $2C = 51.53$  pg) in these two genera (Leitch & Moscardo, Personal communication).

The karyotype analysis of *C. mirabilis* does not support the ancestral position of this species, since it has a derived absence of DAPI banding at the centromere. The explanation for this can be the loss or gain of chromosome bands that have been associated with other angiosperm genera (Murray *et al.*, 2011).

Meerow & Clayton (2004) suggested the use of a population genetic marker approach, such as microsatellite DNA to resolve the phylogeny in the genus. Swanevelder (2003) as well as Van der Westhuizen (2010), resorted to microsatellites to infer the phylogeny in *Clivia*, but both studies were unsuccessful. Genome size has an influence on the success of techniques such as microsatellites (Costa *et al.*, 2000; Fay *et al.*, 2005) and that a large genome ( $1C > 15$  pg) usually yields uninformative results (Fay *et al.*, 2005). The unsuccessful use of microsatellites in *Clivia* probably is thus most likely due to the large genome size.

### 3.3.6 The implication of the 2C value of *Clivia*

The average genome size ( $2C$  in pg) of the six *Clivia* species is 37.12 pg, thus it can be considered to be a large genome. Several studies have indicated a correlation between genome size and many phenotypic characters, such as pollen volume (Bennett, 1972), radiation sensitivity (Sparrow & Miksche, 1961) and seed size (Bennett & Leitch, 2005). The main implication of a large genome is however the inability to adapt due to restricted plant development. This in turn has a restriction on the ecological distribution of the species (Knight *et al.*, 2005). In a study, the genome sizes of 421 species were compared with various



ecological parameters (Knight & Ackerly, 2002; Knight *et al.*, 2005). These authors concluded that species with a large genome cannot tolerate environments with extreme temperatures. Another concern is the sensitivity of species with a large genome to metal pollution in the soil (Vidic *et al.*, 2003).

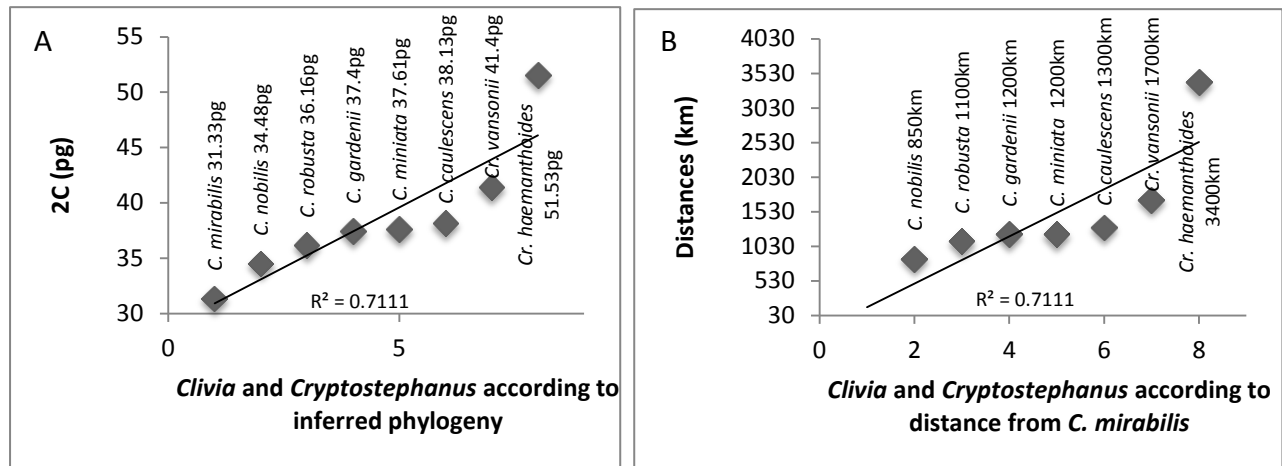


**Figure 3.4.** Correlations within and between *Clivia* and *Cryptostephanus* based on (A) genome size and inferred phylogeny. The same positive correlation is found based on (B) the approximate geographical distances (in km) between *C. mirabilis* in the west and the rest of the *Clivia* and *Cryptostephanus* species. Numbers on the x-axis is numbers allocated to the species from 1-8 based on the inferred phylogeny from most primitive on the left, to most recent on the right (A) and based on the geographical distribution of the species from west to east in (B).

Since the duration of mitosis and meiosis (Bennett, 1977) and stomatal cell size (Masterson, 1994) are some of the morphological aspects influenced by the genome size, the life cycle and production speed of a plant with a small genome will be much faster compared to a species with a large genome (Bennett *et al.*, 1998). Species with a large genome will thus also have a slow diversification rate (Vinogradov, 2003; Knight *et al.*, 2005). Species with large genomes also produces large seeds which have more limitations to its dispersal abilities. Any environmental changes will have a much greater influence on species with a large genome since it will not be able to colonize as easily as species with a small genome (Knight *et al.*, 2005).

Species with a large genome, including *Clivia*, have a much greater risk of extinction because of all the above mentioned factors. Vinogradov (2003) found a direct link between genome size and threat of extinction, and determined that species with a small genome and a mean genome size of  $1C = 7.4$  pg, had no threat, compared to species with a large genome (with a mean size of  $1C = 12.5$  pg) that have a local risk of extinction. *Clivia* has an average

genome of 1C = 18.56 pg (2C = 37.12 pg), and, when considering factors such as habitat loss, the large size of the genome will have an impact on the survival of the genus.

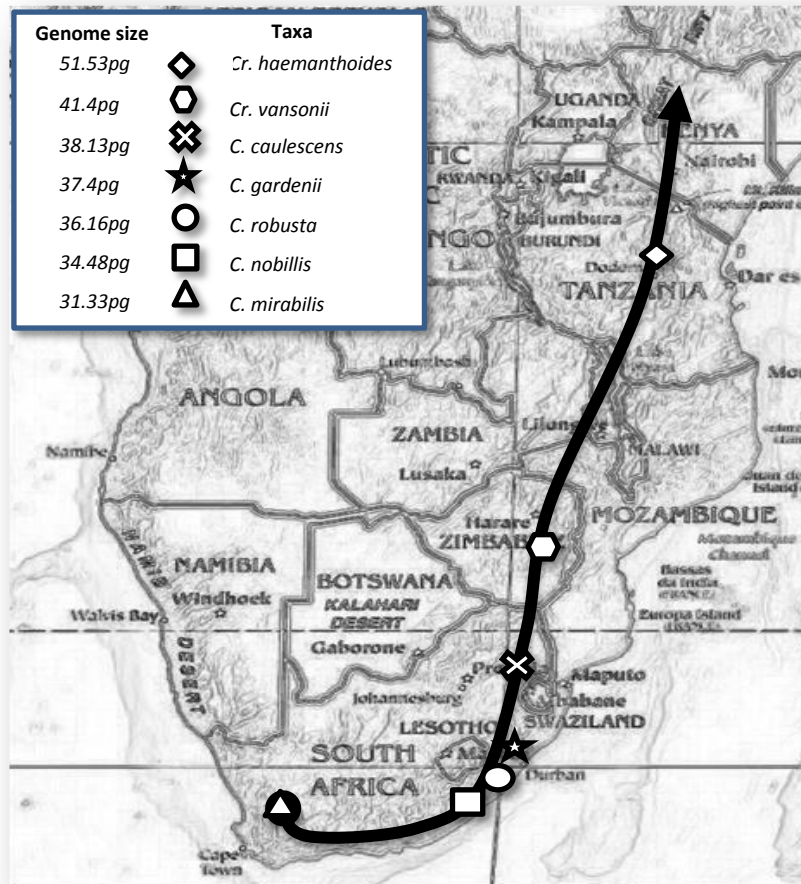


**Figure 3.5.** Correlation between geographical distribution and genome sizes indicating the possible direction of evolution.

### 3.4 SURVIVAL THREATS

Data on conservation status show that all these species, except *C. caulescens* are vulnerable (*C. caulescens* is near threatened). *Clivia robusta* should be categorised as endangered (Swanevelder, 2003), and all the other species should rather be regarded as endangered or critically endangered (Swanevelder & Fisher, 2009). Fortunately, although *C. mirabilis* is regarded as vulnerable, it is one of the six vulnerable species in the Western Cape that is preserved in national, provincial or privately owned reserves (Snijman & Victor, 2003).

Habitat destruction and plant removal by traditional healers for use as muthi, as well as the removal of beautiful or rare plants for horticultural purposes, poses as a threat for the survival of the genus in nature (Swanevelder & Fisher, 2009).

In traditional medicine, *Clivia* have an important role in protection, where the roots are used as a charm to ward off evil and to be used as a love-charm emetic (Gerstner, 1941). Another use of *Clivia* is as one of the components in intelezi mixes, which are then traded in medicinal markets in Durban (Cunningham, 1988). It is believed that *Clivia* (or the Zulu name umayime) will halt the course of a disease (Pujol, 1990).

Williams *et al.* (2001) found clivias to be one of the most common plants in medicinal shops on the Witwatersrand. Seventy percent of the traders supply *Clivia*, and 42% of these *Clivia* plants are removed from populations in KwaZulu-Natal. It can be problematic to identify the species confiscated (Williams *et al.*, 2001) and these plants are likely to be *C. gardenii* species, which are also traded in the medicinal plant markets of KwaZulu-Natal (Cunningham, 1988). *Clivia* plants are also traded in Mpumalanga (Mander, 1997). Williams *et al.* (2001) identified the *Clivia* plants confiscated from 35 herbal dealers in Witwatersrand as *C. miniata* and *C. nobilis*. In all three these localities (Witwatersrand, KwaZulu-Natal and Mpumalanga), the species in the markets have been identified as *C. nobilis*. It is very unlikely that the most southern species would have made it so far north into the markets that are much closer to the northern species. The distribution area of *C. caulescens* is situated in the Mpumalanga Province (Arnold *et al.*, 2002), and there is a significant probability that plants in these markets will belong to the latter species (Crouch *et al.*, 2003). A study on DNA-barcoding in the genus will be able to identify the species confiscated from medicinal markets. Such a study is currently in progress.

### 3.5 BIOCHEMICAL COMPOSITIONS AND MEDICINAL PROPERTIES OF *CLIVIA*

As already mentioned, *Clivia* plants are harvested from its natural environment to be used as traditional medicine. Ironically the medicinal properties of *Clivia* poses as a threat to the survival of the genus in nature, since traditional healers ascribe to the believe that a plant loses its healing power when it is cultivated in areas other than the natural environment. It is therefore unlikely that traditional doctors will be persuaded to cultivate clivias and to protect the plants in nature. Numerous studies have investigated the medicinal ingredients of the Amaryllidaceae and *Clivia*, and these have determined that the main medicinal substance in the Amaryllidaceae is alkaloids (Boit & Mehlis, 1961; Mehlis, 1965; Bienert, 1969; Leven, 1981; Ali *et al.*, 1983; Jeffs *et al.*, 1988; Veale *et al.*, 1989; Viladomat *et al.*, 1997; Sewram *et al.*,

1998; Evidente *et al.*, 1999; Darwish *et al.*, 2002; Crouch *et al.*, 2003). Alkaloids are found in “natural” and cultivated plants and the latter will have the same healing properties as plants removed from nature. The believe system of traditional healers will unfortunately not easily be changed.

Some alkaloids are known for their anti-bacterial, anti-fungal, anti-tumour, anti-viral, anti-leukemic, anti-malarial, insecticidal and analgesic properties (Ghosal *et al.*, 1985; Pohl, 1999; Pelletier, 1999). Traditional healers use 156 plant species from 73 families for the treatment of gynaecological conditions (Steenkamp, 2003). Some alkaloids even induce reversible infertility in mice (Pelletier, 1999). The pharmacological properties of the Amaryllidaceae are mainly due to the presence of a group of isoquinoline alkaloids.

The first publication, to our knowledge, that mentioned the use of *Clivia* rhizomes for the cure for the bite of a puff-adder snake is that of Smith (1895). Bryant (1909) investigated Zulu medicine and recorded the use of *Clivia* rhizomes in a remedy for snake bites. *Clivia miniata* is used by the Zulu people for the relief of fevers (Bryant, 1909), and the Xhosa people use it for barrenness and urinary problems (Broster & Bourn, 1982; Hutchings *et al.*, 1996). The Swazi know *C. caulescens* as hlakahla-wemahlatsi and also uses it in medicinal practices (Dlamini, 1981).

Other uses for *Clivia* are as an antenatal tonic for a healthy foetal growth, or for inducing or augmenting labour (Bryant, 1909; Gerstner, 1941; Veale *et al.*, 1992). Veale *et al.* (1989) experimented with isolated rat uteri to test the working of the *Clivia* extract on the uterus and the active ingredient responsible for these medicinal properties. The active uterotonic compound was identified as a linolenic acid (Sewram *et al.*, 2001).

The main function of alkaloids in organisms is the defence mechanism it provides for the organism against predators, herbivores (animals and insects). This is due to the bitter taste that most alkaloids have (Wink, 2008). The alkaloids from Amaryllidaceae has been used as traditional medicine as long ago as the 4th century B.C., when Hippocrates advised the use of Amaryllidaceae preparations for the control of uterine tumours (Pelletier, 1999). Many of the alkaloids of the family Amaryllidaceae are harmful or poisonous to humans (Ghosal *et al.*, 1985; Pohl, 1999), resulting in excessive saliva production and diarrhoea, whereas higher

doses can be fatal (Pohl, 1999).

Twenty six alkaloids have been extracted from four *Clivia* species; *C. miniata*, *C. gardenii*, *C. caulescens* and *C. nobilis* (Table 3.5). Most of the studies focused on extracting alkaloids from *C. miniata*.

**Table 3.5.** Alkaloids isolated from *Clivia* (table adapted from Crouch *et al.* (2003). The absence of a (+) sign does not necessarily indicate the absence of the alkaloid in a species, but may indicate that researchers have not investigated the presence of the alkaloid in the species.

| Class of Alkaloid | Alkaloid trivial name | Activity                                                       | <i>C. miniata</i>       | <i>C. gardenii</i> | <i>C. caulescens</i> | <i>C. nobilis</i> |
|-------------------|-----------------------|----------------------------------------------------------------|-------------------------|--------------------|----------------------|-------------------|
| Lycorine          | caranine              | Antiviral, analgesic, toxicity, apoptosis, virostatic          | <sup>6</sup>            |                    |                      |                   |
|                   | cliviasindhine        |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | lycorine              |                                                                | <sup>1,2,3,4,6,10</sup> |                    | <sup>7,9</sup>       | <sup>6,10</sup>   |
|                   | sternbergine          |                                                                |                         |                    | <sup>7,9</sup>       |                   |
| Homolycorine      | clivacetine           | No Antiviral                                                   | <sup>6</sup>            |                    |                      |                   |
|                   | clivatine             |                                                                | <sup>1,4,6</sup>        |                    |                      | <sup>6</sup>      |
|                   | cliviamartine         |                                                                | <sup>3,6</sup>          |                    |                      |                   |
|                   | cliviasine            |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | cliviasyaline         |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | clividine             |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | clivimine             |                                                                | <sup>1,3,6</sup>        |                    |                      |                   |
|                   | clivonidine           |                                                                | <sup>4,6</sup>          |                    |                      |                   |
|                   | clivonine             |                                                                | <sup>1,3,4,6</sup>      |                    |                      |                   |
|                   | hippeastrine          |                                                                | <sup>1,6</sup>          |                    | <sup>7,9</sup>       |                   |
|                   | miniature             |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | nobilisine            |                                                                |                         |                    |                      | <sup>6,5</sup>    |
| Haemanthamine     | haemanthamine         | anti-viral, anti-tumoural, analgesic, insecticidal             | <sup>6</sup>            | <sup>9</sup>       | <sup>7,9</sup>       |                   |
|                   | haemanthidine         | virostatic, anti-viral, anti-tumoural, analgesic, insecticidal |                         | <sup>9</sup>       |                      |                   |
|                   | 11-hydroxyvittatine   | anti-viral, anti-tumour, analgesic, insecticidal               |                         | <sup>9</sup>       | <sup>7,9</sup>       |                   |
| Miscellaneous     | cliviahaksine         |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | clivialine            |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | clivojuline           |                                                                | <sup>6</sup>            |                    |                      |                   |
|                   | homolycorine          |                                                                | <sup>10</sup>           |                    |                      | <sup>10</sup>     |
|                   | galanthamine          |                                                                | <sup>10</sup>           |                    |                      | <sup>10</sup>     |
|                   | nobilisine A & B      |                                                                | <sup>8</sup>            |                    |                      | <sup>6</sup>      |

Authors: <sup>1</sup>Boit & Mehlis (1961); <sup>2</sup>Leven (1981); <sup>3</sup>Leven *et al.* (1982); <sup>4</sup>Ali *et al.* (1983); <sup>5</sup>Jeffs *et al.* (1988); <sup>6</sup>Viladomat *et al.* (1997); <sup>7</sup>Pohl (1999); <sup>8</sup>Darwish *et al.* (2002); <sup>9</sup>Crouch *et al.* (2003); <sup>10</sup>Bay-Smidt *et al.* (2011)

Lycorine found in *C. miniata*, *C. caulescens* and *C. nobilis*, and haemanthidine found in *C. gardenii*, are virostatic alkaloids that reduce viral DNA synthesis (Pelletier, 1999). Lycorine also has analgesic properties, and on the other hand toxicity (to animals), apoptosis and cytotoxicity (Pelletier, 1999). Galanthamine found in *C. miniata* and *C. nobilis* selectively inhibit human acetylcholinesterase. This activity can be helpful in the treatment of Alzheimer

disease and this alkaloid is therefore currently being tested in trial studies (Pelletier, 1999). In a recent study, Bay-Smidt *et al.* (2011) tested four *Clivia* species for acetylcholinesterase inhibition and affinity to the serotonin reuptake transport protein. *Clivia miniata* had a low inhibition (IC<sub>50</sub> = 18) of the enzyme acetylcholinesterase (AChE) due to the extract containing the alkaloids types crinine, galanthamine and lycorine. *C. nobilis* had an IC<sub>50</sub> of 41.

Nobilisine is an alkaloid only observed in *C. nobilis* (Jeffs *et al.*, 1988; Viladomat *et al.*, 1997). As already mentioned, many alkaloids act as a defence mechanism for the plant due to a bitter taste that repels animals and insects from eating the plant. An interesting observation was made amongst *Clivia* species (J.J. Spies, UFS, personal communication). *Clivia nobilis* plants were brought from East London to Bloemfontein and kept amongst other *Clivia* species. The mouse species *Mus orangiae* (or the Free State Pygmy mouse) is a species found in the Free State area in high altitudes and short, open grassland. Mice, probably from this group or another rodent species, fed on the entire *C. nobilis* collection, leaving only the underground rhizomes and not harming any of the other species. This phenomenon has not previously been reported and further studies on the chemical composition in *C. nobilis* might shed light on the preference that the mice had in eating the leaves of *C. nobilis*.

Despite of all the alkaloids prevalent in *Clivia*, there are several pests that escapes unharmed from the possible toxic effects of alkaloids or are not repelled by their bitterness. These include *Brithys crini*, *B. pancratii* (both known as the lily borer), mealy bugs, grasshoppers, leaf miners, scale insects, snails and slugs, snout beetles and rodents, which normally attack plant roots or remove the berries from the plant (Swanevelder & Fisher, 2009) to eat the fleshy endocarp.

Uterotonic activity tests on mice revealed that the aqueous extract of *Clivia* initiates and maintains contraction in the outer layer of the uterus (the layer responsible for contractions during labour) (Veale *et al.*, 1989, 2000; Sewram *et al.*, 2001). Lycorine (isoquinoline alkaloid) is a prevalent alkaloid in the Amaryllidaceae, and although it has antiviral properties, it is toxic and can lead to paralysis and collapse (Steenkamp, 2003).

*Clivia miniata* was tested for antischistosomal activity which can be used as a natural drug against schistosomiasis, a tropical disease that is increasing worldwide and affecting

more than 200 million people. Of 281 species tested, there are only 15 effective plant species with in vitro antischistosomal activity. *Clivia* does not have this activity (Yousif *et al.*, 2007).

Plant indigestion is a problem among children under 15 years of age. A report on paediatric plant exposures in Germany during 1998 to 2004 where possible harmful plants were digested, reported a total of 310 *Clivia* indigestions with possible minor effects (Pietsch *et al.*, 2008). A study in Spain reported 1 590 plant or herb exposures in humans and the indigestion of *Clivia miniata* accounts for less than 2% (Ramón *et al.*, 2003).

Lectins are a group of proteins (Kai *et al.*, 2003) with a high degree of conservatism in the Amaryllidaceae (Damme *et al.*, 1994) and may therefore have an important role. Agglutinin in snowdrop acts as a defence mechanism against chewing and sucking insects (Powell *et al.*, 1993), and there have been more evidence that other lectins also have a defence mechanism against plant-eating organisms (Murdock & Shade, 2002). Lectins are non-toxic to higher animals (Pusztai *et al.*, 1990) and Damme *et al.* (1994) suggests that the genes coding for lectins can be good candidate for transfer to crops to give resistance to insects.

*Clivia* has a high expression of lectin in the seed endosperm as well as ovaries. Lectin expression in the endosperm has not been detected in other Amaryllidaceae (Damme *et al.*, 1994). If lectins are toxic to insects, this phenomenon will be a good defence mechanism to protect the seeds and therefore the survival of the plant. This can be supported by observation (JJ Spies, pers. comm.) of caterpillars eating the seeds of *Brunsvigia*, which has similar seeds to those of *Clivia*, but caterpillars have never been observed on the seeds of *Clivia*. Lectins occur in the entire *Clivia* plant, but very low concentrations are expressed in the leaves, stems and petals. This might explain caterpillars and snails feeding on the leaves, stems and flowers of *Clivia* and not the seeds.

Bay-Smidt *et al.* (2011) constructed a phylogeny from combined analysis of *ITS* and *trnL-F* sequences, tested 14 taxa of the tribe Haemantheae for different alkaloids and compared it with their cladogram. Seven different alkaloid types were extracted: (A) lycorine, (B) homolycorine, (C) galanthamine, (D) montanine, (E) crinine, (F) tazettine and (G) cherylline. Only two *Gethyllis* species were tested and no representatives of the genus *Apodolirion*. None of these alkaloids were present in the *Gethyllis* species. Unfortunately, it would be unreliable

to conclude that alkaloids are absent in all species of the subtribe Gethyllidinae, but for the sake of this article we will exclude the Gethyllidinae from the rest of this discussion.

The question arises on whether the presence or absence of alkaloids can be used to assist in the phylogeny of the Haemantheae. There is no clear pattern of alkaloids that are genus specific (Table 3.6). For instance, (A) lycorine is present in *Clivia* and *Haemanthus*, but only in some and not all of the *Haemanthus* species. The only possible correlation of alkaloids with the Haemantheae phylogeny might be that the alkaloid (D) montanine developed after the divergence of *Clivia* from the rest of the genera in Haemantheae since it is absence in the basal *Clivia* clade, but present in 91% of the species from the other genera and thus synapomorphic for *Haemanthus*, *Scadoxus* and *Cryptostephanus*. *Clivia* also do not have the alkaloids (E) crinine, (F) tazettine and (G) cherylline which are present in other genera.

If only the more general alkaloids from this study are taken into account and the unique alkaloid (G) cherylline found only in *Cryptostephanus* is not considered, the *Clivia* has three of the six alkaloids present, *Scadoxus* and *Cryptostephanus* four and *Haemanthus* six. The number of alkaloids present increases from three in the basal clades to six in the more advanced *Haemanthus* clade.

**Table 3.6.** Summary of the percentage of species in the genera of the tribe Haemantheae that contain the types of alkaloids studied by (Bay-Smidt *et al.*, 2011).

|                        | number of species | Percentage (%) of species that contain the alkaloid types A, B, C, D, E, F & G |     |     |     |     |    |     |
|------------------------|-------------------|--------------------------------------------------------------------------------|-----|-----|-----|-----|----|-----|
|                        |                   | A                                                                              | B   | C   | D   | E   | F  | G   |
| <i>Clivia</i>          | 2                 | 100                                                                            | 100 | 100 | 0   | 0   | 0  | 0   |
| <i>Cryptostephanus</i> | 1                 | 100                                                                            | 0   | 100 | 100 | 100 | 0  | 100 |
| <i>Scadoxus</i>        | 3                 | 33                                                                             | 0   | 0   | 66  | 66  | 33 | 0   |
| <i>Haemanthus</i>      | 8                 | 25                                                                             | 63  | 25  | 88  | 50  | 13 | 0   |
| TOTAL                  | 14                | 6                                                                              | 7   | 5   | 10  | 7   | 2  | 1   |

Key to alkaloids: (A) lycorine, (B) homolycorine, (C) galanthamine, (D) montanine, (E) crinine, (F) tazettine and (G) cherylline.

### 3.6 POLLEN AND POLLINATION

The exact details of pollination in *Clivia* have not been sufficiently studied in their natural environment, so it is speculated that the natural pollinators of the pendulous species are bird pollinators or that these species self-pollinate. The open *C. miniata* flowers might be



moth or butterfly pollinated due to the yellow centre of many *C. miniata* taxa (Swanevelder & Fisher, 2009). The UV reflection patterns in flowers can have an influence on how pollinator visually perceive the flowers, but both the human visual spectrum as well as the UV spectrum can have an influence on attracting pollinators. Bees (Adendorff, 2008), Lepidoptera (Brunton & Majerus, 1995; Adendorff, 2008) as well as birds (Burkhardt & Maier, 1989; Bennett & Cuthill, 1994; Ödeen & Håstad, 2010) have the ability to perceive UV reflection patterns. Some genera of the Amaryllidaceae exhibit a bull's-eye or target UV patterning, but ultraviolet reflectance patterns revealed that the flowers of *Clivia* do not have a targeted UV pattern. The flowers do however have very strong overall UV reflection. *Clivia gardenii*, *C. miniata* and the cultivar *C. miniata* 'Aurea' are strongly UV reflective, but have absorptive tips and anthers. Although the UV reflection in *C. miniata* is very strong, there are contrasts between the tepals and anthers. The hybrid *Clivia* × *cyrtanthiflora* also has a strong UV reflection, but only the anthers and not the tips are absorptive. *Clivia* has a clear contrast in both the human visual spectrum (HVS) and the UV spectrum. These strong UV and HVS patterns make the flowers more attractive and visible to pollinators in the shaded environment (Conran, 2005).

From the cladogram of Conrad *et al.* (2003), these authors concluded that the open, upright flowers of *C. miniata* are a derived morphological characteristic. Since butterflies pollinate the open flowers and sunbirds the pendulous flowers, it was subsequently suggested that butterfly pollination is derived from the ancestral sunbird pollination state. The topology of the *ITS1-2* cladogram for the current paper is congruent with this theory, when considering the "overall" flower types for each genus. *Clivia* is basal in this cladogram, and the majority species have pendulous, tubular flowers. The flowers of *Cryptostephanus vansonii* are tubular, but have spreading to suberect tepals (Duncan, 2010). *Apodolirion* and *Gethyllis* have very similar small, open flowers. The species of *Haemanthus* either have flowers in the form of a compact head, or spreading, but the majority of the species have a compact form. The flowers of the South African *Scadoxus* species has the appearance of a large, single flower, but are in fact an umbel cupped with four or more erect bracts (Goldblatt & Manning, 2000). This flower morphology can be more advantageous since the pollinator needs to visit only as single "flower" but in affect will be pollinating multiple flowers from only one visit. This flower type seems to be a derived adaptation based on the cladogram topology.

Other studies investigated pollen viability (Shen *et al.*, 2007), in vitro placental pollination (Zůbková & Sladký, 1975) and the natural segregation of the nucleolar components in pollen tubes (Franke *et al.*, 1972; Tang, 1973, 1979; Knox & Singh, 1987). Chemotropism of pollen tubes studied in *C. nobilis* indicates a positive chemotropism to one or more segments of the floral organs of the same species (Linck & Blaydes, 1960). Unfortunately this author did not investigate the chemotropism of the pollen of one *Clivia* species towards the floral parts of another *Clivia* species. Further studies on the chemotropism of the pollen tubes toward plant parts of the same plant as well as towards different plants or species might possibly yield valuable new data on fertilization in *Clivia*.

### 3.7 CONCLUSION

*Clivia* is a member of the family Amaryllidaceae and the tribe Haemantheae and is closest related to the genus *Cryptostephanus*. These two genera are basal to the tribe and have the highest chromosome numbers of the genera in the tribe. Only one polyploid has been reported for *Clivia* ( $2n = 44$ ) and all six species have a somatic chromosome number of  $2n = 22$ . This chromosome number is plesiomorphic for the Amaryllidaceae, and it is suspected that dysploid reduction resulted in the lower chromosome numbers in the tribe.

*Clivia* has the smallest mean genome size (2C measured in pg) in the Haemantheae and the mean genome sizes for the genera increases with the evolutionary direction in the tribe. There is a negative correlation between the mean genome size in *Clivia*, *Scadoxus* and *Haemanthus*, and the chromosome numbers. *Cryptostephanus* and *Apodolirion* have somatic chromosome numbers of  $2n = 24$  and  $2n = 12$  respectively and have very similar genome sizes.

The exact phylogenetic position of the *Clivia* species within the genus is inconclusive due to the differences in results between studies. There are several factors contributing to the inconclusive results. *Clivia mirabilis* and *C. robusta* were not included in all the studies due to its late discovery and relatively recent descriptions respectively. The interspecific variation due to overlapping geographical distribution has not been taken in consideration in all the studies, and possible misidentification of species also seemed to have influenced the phylogenetic pattern in some studies.

An in depth study of the genus is in progress, but from a combination of published and unpublished data it seems as if *C. mirabilis*, *C. nobilis* and *C. caulescens* forms genetically and geographical distinct groups (with minor geographical overlapping in some areas). *Clivia miniata*, *C. gardenii* and *C. robusta* overlap in larger geographical areas, and the possible hybridization and introgression events are reflected in the interspecific variation as well as intraspecific similarities.

The most primitive species is *C. mirabilis*. Evolution in the genus has a correlation with geographical distribution from west to east and genome size from small to large. The relative large genome size of *Clivia*, together with the medicinal properties, contributes to the vulnerability of the genus.

An in-depth article on phylogeny, inter- and intraspecific variation and a barcode system to identify the species and their geographical origin is in progress. There are still scope for further research in the genus, and future studies might include an in depth relationship study between alkaloids and the evolution of *Clivia* and its relatives.

### 3.8 STATEMENT OF RESEARCH QUESTIONS

Based on this literature review the following research questions should be addresses in this thesis.

1. How effective can DNA-barcoding regions be applied in *Clivia*? This question will be answered by investigating the genetic variation within all the *Clivia* species by comparing five different gene regions (*psbA-trnH*, *trnL-F*, *matK*, *atpH* and *ITS2*). At least five samples per species will be included for the comparison. Can the DNA-barcoding regions suggested by CBOL be effectively applied on *Clivia* to identify species? The effectiveness in the barcoding regions will be tested statistically using the computer software Geneious R6, jMOTU, MEGA 5.05 as well as minor supporting programs. This will be discussed in Chapter 4.
2. *Clivia* is an ancient polyploidy. What are the influences of the stable chromosome number on the DNA-sequences? The same gene regions will be compared between *Clivia* (a “stable” ancient polyploidy) and *Lachenalia* (with variable chromosome numbers and

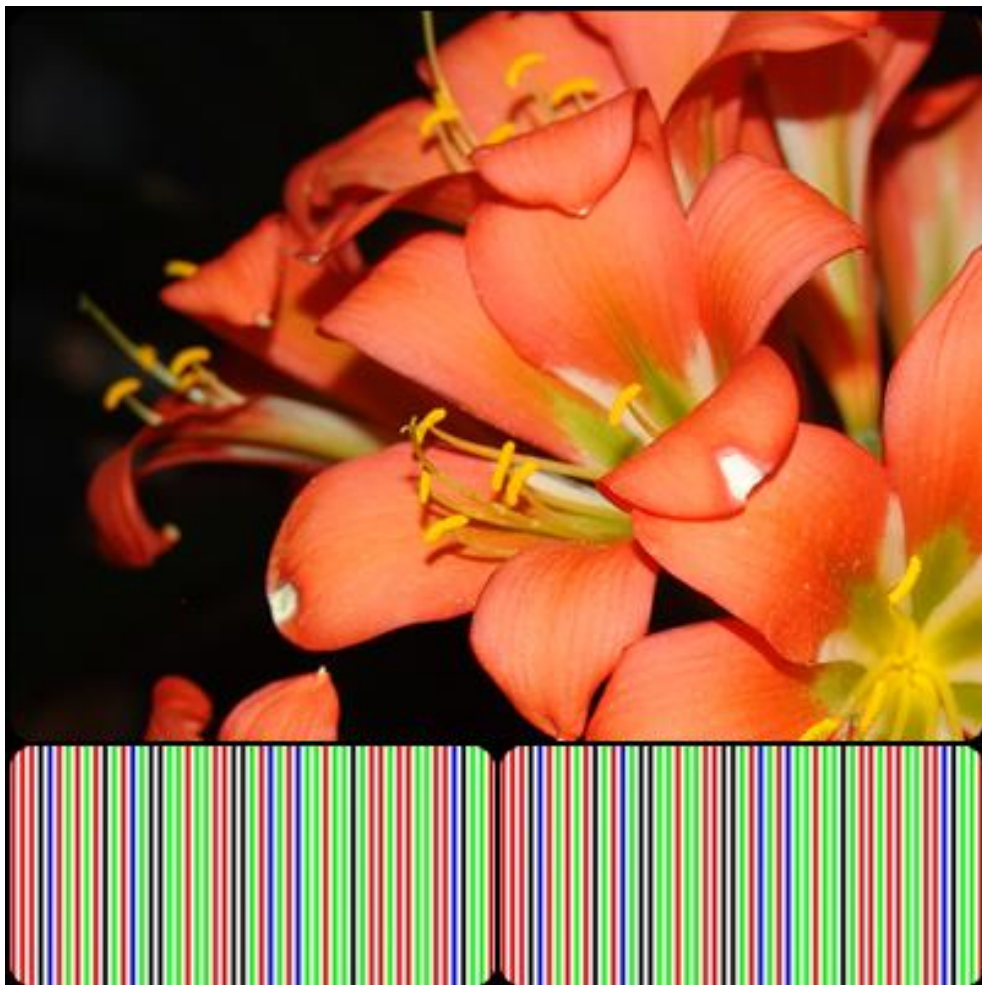
polyploidy levels) to test the effects of the difference in chromosome constitutions on the DNA. The specific focus will be on Tandem repeats, SNPs and INDELS in the sequences. This will be discussed in Chapter 4.

3. What is the extent of phylogeographical evolution in *Clivia*? Sequence data from species representing the total geographical distribution area, will be analysed to determine the phylogeography in the genus. This will be discussed in Chapter 4.

## PART B: *Clivia*

### *Chapter 4*

#### DNA-barcoding in the genus *Clivia*



**modified from** Spies, P., van der Westhuizen, H., Stegmann, S., Watson, M. & Spies, J.J. 2012 *Clivia* 13: 26-37

## ABSTRACT

*Clivia* is a member of the family Amaryllidaceae and consists of six species and one natural hybrid. The horticultural potential and medicinal properties of the genus, and habitat destruction due to property developments, lead to illegal removal of plants from nature. These factors contribute to the threatened status of the genus. Eight chloroplast regions (*atpH-atpI*, *matK*, *trnH-psbA*, *rbcL*, *rpL16*, *rpoB*, *rpoC1* and *trnL-F*) and one nuclear region (*ITS2*) were sequenced and tested for their efficacy as DNA-barcodes to identify unknown *Clivia* plants. Each species (and where possible different colour variants) were represented by at least three specimens. The sequencing data were analysed with tree-based, distance-based and character-based analyses. Both the tree-based and character-based analyses are effective in the identification of a sample. Four of the six *Clivia* species can be identified with DNA-barcodes, i.e. *C. mirabilis*, *C. nobilis*, *C. miniata* and *C. caulescens* / *C. xnimbicola*. The two closely related species *C. robusta* and *C. gardenii* do not have unique barcodes to separate them, and identification of these species need to be revised. From the current results, the best barcoding regions with best discriminatory power are the *trnL-F* chloroplast region, the *matK* chloroplast gene and the *ITS2* nuclear spacer region. The two-barcode regions, *matK* and *trnL-F* are sufficient to distinguish four *Clivia* species.

## PREFACE

Parts of this chapter will be submitted for publication as a barcode data release paper. Careful attention will be given to the review criteria<sup>17</sup>, e.g. that the data quality is up to standard, that the data records “represent a significant addition to the public knowledge base”, that the release manuscript is relevant to other research programs and “societal applications” and that all the taxonomic and barcoding data is accessible for the research community.

---

<sup>17</sup>[http://bol.uvm.edu/docs/data\\_release\\_guidelines.pdf](http://bol.uvm.edu/docs/data_release_guidelines.pdf)

## 4.1 INTRODUCTION

### 4.1.1 The need for DNA-barcoding in *Clivia*

The majority of *Clivia* species can be easily and correctly identified based on morphological traits, but in the vegetative state identification by an inexperienced person become problematic, especially since some of the leaf-characteristics overlap between species. One such example is the median lines on the leaves, that are characteristic of *C. mirabilis* and *C. nobilis*, but that may not only also be absent in both species but also sometimes occur in other species. The leaf edges of both these species have a very fine serrated edge. These two species, as well as many of the other species, can very easily be miss-identified, especially when seedlings are sold. For example, seedlings can easily be exported as the rare *C. mirabilis*, but in reality it may be *C. nobilis* plants being sold. For this reason it is necessary to identify the appropriate DNA-barcoding regions in *Clivia*, to explore the most appropriate analysis method and to create a proper database for the genus.

The seeds of *Clivia* are too heavy for wind dispersal and usually get distributed by rodents or by rolling down slopes. Taxa with limited dispersal ability are expected to have substantial phylogeographical structure (Moritz & Cicero, 2004), since the chloroplast is maternally inherited through the seeds. Conversely, the distribution of pollen should be much broader and may even span geographical barriers. The patterns of geographical structure may therefore differ when studying chloroplast and nuclear regions.

In theory, it should be possible to distinguish between populations if the populations are isolated from each other and if the populations have adapted to new environments by means of selection of favourable mutations. Pollination from nearby populations, areas or other species should not influence the results, since the DNA in pollen does not contribute to the DNA studied.

For this study on *Clivia*, the ideal was to find DNA that will distinguish perfectly between the different species. We analysed eight different DNA regions, all of which have different mutation rates and which have a different number of mutation sites. The nuclear *ITS2* region is biparently inherited and may ultimately result in different barcoding patterns

than the chloroplast gene regions. The aim was to find a region with enough mutation sites to distinguish between the different species.

Moritz & Cicero (2004) emphasized the need to include groups with frequent hybridization and recent radiations in studies to satisfy DNA-barcoding sceptics. Some *Clivia* species (*C. mirabilis* and to a large degree *C. caulescens* and *C. nobilis*) are geographically isolated by distance, mountains and rivers, but there are overlapping areas with co-occurrence of species (*C. miniata* & *C. gardenii*; *C. miniata* & *C. caulescens*; and *C. miniata* & *C. robusta*). Because *Clivia* can freely hybridize and there may be issues of incomplete lineage sorting, this is an interesting genus to investigate and a good candidate for a DNA-barcoding study.

*Clivia* has been included in various phylogenetic or phylogeographic studies (Meerow & Snijman, 1998; Meerow *et al.*, 1999; Conrad *et al.*, 2003; Meerow & Clayton, 2004; Conrad, 2008). A DNA-barcoding study has however never been performed on the entire genus and its closest relative, *Cryptostephanus*. Thus, very little data for the suggested DNA-barcoding regions are available on GenBank (Table 4.1). All of the species are rarely represented in any published studies. *Clivia* and *Cryptostephanus* are both members of the tribe Haemantheae<sup>18</sup> (see Chapter 3), and a small number of samples from the genus *Gethyllis* (from the same tribe as *Clivia*) were also included during the study of some of the gene regions.

#### 4.1.2 Aims and objectives

The aim of this study was to evaluate various DNA regions as putative DNA-barcoding regions in *Clivia*.

These aims will be accomplished through the following objectives:

1. The putative new regions for barcoding *Clivia* (*atpH-I*, *ITS2*, *trnH-psbA*, *rpL16*, *rpoB*, *rpoC1* and *trnL-F*) will be evaluated by analysing levels of inter- and intraspecific variation in all the *Clivia* species and calculating the barcoding gap.

---

<sup>18</sup>Other members of this tribe are the genera *Gethyllis*, *Haemanthus*, *Scadoxus* and *Apodolirion*.



2. The data sets of all the regions will be aligned and then their efficacy will be tested by performing tree-based, distance-based and character-based analyses.

**Table 4.1** Availability of different barcoding markers of the closely related genera *Clivia* and *Cryptostephanus* on NCBI, GenBank (accessed November 2012)

| Marker    | Genomic source | Type                              | Data on GenBank      |                      |                                                             |
|-----------|----------------|-----------------------------------|----------------------|----------------------|-------------------------------------------------------------|
|           |                |                                   | Number of accessions | Data for both genera | *Number of <i>Clivia</i> ( <i>Cryptostephanus</i> ) species |
| nrlTS     | Nuclear        | Transcribed spacers and 5.8S gene | 20                   | Yes                  | 5 (2)                                                       |
| nrlTS2    | Nuclear        | Transcribed spacer                | 1                    | No                   | 1 (0)                                                       |
| matK      | Plastid        | Protein coding                    | 15                   | Yes                  | 4 (1)                                                       |
| rbcLa     | Plastid        | Protein coding                    | 8                    | Yes                  | 3 (1)                                                       |
| rpoB      | Plastid        | Protein coding                    | 0                    | 0                    | 0 (0)                                                       |
| rpoC1     | Plastid        | Protein coding                    | 0                    | 0                    | 0 (0)                                                       |
| trnH-psbA | Plastid        | Intergenic spacer                 | 0                    | 0                    | 0 (0)                                                       |
| trnL-F    | Plastid        | Intron and intergenic spacer      | 24                   | Yes                  | 5 (2)                                                       |
| trnT-L    | Plastid        | Intergenic spacer                 | 0                    | 0                    | 0 (0)                                                       |
| atpH-atpI | Plastid        | Intergenic spacer                 | 17                   | No                   | 2 (0)                                                       |
| rpL16     | Plastid        | Intron                            | 0                    | 0                    | 0 (0)                                                       |

\*Based on the names listed in GenBank

## 4.2 MATERIALS AND METHODS

### 4.2.1 Collection and Material

All the plant materials used in this study (Table 4.2) were supplied by collectors or institutes (see acknowledgements at the beginning of this thesis) to ensure that collection of material is non-invasive to the fragile ecological areas. The *Clivia* samples represent all the species in the genus (with at least three representatives per species). Different colour variants (e.g. yellow and orange flowers) were included where possible. Samples from a broad geographical range were collected by various collectors to represent the geographical coverage of the genus (Figure 4.1). Each leaf sample was collected with a unique collection number assigned to it. The collection site was notated, though in some instances samples without the exact collection site were also included and only the province from which it was collected was documented.

The leaf material were either collected in a saturated NaCl-CTAB solution and stored at 4°C or in silica and stored at room temperature. Since non-invasive techniques were used (collection of leaf samples), plants were photographed for identification purposes. *Clivia* species were identified by Prof. J.J. Spies (UFS).

**BOX 4.1: *Affinis* (aff.) species**

Six *Clivia* species have formally been described (see Chapter 3). There is, however, controversy in the *Clivia* community regarding the delimitation of some species. Some of the questions that have been raised is, e.g. “Should the robust form of *C. gardenii* (*C. robusta*) be regarded as a separate species?” “Should other forms such as *C. miniata* with stems be considered as putative new species?” “If *C. xnimbicola* has been recognised as a natural hybrid, should other natural hybrids not also be described and recognised”.

For this study, some atypical samples were included. The species it resembles is indicated by the taxonomical nomenclature *aff.* (Latin for *Affinis*) followed by the species. These samples however have distinct, uncharacteristic morphologies.

***Clivia* aff. *caulescens***

These plants grow in Swaziland and morphologically resemble a cross between *C. caulescens* and *C. gardenii*. The distribution is not included in the range of *C. gardenii* but overlaps with the distribution of *C. caulescens* and *C. miniata*.

***Clivia* aff. *gardenii*<sup>1</sup>**

These *C. gardenii*'s grow in north-eastern KwaZulu-Natal, the flowers atypical from other *C. gardenii*'s, the leaves are greyish and these plants have a stem.

***Clivia* aff. *gardenii*<sup>2</sup>**

Is found in and around the Ngome forest in northern KwaZulu-Natal in/adjacent to areas where *C. miniata* also occurs. These plants have a unique round thickening at the base of the stem and the rest of the plant is morphologically similar to *C. gardenii*.

***Clivia* aff. *miniata***

These plants occur in the area of the Mzamba River (Eastern Cape). They have greyish leaves, stems are present and they have either upright or drooping flowers resembling that of *C. miniata* and *C. gardenii* respectively.

***Clivia* aff. *robusta*** is a very large type of *C. robusta* and differs morphologically from the type form by resembling *C. nobilis* (Fred van Niekerk's 'maxima').

***Clivia gardenii* vs. *C. robusta*** (previously known as “swamp” *gardenii*) are distinguished from each other by the geographical range and morphological differences. *Clivia gardenii* occurs in central and north-eastern KwaZulu-Natal (north of Durban) in well drained soil, whereas *C. robusta* grows in southern KwaZulu-Natal and in the Eastern Cape (in the Pondoland centre of endemism) in swampy conditions. *Clivia robusta* resembles *C. gardenii* with a few morphological differences, i.e. it is larger with a prominent stem, it has more flowers (15-40) compared to the 14-20 of *C. gardenii* and the leaves differ slightly (Swanevelder & Fisher, 2009). Deviations from the described forms have been found (Prof. Spies, pers. comm.), such as the *C. robusta* growing at high altitudes in dry conditions on the cliffs of the Oribi Gorge (KwaZulu-Natal). Although one of the distinguishing characteristics of *C. robusta* is the robustness of this species, small adult *C. robusta* specimens that are the same size of *C. gardenii* have been observed. *Clivia gardenii* usually grow in dry conditions (Swanevelder & Fisher, 2009), but exceptions have been found where *C. gardenii* grows in the same swampy conditions as *C. robusta*. Because of these overlapping conditions and traits between *C. gardenii* and *C. robusta*, both will be considered as part of the *C. gardenii*-complex.

***Clivia gardenii*-complex**

This is a term referring to the species *C. gardenii*, *C. robusta*, *C. aff. gardenii*<sup>1</sup> & <sup>2</sup>, *C. aff. robusta* and *C. aff. miniata*. This term was created since this is a group with many morphological and genetic variation, and is may be difficult to delimit members in this group based on the morphology and molecular data. The key factors for this taxonomical confusion may lay in the geographical overlap or close proximity of different species leading to hybridization (ancient or recent). Other factors are adaptation to e.g. swampy areas leading to stem formation. It is not part of this study to comprehensively study this complex, but specimens of this complex were included in the molecular analysis.

**Table 4.2** Samples used in the current study with the collection numbers, original collection localities and approximate GPS coordinates (to the nearest degree).

| Species                                               | Collection number                                                                                                                                                                                                              | Locality                                 | Latitude | Longitude |
|-------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|----------|-----------|
| <i>Clivia xnimbicola</i>                              | Spies 8552 <sup>BA</sup> , 8553 <sup>B</sup> , 8554 <sup>B</sup> ,<br>8555 <sup>BA</sup> , 8556 <sup>BA</sup> , 8566 <sup>BA</sup> , 8570 <sup>BA</sup> ,<br>8578 <sup>B</sup> , 8594, 8595, 23 <sup>S</sup> , 29 <sup>S</sup> | Bearded Man, Mpumalanga                  | -25      | 31        |
| <i>C. aff. caulescens</i>                             | Spies 8575 <sup>B</sup>                                                                                                                                                                                                        | Swaziland                                | -25      | 31        |
| <i>C. aff. gardenii</i> <sup>1</sup>                  | Spies 8576, 8577                                                                                                                                                                                                               | East of Greytown, KwaZulu-Natal          | -29      | 31        |
| <i>C. aff. gardenii</i> <sup>1</sup>                  | Spies 8325, 9000, 9001                                                                                                                                                                                                         | Mzamba Estuary, Eastern Cape             | -31      | 30        |
| <i>C. aff. gardenii</i> <sup>2</sup>                  | Spies 8781                                                                                                                                                                                                                     | Ngome Forest Reserve, KwaZulu-Natal      | -27      | 31        |
| <i>C. aff. miniata</i>                                | Spies 8318 <sup>W</sup> , 8469 <sup>W</sup> , 8470 <sup>W</sup> ,<br>8780, 9002                                                                                                                                                | Mzamba Estuary, Eastern Cape             | -29      | 31        |
| <i>C. aff. robusta</i>                                | Spies 8579, 8582                                                                                                                                                                                                               | Port St. John's, Eastern Cape            | -31      | 29        |
| <i>C. aff. robusta</i>                                | Spies 8804 <sup>W</sup> , 8805 <sup>W</sup> , 8806 <sup>W</sup>                                                                                                                                                                | Mtentu River, Eastern Cape               | -31      | 28        |
| <i>C. aff. robusta</i>                                | Spies 8585                                                                                                                                                                                                                     | Umtamvuna Nature Reserve, KwaZulu-Natal  | -31      | 30        |
| <i>C. aff. robusta</i>                                | Spies 8593                                                                                                                                                                                                                     | Unknown, KwaZulu-Natal                   |          |           |
| <i>C. caulescens</i>                                  | 38 <sup>S</sup>                                                                                                                                                                                                                | Soutpansberg, Mpumalanga                 | -22      | 29        |
| <i>C. caulescens</i>                                  | Spies 8417 <sup>BA</sup>                                                                                                                                                                                                       | Near Mbabane, Swaziland                  | -26      | 31        |
| <i>C. caulescens</i>                                  | Spies 8487 <sup>BA</sup> , 8488 <sup>BA</sup>                                                                                                                                                                                  | Wolkberg, Limpopo                        | -24      | 29        |
| <i>C. caulescens</i>                                  | Spies 8494 <sup>BA</sup> , 8495, 8496 <sup>BA</sup> ,<br>8497 <sup>BA</sup> , 8499 <sup>BA</sup> , 8500 <sup>BA</sup> , 8501 <sup>BA</sup> ,<br>8502 <sup>BA</sup> , 8503 <sup>BA</sup> , 8504 <sup>BA</sup>                   | Mariepskop Forest Reserve, Mpumalanga    | -24      | 30        |
| <i>C. caulescens</i>                                  | Spies 9049 <sup>B</sup> , 9056 <sup>BA</sup> , 9057 <sup>BA</sup> ,<br>9058 <sup>BA</sup>                                                                                                                                      | Wonderview, Mpumalanga                   | -24      | 30        |
| <i>C. caulescens</i>                                  | Spies 8609 <sup>BA</sup> , 8479, 10 <sup>S</sup> , 25 <sup>S</sup>                                                                                                                                                             | God's Window, Mpumalanga                 | -24      | 30        |
| <i>C. caulescens</i>                                  | Spies 8490                                                                                                                                                                                                                     | Pinnacle, Mpumalanga                     | -24      | 30        |
| <i>C. caulescens</i>                                  | 40 <sup>S</sup> , Spies 8557 <sup>BA</sup> , 8561 <sup>BA</sup> , 8571 <sup>BA</sup>                                                                                                                                           | Bearded Man, Mpumalanga                  | -25      | 31        |
| <i>C. caulescens</i>                                  | 39 <sup>S</sup> , Spies 8562 <sup>BA</sup>                                                                                                                                                                                     | Swaziland                                | -26      | 31        |
| <i>C. caulescens</i>                                  | 6 <sup>S</sup> , 13 <sup>S</sup> , 14 <sup>S</sup> , 15 <sup>S</sup> , 17 <sup>S</sup> , 20 <sup>S</sup> , 22 <sup>S</sup> ,<br>24 <sup>S</sup> , 31 <sup>S</sup> , 32 <sup>S</sup> , 41 <sup>S</sup> , 43 <sup>S</sup>        | Unknown, Mpumalanga                      |          |           |
| <i>C. gardenii</i>                                    | Spies 8367 <sup>BA</sup> , 8368 <sup>BA</sup> , 8369 <sup>B</sup> ,<br>8523                                                                                                                                                    | Ngome Forest Reserve, KwaZulu-Natal      | -27      | 31        |
| <i>C. gardenii</i>                                    | Spies 8405 <sup>B</sup>                                                                                                                                                                                                        | Entumeni Nature Reserve, KwaZulu-Natal   | -28      | 31        |
| <i>C. gardenii</i>                                    | Spies 8418 <sup>B</sup>                                                                                                                                                                                                        | Greytown, KwaZulu-Natal                  | -29      | 30        |
| <i>C. gardenii</i>                                    | Spies 8403 <sup>B</sup>                                                                                                                                                                                                        | Harburg, KwaZulu-Natal                   | -29      | 30        |
| <i>C. gardenii</i>                                    | Spies 8444 <sup>B</sup>                                                                                                                                                                                                        | Port Shepstone, KwaZulu-Natal            | -30      | 30        |
| <i>C. gardenii</i>                                    | Spies 8374 <sup>B</sup> , 8376 <sup>BA</sup>                                                                                                                                                                                   | Unknown, KwaZulu-Natal                   | -28      | 30        |
| <i>C. gardenii</i> x <i>C. miniata</i> natural hybrid | Spies 8658 <sup>W</sup>                                                                                                                                                                                                        | Unknown                                  |          |           |
| <i>C. miniata</i>                                     | Spies 8558 <sup>B</sup> , 18 <sup>S</sup> , 21 <sup>S</sup> , 22 <sup>S</sup> , 33 <sup>S</sup> ,<br>34 <sup>S</sup> , 36 <sup>S</sup> , 37 <sup>S</sup> , 42 <sup>S</sup>                                                     | Bearded Man, Mpumalanga                  | -25      | 31        |
| <i>C. miniata</i>                                     | 35 <sup>S</sup>                                                                                                                                                                                                                | Swaziland                                | -26      | 31        |
| <i>C. miniata</i>                                     | Spies 8526                                                                                                                                                                                                                     | Ngome Forest Reserve, KwaZulu-Natal      | -27      | 31        |
| <i>C. miniata</i>                                     | Spies 8406 <sup>B</sup>                                                                                                                                                                                                        | Eshowe, KwaZulu-Natal                    | -28      | 31        |
| <i>C. miniata</i>                                     | Spies 8992                                                                                                                                                                                                                     | Blinkwater Nature Reserve, KwaZulu-Natal | -29      | 30        |
| <i>C. miniata</i>                                     | Spies 8394 <sup>B</sup> , 8667 <sup>W</sup>                                                                                                                                                                                    | Umtamvuna Nature Reserve, KwaZulu-Natal  | -31      | 30        |
| <i>C. miniata</i>                                     | Spies 8391 <sup>B</sup> , 8408 <sup>B</sup>                                                                                                                                                                                    | Dwesa, Eastern Cape                      | -32      | 28        |
| <i>C. miniata</i>                                     | Spies 8419 <sup>B</sup>                                                                                                                                                                                                        | Kentani, Eastern Cape                    | -32      | 28        |
| <i>C. miniata</i>                                     | Spies 8396 <sup>B</sup>                                                                                                                                                                                                        | Kei River, Eastern Cape                  | -32      | 28        |
| <i>C. miniata</i>                                     | Spies 8410 <sup>B</sup>                                                                                                                                                                                                        | Peddie, Eastern Cape                     | -33      | 27        |
| <i>C. miniata</i>                                     | Spies 8573, 8574                                                                                                                                                                                                               | Unknown, KwaZulu-Natal                   | -28      | 30        |
| <i>C. miniata</i>                                     | Spies 8637 <sup>BA</sup>                                                                                                                                                                                                       | Squebez, KwaZulu-Natal                   | -27      | 31        |
| <i>C. mirabilis</i>                                   | Spies 8963                                                                                                                                                                                                                     | Oorlogskloof, Northern Cape              | -34      | 19        |
| <i>C. mirabilis</i>                                   | Spies 8942, 8945, 8943 <sup>vdW</sup> ,<br>8944 <sup>vdW</sup>                                                                                                                                                                 | Locality 1, population 1, Western Cape   | -31      | 19        |
| <i>C. mirabilis</i>                                   | Spies 8946 <sup>vdW</sup> , 8947 <sup>B</sup> , 8948 <sup>vdW</sup> ,<br>8949 <sup>vdW</sup> , 8950 <sup>vdW</sup> , 8951 <sup>vdW</sup> , 8952                                                                                | Locality 1, population 2, Western Cape   | -31      | 19        |
| <i>C. mirabilis</i>                                   | Spies 8953 <sup>B</sup> , 8954 <sup>vdW</sup> , 8955 <sup>B</sup> ,<br>8956 <sup>vdW</sup> , 8957 <sup>vdW</sup> , 8958 <sup>B</sup> ,<br>8959 <sup>vdW</sup> , 8960 <sup>vdW</sup> , 8961 <sup>vdW</sup>                      | Locality 2, Western Cape                 | -31      | 19        |
| <i>C. nobilis</i>                                     | Spies 8634, 8936 <sup>B</sup> , 8962 <sup>vdW</sup>                                                                                                                                                                            | Qora, Eastern Cape                       | -32      | 28        |

|                                        |                                                             |                                |     |    |
|----------------------------------------|-------------------------------------------------------------|--------------------------------|-----|----|
| <i>C. nobilis</i>                      | <i>Spies 8938</i> <sup>vdW</sup>                            | Keiskammahoek, Eastern Cape    | -32 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8939</i> <sup>vdW</sup> , <i>8940</i> <sup>B</sup> | Bonza Bay, Eastern Cape        | -32 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8932</i> <sup>B</sup>                              | Horseshoe Valley, Eastern Cape | -32 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8941</i> <sup>vdW</sup>                            | Fish River, Eastern Cape       | -33 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8934</i>                                           | Rocklands, Eastern Cape        | -33 | 25 |
| <i>C. nobilis</i>                      | <i>Spies 8935</i>                                           | Birna River, Eastern Cape      | -33 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8937</i> <sup>B</sup>                              | Chalumna, Eastern Cape         | -33 | 27 |
| <i>C. nobilis</i>                      | <i>Spies 8933</i> <sup>B</sup>                              | Unknown, Eastern Cape          |     |    |
| <i>C. robusta</i>                      | <i>Spies 8440</i> <sup>B</sup>                              | Port Shepstone, KwaZulu-Natal  | -30 | 30 |
| <i>C. robusta</i>                      | <i>Spies 8415</i> <sup>B</sup>                              | Port Edward, KwaZulu-Natal     | -31 | 30 |
| <i>C. robusta</i>                      | <i>Spies 8841</i>                                           | Lupatana, Eastern Cape         | -31 | 29 |
| <i>C. robusta</i>                      | <i>Spies 8442</i> <sup>B</sup>                              | Port St. Johns, Eastern Cape   | -31 | 29 |
| <i>C. robusta</i>                      | <i>Spies 8462</i> <sup>B</sup>                              | Unknown, KwaZulu-Natal         | -30 | 30 |
| * <i>Cryptostephanus haemanthoides</i> | AY280350                                                    |                                |     |    |
| <i>Cr. vansonii</i>                    | <i>Spies 8964</i>                                           | Bvumba Mountains, Zimbabwe     | -19 | 32 |
| * <i>Cr. vansonii</i>                  | HM140800, HM140800, AY280351.1                              |                                |     |    |
| * <i>Gethyllis afra</i>                | HM140801                                                    |                                |     |    |
| <i>G. britteniana</i>                  | <i>Spies 9050</i> <sup>B</sup>                              | Nardouwsberg, Western Cape     | -31 | 18 |
| * <i>G. britteniana</i>                | AY280352                                                    |                                |     |    |
| * <i>G. ciliaris</i>                   | AY280353                                                    |                                |     |    |
| * <i>G. grandiflora</i>                | HM140802                                                    |                                |     |    |
| * <i>G. namaquensis</i>                | EU543627                                                    |                                |     |    |
| * <i>G. verticillata</i>               | AY28035                                                     |                                |     |    |
| <i>G. lanuginosa</i>                   | <i>Spies 9051</i> <sup>B</sup>                              | Perdekraal, Western Cape       | -32 | 20 |
| <i>G. lanuginosa</i>                   | <i>Spies 9055</i> <sup>B</sup>                              | Engelse punt, Western Cape     | -31 | 19 |
| <i>G. multifolia</i>                   | <i>Spies 9054</i> <sup>B</sup>                              | Rawsonville, Western Cape      | -33 | 19 |
| <i>G. setosa</i>                       | <i>Spies 9052</i> <sup>B</sup>                              | Unknown, Western Cape          | -30 | 18 |
| <i>G. sp. nov.</i>                     | <i>Spies 9053</i> <sup>B</sup>                              | Cederberg, Western Cape        | -32 | 19 |
| * <i>Apodolirion cedarbergense</i>     | AY280344                                                    |                                |     |    |
| * <i>A. lanceolatum</i>                | AY280345                                                    |                                |     |    |

<sup>vdW</sup> Sequences from co-author (van der Westhuizen, 2010)

<sup>S</sup> Sequences from co-author (Stegmann, 2011)

<sup>W</sup> Sequences from co-author (Watson)

<sup>B</sup> Samples send to CCDB for sequencing *matK* and *rbclA* (the other regions were sequenced at the Dept. of Genetics, UFS)

<sup>BA</sup> Additional samples send to CCDB for sequencing *matK* and *rbclA* (no other region was sequenced for these samples)

\* Sequences obtained from GenBank

All the specimen records and sequences are maintained in a database at the Department of Genetics, UFS. Sequences generated by CCDB are deposited in the publically available database BOLD, together with all the data associated with each specimen (e.g. GPS coordinates, photographs and collection information). After publication the data will be released and made available on NCBI, GenBank.

## 4.2.2 Methods

Refer to Chapter 2 for a detailed explanation of all the methods used in this study.

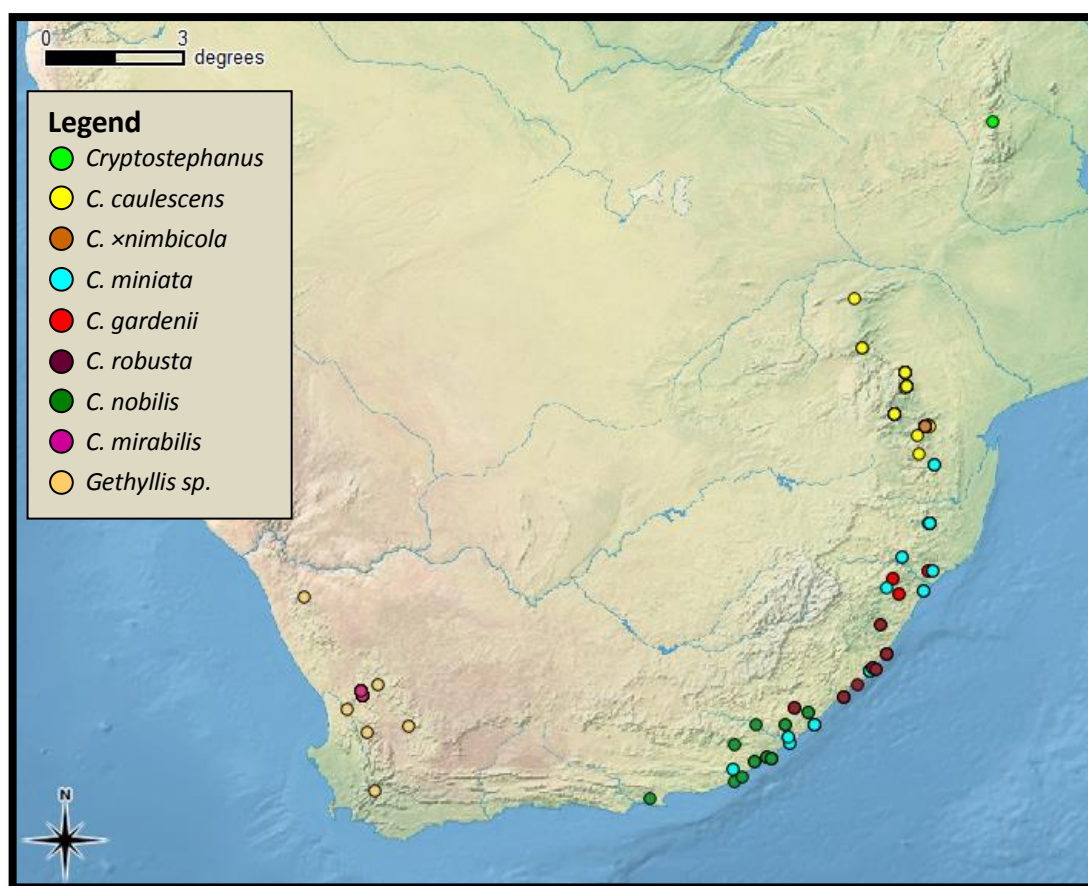


**Figure 4.1** The geographical distribution of the *Clivia* and *Gethyllis* samples used in this study (map was created in Quantum GIS 1.8.0).

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Extraction, amplification and sequencing

The leaf material used in this study was mainly sampled by collectors and breeders. It was mainly the older, harder leaves with a higher level of secondary compounds that were collected. The young leaves of *Clivia* yielded a better quality DNA than the older, outer leaves of the plants. When visualised on an agarose gel, the DNA from the young leaves had less or no smears. Even by adapting the extraction protocol by adding recommended chemicals (such as PVP and SDS) at different stages in the process (see Chapter 4), the secondary compounds (e.g. polysaccharides and polyphenolic compounds) could not be removed completely. The polysaccharides co-precipitate with the DNA (Kumari *et al.*, 2012), resulting in a viscous solution. Some leaf samples, where the direct PCR approach was followed, seemed to contain PCR inhibitory components, and were excluded from the study after 3-4 amplification attempts. Kumari *et al.* (2012) developed an effective DNA-extraction technique

yielding good quality DNA from a broad spectrum of plants. Even in potatoes, with high polysaccharide content, DNA were successfully isolated and used in numerous downstream applications. For future studies on *Clivia* it is suggested that the young leaves are used for DNA extraction, or alternatively that the method of Kumari *et al.* (2012) be implemented as an extraction protocol.

The quality and quantity of PCR success differed for the different gene regions (Table 4.3). Each unsuccessful PCR reaction for a particular sample was followed by two to three more attempts, often adapting the protocol to optimise the reaction. Thereafter that specific sample was excluded from further analyses for that gene region. In this study it was attempted to obtain successful PCR amplification for an overall total of 393 reactions (of which several were repeated up to three times). Successful PCR amplification was obtained for 310 reactions (78.9%). The sequencing success of the successful PCR reactions was slightly higher (87.0%) and successful sequences were thus obtained for 261 sequences in this study. A total number of 163 additional sequences from the co-authors, ten from GenBank and three from CCDB were added to the sequences generated in this study, aligned (Appendix B) and used in the analysis. The DNA from leaf material was also sequenced by the Canadian Centre for DNA-Barcoding (CCDB) for *matK* and *rbclLa*.

**Table 4.3** Summary of PCR and sequence amplification success per DNA-barcoding locus in *Clivia*. Statistics for each gene region after alignment and trimming were obtained from Geneious R6 and MEGA 5.05. Only the results generated in this study are represented in this Table.

| Gene region      | PCR statistics                     |                 | Sequencing statistics              |                      | Gene region statistics |                     |                    |                |            |
|------------------|------------------------------------|-----------------|------------------------------------|----------------------|------------------------|---------------------|--------------------|----------------|------------|
|                  | Number of specimens amplified* / n | PCR Success (%) | Number of specimens sequenced / ns | Sequence Success (%) | Aligned length (bp)    | Identical sites (%) | Variable sites (%) | GC content (%) | INDELS (%) |
| <i>atpH-I</i>    | 59/71                              | 83.1            | 52/71                              | 73.24                | 430                    | 94.9                | 1.4                | 30.9           | 2.2        |
| <i>ITS2</i>      | 28/39                              | 71.8            | 20/39                              | 51.28                | 262                    | 76.0                | 4.2                | 69.5           | 1.7        |
| <i>matK</i>      | 58/58                              | 100             | 58/58                              | 100                  | 826                    | 94.1                | 2.43               | 31.8           | 0          |
| <i>rbclLa</i>    | 58/58                              | 100             | 58/58                              | 100                  | 548                    | 77.9                | 1.46               | 43.3           | 0          |
| <i>rpl16</i>     | 6/7                                | 85.7            | 6/7                                | 85.71                | 886                    | 97.5                | 1.92               | 33.5           | 0.3        |
| <i>rpoB</i>      | 49/49                              | 100             | 39/47                              | 79.59                | 387                    | 97.2                | 1.55               | 39.6           | 0          |
| <i>rpoC1</i>     | 49/49                              | 100             | 46/49                              | 93.88                | 264                    | 95.1                | 1.89               | 40.6           | 0.7        |
| <i>trnH-psbA</i> | 20/33                              | 60.6            | 12/33                              | 36.36                | 554                    | 78.6                | 2.173              | 29.6           | 15.3       |
| <i>trnL-F</i>    | 44/58                              | 75.9            | 37/58                              | 63.79                | 793                    | 72.3                | 2.2                | 32             | 2.6        |
| <i>trnT-L</i>    | 1/24                               | 4.2             | 0/24                               | 0                    | n/c                    | n/c                 | n/c                | n/c            | n/c        |

\*samples with a low quality amplification (thus with a smear) were included in the analyses

To compare the different loci, only the specimens that amplified across all the loci should be considered (Ford *et al.*, 2009). For the initial screening of the gene regions, each

dataset will be analysed individually whereafter the best combinations of two or more regions will be identified (see Chapter 7).

*Cryptostephanus vansonii* was included as an outgroup species in some of the datasets to determine the intergeneric gap between *Clivia* and its closest relative.

#### 4.3.2 Pre-screening for candidate DNA-barcoding regions in *Clivia*

Pre-screening the potential of DNA-barcodes will be based on the sequencing success and percentage of variable sites per gene region. A gene region that is easy amplifiable, may not yield enough variable sites and thus not enough discriminatory power. The latter will be investigated in the sections following the pre-screening.

The sequencing success and variable sites have each been divided into three categories; high/best, moderate and low. The cut-off value for each category was determined by dividing the range by the number of categories. For example, the range for the variable sites is 1.4%-4.2%, therefore 2.8% interval between the minimum and the maximum. Each category covers 0.93% variable sites. The range for the percentage of PCR success is 36.36% to 100%.

The five gene regions with the best sequencing success are *rpoB*, *rpoC1*, *rpl16*, *matK* and *rbcLa*. None of these regions fall into the category of high percentage variable sites (Table 4.4), but, with the exception of *matK*, falls into the low percentage (1.4-2.33%) variable sites. This implies that *rpoB*, *rpoC1*, *rpl16* and *rbcLa* will have a lesser chance to be successful barcoding regions.

The *ITS2* and *psbA-trnH* regions perform similar in the sense that these regions have the highest percentage variable site (between 3.27-2.33%) and fall into the 'low sequencing success' group (between 36.36-57.58% successes). Sequencing success is a variable factor that can be improved. When eliminating the amplification and success rate as a possible factor, there remain three candidate loci, namely *matK*, *psbA-trnH* and *ITS2*. The latter region is known to have amplification difficulties (CBOL Plant Working Group, 2009), which are mainly due to the high GC rich content in this areas (Baldwin *et al.*, 1995; Álvarez & Wendel, 2003).

**Table 4.4** A comparison between the sequencing successes vs. variable sites (%) for the gene regions. The sequencing success are divided into three categories, best, moderate and low according to the percentage of sequences obtained from the dataset for each region. The percentages of variable sites for each gene region are divided in high, moderate and low.

| Success                        | Variable sites                  |                       |                 |
|--------------------------------|---------------------------------|-----------------------|-----------------|
|                                | High (3.27-4.2%)                | Moderate (2.34-3.26%) | Low (1.4-2.33%) |
| <b>Best (78.8-100%)</b>        | <i>matK</i>                     |                       |                 |
| <b>Moderate (57.59-78.79%)</b> | <i>rpoB, rpoC1, rpL16, rbcL</i> |                       |                 |
| <b>Low (36.35-57.58%)</b>      | <i>atpH-I, trnL-F</i>           |                       |                 |
|                                | <i>ITS2, psbA-trnH</i>          |                       |                 |

The *trnH-psbA* region has the lowest GC content (29.9%) of the regions studied (Table 4.3). Based on results from other studies, the *trnH-psbA* region seems to be a good barcoding candidate (Kress *et al.*, 2005; Chase *et al.*, 2005, 2007; Kress & Erickson, 2007; De Mattia *et al.*, 2011; Hollingsworth *et al.*, 2011; Wang *et al.*, 2011; Armenise *et al.*, 2012; Theodoridis *et al.*, 2012). However, with the very poor sequencing success (36.35%) as well as the high degree of INDELS (15.3%), the use of this region should be carefully considered. A region with many INDELS may be difficult to align. Thus, although *rpoB*, *rpoC1*, *rbcLa* and *trnL-F* have been considered as DNA-barcodes in previous studies (Chase *et al.*, 2007; Lahaye *et al.*, 2008; Hollingsworth *et al.*, 2011), Kress & Erickson(2007) rejected the *trnL-F* region based on the low degree of interspecific variation,

This is, to our best knowledge, the first time that *rpL16* and *atpH-I* is tested in a barcoding study. The species are underrepresented in the final dataset of *rpL16*. For this reason the true degree of variation may not be reflected. Further studies should represent more species for *rpL16*. The chloroplast *atpH-I* gene region have sequencing success of 73.24% and 2.2% INDELS.

The lowest PCR success rate was obtained with *trnT-L*, with successful amplification of only a single sample. Universal primers (Taberlet *et al.*, 1991) did not result in successful amplification in *Clivia*, thus new primer pairs should most probably be designed for *Clivia* for future studies. This region has been rejected as a barcode based on low interspecific variation (Kress & Erickson, 2007), and is rejected in this study based on the low PCR success in *Clivia*.



In summary, the most variable regions are *ITS2* and *trnH-psbA* and both these regions have low sequencing success. The next most variable regions are *matK* and *trnL-F* (2.42% and 2.2% respectively). The higher variable regions may potentially be better barcoding candidates. The determining factor will however be the discriminatory power.

#### 4.3.3 Assessing the analysis methods for selecting a barcode

The screening of the gene regions that can serve as possible DNA-barcodes in *Clivia* was done on the individual dataset for each locus.

As discussed in detail (Chapter 2), the statistical approach used during analysis is an important component in DNA-barcoding. All three generally accepted types of analysis were tested i.e. the tree-based analysis, distance-based analysis and character-based analysis.

##### 4.3.3.1 Tree-based analysis

Five trees were drawn (Appendix C) for each region using character-based (MP, ML and BI) and distance methods (NJ and UPGMA). MEGA5.05 software was used to determine the model that best fit the data for the ML, NJ and UPGMA analyses. The Tamura 3-parameter was the best model for *atpH-I*, *ITS2*, *trnH-psbA*, *rpoB* and *rpoC1*. The *trnL-F* cladograms were obtained using the Tamura 3-parameter with Gamma distribution. The Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) was used as the best fit model for the region *rpl16*. Clades with a bootstrap value of at least 70% has been considered as a proper cut of value for a monophyletic grouping (Fazekas *et al.*, 2008). The different trees for each gene region were compared to determine which regions and analysis methods will be the most suitable for DNA-barcoding of *Clivia* (Table 4.5).

The *atpH-I* gene region was not able to differentiate between the closely related genera *Clivia* and *Cryptostephanus*. The more distantly related genus *Gethyllis* forms a monophyletic group with the highest bootstrap support obtained in the ML tree (83%) and can clearly be distinguished from the other two genera. Three specimens from the Port St. Johns area [*C. aff. robusta* (Spies 8579, 8582); *C. robusta* (Spies 8442)] have a monophyletic grouping with bootstrap support of 70% in the MP cladogram, suggesting that *atpH-I* may

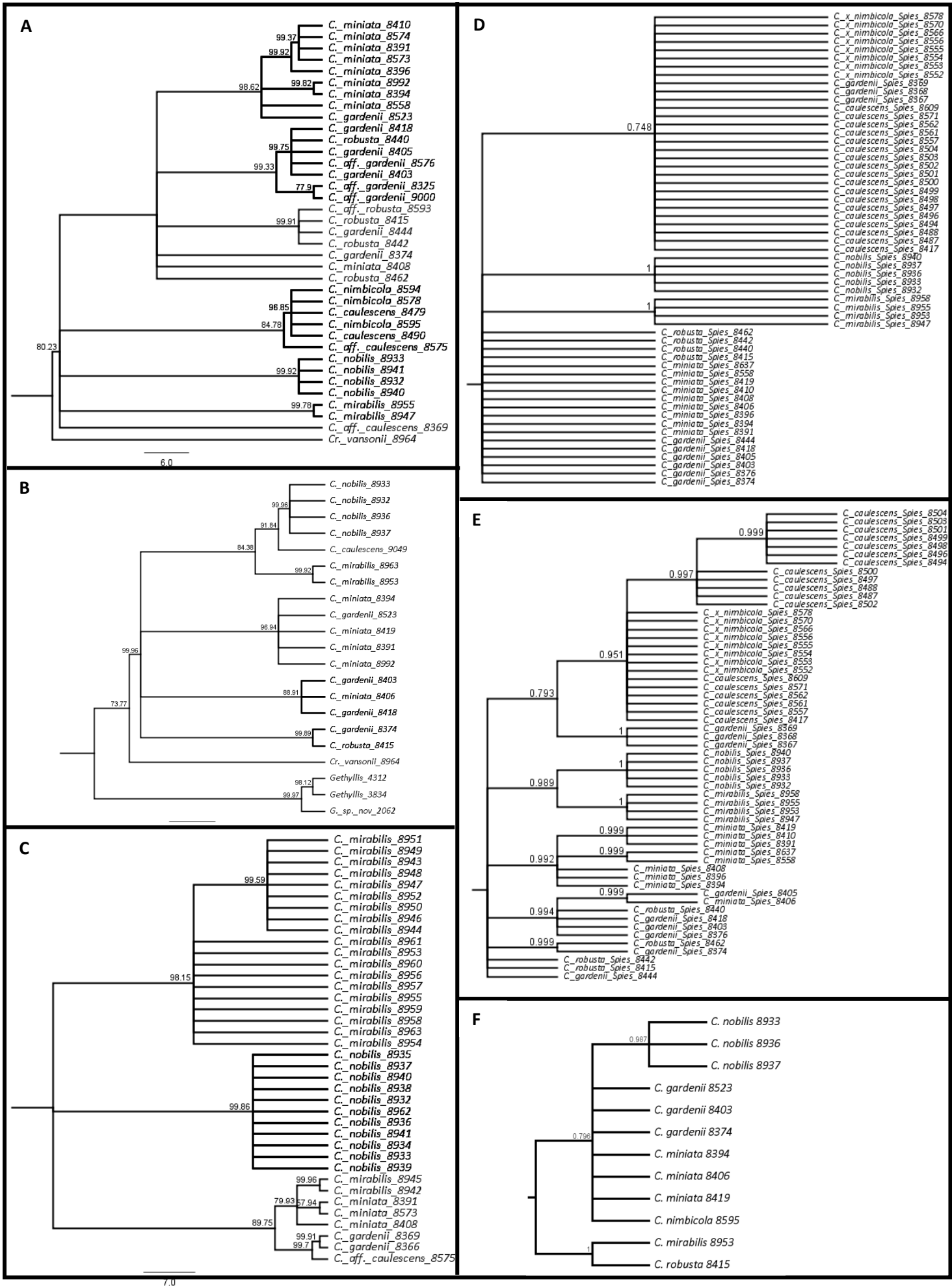
have the potential to identify specimens collected from that area. *Clivia mirabilis* has a tetra-nucleotide repeat (TACA) in the *atpH-I* region that is unique to this *Clivia* species. None of the tree building methods distinguishes *C. mirabilis* as a monophyletic group. Nevertheless, this unique repeat should be able to distinguish *C. mirabilis* using character-based analysis and should therefore be used as a method to identify *C. mirabilis* from unknown samples.

**Table 4.5** A summary of all five cladograms drawn for each gene regions. For each species, the candidate barcoding regions were chosen based on whether or not that species is monophyletic in any of the trees. The different trees (NJ, ML, UPGMA, MP & BI) for each of these chosen candidate regions were screened. The best tree building method was determined by the tree with the highest branch support for the species in question.

| Genus                                                        | Species                       | Candidate Barcoding region(s)          | Best tree building method         | Branch support for the monophyletic grouping |
|--------------------------------------------------------------|-------------------------------|----------------------------------------|-----------------------------------|----------------------------------------------|
| Genus <i>Clivia</i>                                          |                               | <i>ITS2</i>                            | Any                               | 92-100                                       |
|                                                              | <i>miniata</i>                | <i>ITS2</i>                            | Distance (NJ)                     | 72                                           |
|                                                              |                               | <i>rpoB</i>                            | Distance (NJ)                     | 65                                           |
|                                                              |                               | <i>trnL-F</i>                          | Distance (NJ) / character (MP)    | 87 / 71                                      |
|                                                              |                               | <i>matK</i>                            | Character (BI)                    | 99.7%                                        |
|                                                              |                               | <i>rbcLa</i>                           |                                   |                                              |
|                                                              | <i>gardenii, robusta</i>      | <i>None</i>                            | None                              | None                                         |
|                                                              | <i>caulescens / ximbicola</i> | <i>rbcLa</i>                           |                                   | %                                            |
|                                                              |                               | <i>matK</i>                            | Character (MP / BI)               | 64 / 97.5%                                   |
|                                                              |                               | <i>trnL-F</i>                          | Character (ML / BI)               | 62 / 96.85%                                  |
|                                                              | <i>mirabilis</i>              | <i>ITS2</i>                            | Distance (UPGMA) / character (MP) | 73 / 80                                      |
|                                                              |                               | <i>rbcLa</i>                           |                                   |                                              |
|                                                              |                               | <i>rpL16</i>                           | Any                               | 93-97                                        |
|                                                              |                               | <i>matK</i>                            | Distance (UPGMA) / character (BI) | 100                                          |
|                                                              | <i>nobilis</i>                | <i>ITS2</i>                            | Distance (UPGMA)                  | 96                                           |
|                                                              |                               | <i>trnH-psbA</i>                       | Character (ML / BI)               | 73 / 99.16%                                  |
|                                                              |                               | <i>matK</i>                            | Distance (UPGMA) / character (BI) | 96; 100%                                     |
|                                                              |                               | <i>rpoC1</i>                           | Distance (UPGMA)                  | 57                                           |
|                                                              |                               | <i>trnL-F</i>                          | Character (MP / BI)               | 92 / 99.92%                                  |
|                                                              |                               | <i>rpL16</i>                           | Any                               | 88-97                                        |
|                                                              |                               | <i>matK</i>                            | Any                               | 99-100                                       |
|                                                              |                               |                                        |                                   |                                              |
|                                                              | Genus <i>Cryptostephanus</i>  | <i>ITS2</i>                            | Any                               | -                                            |
|                                                              | Genus <i>Gethyllis</i>        | <i>ITS2</i>                            | Any                               | 95-100                                       |
|                                                              |                               | <i>atpH-I</i>                          | Character (ML)                    | 83                                           |
| <i>trnH-psbA</i>                                             |                               | Distance (UPGMA) / character (MP / BI) | 89 / 89 / 99.98%                  |                                              |
| <i>*matK, rbcLa, rpoB, rpoC1 and trnL-F not investigated</i> |                               |                                        |                                   |                                              |

The *ITS2* is the only region that can clearly distinguish among the three genera *Gethyllis*, *Cryptostephanus* and *Clivia* using any of the tree building methods (Figure 4.2 & Appendix C). The species *C. nobilis* and *C. mirabilis* can each be identified with *ITS2* DNA-barcodes. The highest bootstrap support for these two species was obtained with the

**Figure 4.2** (on following page) Bayesian Inference (BI) phylograms for **A)** *trnL-F*, **B)** *ITS2*, **C)** *rpl16*, **D)** *rbcLa*, **E)** *matK* and **F)** *trnH-psbA* were constructed with Geneious R6. The values on the branches represent the posterior probabilities for the clades.



UPGMA analysis for *C. nobilis* (96% bootstrap support); for *C. mirabilis* the MP and UPGMA trees had the highest support (80% and 73% bootstrap support respectively). *Clivia miniata* forms a monophyletic group in the MP tree with 84% bootstrap support. This would suggest that *ITS2* is a good candidate to identify *C. miniata*. Unfortunately, one *C. aff. gardenii* specimen (*Spies 8523*) was grouped with *C. miniata* and one *C. miniata* specimen (*Spies 8406*) was excluded from this group. Both these specimens were collected from regions where *C. miniata* and *C. aff. gardenii* either co-occur or are at least in close proximity to each other (see the overlapping areas in Figure 4.1). There is thus a strong possibility that hybridization between *C. miniata* and *C. gardenii* followed by introgression may have occurred. Recently diverged species have less variation in the *ITS2* region (Hollingsworth *et al.*, 2011), explaining why *C. gardenii* and *C. robusta* is unresolved.

The ***trnH-psbA*** region groups the genus *Clivia* with a posterior probability of 81.86% in the BI. The *trnH-psbA* region is a poor candidate to distinguish between the close relatives *Clivia* and *Cryptostephanus*. Monophyletic support for *C. nobilis* is good with a bootstrap support of 73% in the ML analysis and 99.16% in the BI. This is therefore a good candidate region to identify *C. nobilis* in a DNA-barcoding study.

The ***matK*** chloroplast region is a very good barcoding candidate to distinguish between all the *Clivia* species except the '*C. gardenii* complex'. All the species are monophyletic with high bootstrap support. The Bayesian Inference (BI) cladogram provides the best support for the majority of the monophyletic groupings. Bases 541 to 550 consist of a tetranucleotide repeat (AAAT), present in all the species except *C. mirabilis* and *C. nobilis*. The repeat could have originated in two ways. First by slipped strand mispairing, thus the creation of the repeat is the oldest event, followed by the SNP formation. This would be the most likely scenario. The second scenario would be that a SNP in a region transformed that region to resemble a repeat, and this would be due to chance alone. Previous phylogenetic studies suggested that *C. mirabilis* and *C. nobilis* are the more primitive species. They contain AAATCAAT versus AAATAAAT present in the rest of the genus. Analysing a single event to conclude on the evolution of an organism may not be reliable. Therefore, the preliminary conclusion that can be made is that all the species shared a common ancestor with the repeat; all the species except *C. mirabilis* and *C. nobilis* maintained the repeat in their

genomes; *C. mirabilis* and *C. nobilis* diverged from the rest of the species.

The ***rpL16*** gene region was only used to investigate the inter- and intraspecific variation in the genus (thus there were no outgroup sequences in the analyses), and yielded unexpected results. The *rpL16* gene region is able to distinguish between different geographical areas in the *C. mirabilis* distribution range. Material was collected from two sites in one of the populations, and even though the support for monophyletic groupings of the two different sites is somewhat low (63% and 64% in the UPGMA), these two sites can be genetically distinguished from each other. *Clivia nobilis* can also be clearly distinguished with a monophyletic grouping supported with a bootstrap of 97% in the UPGMA analysis. It is unfortunate that the rest of the species were not well represented in the data set. These results suggest that the region has the potential to identify *C. gardenii* (with a bootstrap support of 97% in the UPGMA analysis) and *C. miniata* (also a 97% support in the MP analysis), which warrants the preliminary inclusion of *rpL16* as a possible barcoding region.

The ***rpoB*** region is unable to clearly distinguish between *Clivia* and *Cryptostephanus*. It is a good region to identify *C. miniata* (bootstrap support of 69% with the MP analysis) but cannot be used as an identification tool for any of the other species. A combination of the slower mutation rates of the coding regions such as *rpoB* and *rpoC1*, the relative long reproductive cycle of *Clivia* (four to 18 years from seed to flowering), as well as the vegetative reproduction by means of shoot offset in some individual plants are all factors that can contribute to the poor monophyletic groupings in these two regions.

The ***rpoC1*** region is not a good region to distinguish *Clivia* from *Cryptostephanus*, with very poor bootstrap support for the monophyletic grouping of *Clivia*. This gene region has some potential to identify *C. nobilis* based on a monophyletic grouping with 57% bootstrap support in the UPGMA cladogram. Overall, alternative regions may yield higher bootstrap support and this region is not a good candidate barcoding region for *Clivia*.

The ***trnL-F*** region has very low bootstrap support for the *Clivia* clade and cannot be used as a barcoding region on an intergeneric level. Nevertheless, it seems to have great potential as a possible barcode for *Clivia* species. Even though the bootstrap support is not always satisfactory, all of the following species forms monophyletic groupings (with bootstrap

support and best tree drawing method in brackets): *C. nobilis* (92%, MP), *C. mirabilis* (68%, ML), *C. miniata* (87%, NJ) and *C. caulescens/C. xnimbicola* (62, ML).

To summarise the tree-based analyses: 1) It was shown that the tree-based method can be used to identify most of the species in *Clivia* with the exception of the *C. gardenii*-complex (see Box 4.1 p83). 2) The best tree building method to use depends on the gene region that is analysed as well as the species in question (Table 4.5). The distance tree building methods (NJ and UPGMA) are the most effective for the regions *rpoB* and *rpoC1*. The character-based tree drawing method (ML, MP and BI) is the tree building method that, overall, results in the most supportive cladograms for the region *trnL-F*. The *ITS2* gene region has a slight tendency to give better results within *Clivia* with the distance-based tree building methods (specifically the UPGMA). The BI cladogram provides the highest bootstrap support for *matK*. When analysing the trees for genus-level identification, all the tree building methods are equally effective. The character-based tree building method seems to be the superior method to use for analysis of *matK*, although the distance-based methods (NJ and UPGMA) can be equally effective analysis tools to use in most identification cases.

To assess the efficacy of tree-based methods to identify an unknown sample in phylogenetic trees, the Species Delimitation plugin in Geneious software was used. This plugin is a helpful tool in DNA-barcoding, since it determines the barcode gap suggested by Hebert *et al.* (2003), by calculating the probability of observing divergence under the null hypothesis of coalescence by implementing the method of Rosenberg (2007). The findings of Ross *et al.* (2008) are implemented in this study to determine the probability that the unknown species can be identified by using the current alignment of the dataset.

The barcoding gap is determined by the ratio between the intra- and interspecific distances (Intra/Inter). The intraspecific and interspecific distances are calculated based on the average pairwise tree distances. A small ratio of these distances is indicative of more variation between different species than within a given species.

The mean probabilities (at a 95% confidence interval) that an unknown sample will be identified based on the monophyletic grouping in the tree, are calculated [referred to as P ID (Strict) in Table 4.6]. The criterion is that this species must fall within and not as a sister clade

to the species clade. A more liberal calculation [P ID (Liberal)] determines the mean probability at a 95% confidence interval that an unknown sample will be identified using BLAST, DNA-Barcoding (closest genetic distance) or placement on a tree. The criteria are less strict and the species must either fall sister to or within a monophyletic clade (Masters *et al.*, 2010).

The main focus of the analyses performed by this plugin is to calculate the probability of observing divergence under the null hypothesis of coalescence, thus to determine if a monophyletic group solely resulted from random branching. In order to use this plugin, a BI cladogram should contain more than one monophyletic group. All the gene regions with only a single monophyletic grouping were excluded from the analysis in the Species Delimitation plugin.

Bayesian Inference (BI) cladograms for *ITS2*, *matK*, *rbcLa*, *rpL16* and *trnL-F* (Figure 4.2) were constructed in Geneious R6. The BI cladogram for the *atpH-I* region forms a polytomy for all 49 *Clivia* specimens in the dataset. This can be explained by INDELS being the only polymorphisms in the dataset.

Ross *et al.* (2008) analysed the efficacy of the strict tree-based method versus the liberal tree-based method and concluded that one of the important factors for successful identification is to have a large enough number of representative samples per species. The strict base tree analysis is prone to give false negatives since the query sample needs to be monophyletic to the reference samples. If the reference database is complete, e.g. it represents all the species in the genus under question, and if there are enough representative samples per species, then the liberal tree-based approach is the better analysis option to use. Only when the query sample is not represented in the database it is a better option to use the strict based tree option.

To assess the most effective gene region in *Clivia* for this analysis, the barcoding gap (Intra/Inter ratio), the probability based on the liberal tree drawing method [P ID (Liberal)] and the clade support were considered as three important factors. The clade support for *C. nobilis*, *C. mirabilis*, *C. miniata* and *C. caulescens* is the highest in the *ITS2* and *matK* cladograms. The *ITS2* region is a good locus to use to identify *C. miniata* species due to the

small barcoding gap (0.27), the high liberal probability of 0.94 and the high clade support (96.93%). The barcoding gaps for *C. nobilis* and *C. mirabilis* are the smallest in the *ITS2*, *matK* and *trnL-F* regions. The liberal ID probabilities for *C. nobilis* are 0.92, 0.87 and 0.88 in the *ITS2*, *matK* and *trnL-F* regions respectively compared with 0.77, 0.85 and 0.85 for *C. mirabilis* in these regions. Although the barcoding gap is considered as a reliable factor in determining DNA-barcoding regions (Chase *et al.*, 2005; Hajibabaei *et al.*, 2006), other researchers are of the opinion that an overlap in the distance distribution can be as high as 60% to 80% with a successful identification rate of 90% (Ross *et al.*, 2008).

**Table 4.6** The species delimitation in *Clivia* using the Species Delimitation plugin for Geneious. Data was generated from the BI cladograms based on the *ITS2*, *matK*, *rpl16* and *trnL-F* regions. Numbers for the *C. gardenii* groups corresponds with the groupings as it occurs from top to bottom in the BI cladograms (Figure 4.2), thus the first monophyletic group in the cladogram is referred to as group 1 etc. Calculations of the intra- and interspecific distances are based on the average pairwise tree differences. P ID (Strict) and P ID (Liberal) refers to the mean probability (with a 95% confidence interval for the prediction), that a correct identification of an unknown sample will be made based on the placement of the tree.

| Gene region                 | Species              | Closest Species      | Intra Dist | Inter Dist - Closest | Intra / Inter | P ID (Strict)     | P ID (Liberal)    | Clade Support (%) |
|-----------------------------|----------------------|----------------------|------------|----------------------|---------------|-------------------|-------------------|-------------------|
| <b><i>ITS2</i></b>          |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. nobilis</i>    | <i>C. mirabilis</i>  | 0.006      | 0.023                | 0.26          | 0.69 (0.55, 0.84) | 0.92 (0.81, 1.0)  | 99.95             |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 0.008      | 0.023                | 0.34          | 0.42 (0.26, 0.57) | 0.77 (0.62, 0.92) | 99.91             |
|                             | <i>C. miniata</i>    | <i>C. mirabilis</i>  | 0.009      | 0.034                | 0.27          | 0.75 (0.63, 0.88) | 0.94 (0.84, 1.0)  | 96.93             |
| <b><i>rbcl</i></b>          |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. nobilis</i>    | <i>C. mirabilis</i>  | 0.0004     | 0.001                | 0.37          | 0.69 (0.56, 0.81) | 0.91 (0.81, 1.0)  | 100               |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 0.0003     | 0.001                | 0.3           | 0.67 (0.52, 0.81) | 0.90 (0.79, 1.0)  | 100               |
| <b><i>matK</i></b>          |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. caulescens</i> | <i>C. nobilis</i>    | 0.002      | 0.002                | 0.78          | 0.73 (0.68, 0.79) | 0.92 (0.89, 0.95) | 95.1              |
|                             | <i>C. nobilis</i>    | <i>C. mirabilis</i>  | 0.0008     | 0.002                | 0.5           | 0.60 (0.47, 0.73) | 0.87 (0.77, 0.97) | 100               |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 0.0007     | 0.002                | 0.44          | 0.57 (0.43, 0.72) | 0.85 (0.74, 0.96) | 100               |
|                             | <i>C. miniata</i>    | <i>C. nobilis</i>    | 0.002      | 0.002                | 0.68          | 0.57 (0.47, 0.68) | 0.85 (0.79, 0.92) | 99.2              |
| <b><i>rpl16</i></b>         |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 0.002      | 0.007                | 0.31          | 0.90 (0.84, 0.95) | 0.97 (0.94, 1.00) | 98.15             |
|                             | <i>C. nobilis</i>    | <i>C. ssp.</i>       | 0.002      | 0.007                | 0.27          | 0.84 (0.75, 0.93) | 0.95 (0.89, 1.00) | 99.85             |
|                             | <i>C. ssp.</i>       | <i>C. nobilis</i>    | 0.002      | 0.007                | 0.31          | 0.80 (0.69, 0.90) | 0.92 (0.86, 0.99) | 89.74             |
| <b><i>trnL-F</i></b>        |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. miniata</i>    | <i>C. caulescens</i> | 0.002      | 0.008                | 0.29          | 0.83 (0.74, 0.91) | 0.94 (0.89, 1.00) | 1.10E-07          |
|                             | <i>C. caulescens</i> | <i>C. nobilis</i>    | 0.002      | 0.004                | 0.45          | 0.63 (0.51, 0.76) | 0.89 (0.78, 0.99) | 84.77             |
|                             | <i>C. nobilis</i>    | <i>C. mirabilis</i>  | 0.001      | 0.004                | 0.37          | 0.62 (0.48, 0.77) | 0.88 (0.77, 0.99) | 99.91             |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 7.69E-04   | 0.004                | 0.21          | 0.48 (0.33, 0.64) | 0.85 (0.70, 1.0)  | 99.77             |
| <b><i>trnL-F + matK</i></b> |                      |                      |            |                      |               |                   |                   |                   |
|                             | <i>C. miniata</i>    | <i>C. caulescens</i> | 4.222      | 8.444                | 0.5           | 0.73 (0.65, 0.82) | 0.92 (0.86, 0.97) | NA                |
|                             | <i>C. caulescens</i> | <i>C. nobilis</i>    | 2          | 5                    | 0.4           | 0.52 (0.35, 0.70) | 0.78 (0.63, 0.93) | 7.50E-05          |
|                             | <i>C. nobilis</i>    | <i>C. mirabilis</i>  | 2          | 4                    | 0.5           | 0.53 (0.38, 0.68) | 0.82 (0.71, 0.93) | NA                |
|                             | <i>C. mirabilis</i>  | <i>C. nobilis</i>    | 2          | 4                    | 0.5           | 0.34 (0.18, 0.49) | 0.67 (0.52, 0.83) | NA                |



It seems as if the *rpL16* may also be a good candidate region to identify *C. mirabilis* and *C. nobilis* species. More samples from the other species should be investigated to determine the efficacy of this region as a barcode for *Clivia*.

#### 4.3.3.2 Distance-based analysis

Distance-based analysis was tested by performing analyses in jMOTU software. Distance-based analysis in jMOTU does not rely on aligned sequences and uses pairwise distances to cluster taxonomic units. Each dataset was analysed in jMOTU to find MOTU's (Molecular Operational Taxonomic Units) (Blaxter *et al.*, 2005) that may correspond to the OTU's (Operational Taxonomic Units) described by the taxonomical classification of *Clivia*. Six species have been described, thus the ideal barcoding region will group each species into its own MOTU. A cut-off graph was created for each region (Figure 4.3). The cut-off value is the number of nucleotide changes in a MOTU. For each cut-off value a Microsoft Excel spreadsheet with the defined MOTUs was generated (Appendix D). Each spreadsheet went through a screening process and the cut-off values that define MOTUs that best correlates with morphological OTU, were chosen as the optimal values (Figure 4.3).

A higher cut-off value is an indication of more variation in that specific gene region. The three best performing regions regarding the number of MOTUs defined per number of species in the dataset are *rpL16*, *trnL-F* and *matK*. Although *C. gardenii* has a MOTU in the *rpL16* region, only two specimens were used and the degree of variation is undetermined. Based on all the gene regions in this study, *C. gardenii* is a paraphyletic group. More representatives per species should be included in future studies of *rpL16*, thus at this point it will be concluded that *C. gardenii* and *C. robusta* cannot be defined by their own unique MOTUs.

*Clivia mirabilis*, *C. nobilis* and *C. caulescens* (or the hybrid *C. ×nimbicola*) has distinct MOTUs in various gene regions. The sequence of an unknown sample can be added to the data generated in this study, and by choosing the cut-off values suggested in Table 4.7, the data can be analysed in jMOTU and the sample identifiable by the cluster it falls in. The advantages of identifying a sample in this manner is that data analysis do not rely on an aligned sequence and the computational time is very short (most time it is less than a minute).

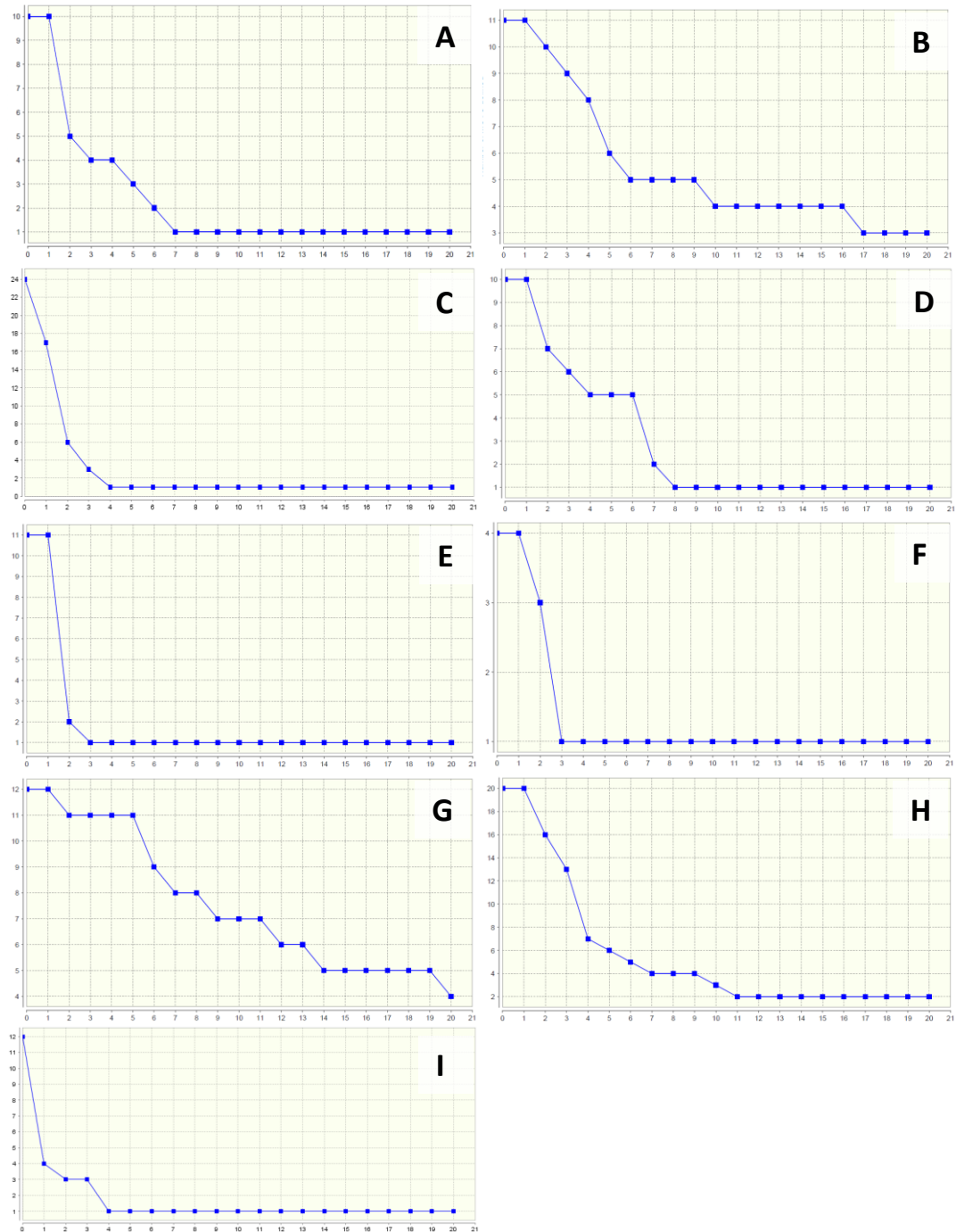


**Figure 4.3** The cut-off distribution graphs for **A)** *atpH-l*, **B)** *ITS2*, **C)** *matK*, **D)** *rpl16*, **E)** *rpoB*, **F)** *rpoC1*, **G)** *trnH-psbA*, **H)** *trnL-F* and **I)** *rbcL*. These graphs were used to determine the best cut-off value for generating MOTUs. The X-axis is the MOTU discrimination in base pairs, therefore the maximum number of nucleotide changes in a MOTU. The Y-axis is the number of MOTUs defined.

*Clivia miniata* is defined in a unique MOTU using the *ITS2* data. Caution should, however, be exercised with the identification of a *C. miniata* specimen. Samples that were provided for this study by third parties contained species that resulted from hybrid speciation. Based on the morphology of the samples, these were identified as *C. miniata* but on molecular level they are not defined as a *C. miniata*. Similar occurrences have been observed

in *C. gardenii* and *C. robusta*. The latter two species seems to have evolved recently and cannot be defined by their own MOTUs.

**Table 4.7** A summary of the data for each gene region generated in jMOTU. The number of species in the dataset is compared with the number of molecular taxonomic units created in jMOTU. Not all the species are defined to a single MOTU. The cut-off values in this table are the recommended values to use to identify an unknown sample.

| Gene region      | No of sequences in the dataset | No. of morphological <i>Clivia</i> OTUs in dataset | Number of <i>Clivia</i> MOTU's generated (cut-off value) | Taxa forming a MOTU                                                                                                         |
|------------------|--------------------------------|----------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| <i>atpH-atpI</i> | 62                             | 6 (+ 3 <i>affinis</i> <i>Clivia</i> 's)            | 9 (1)                                                    | <i>C. mirabilis</i>                                                                                                         |
| <i>ITS2</i>      | 57                             | 6                                                  | 10 (2)                                                   | <i>C. miniata</i> *                                                                                                         |
| <i>matK</i>      | 57                             | 6                                                  | 3 (2)                                                    | <i>C. caulescens</i> / <i>×nimbicola</i> , <i>C. nobilis</i> , <i>C. mirabilis</i>                                          |
| <i>rpl16</i>     | 38                             | 4 (+ one <i>affinis</i> <i>Clivia</i> )            | 6 (3) <sup>#</sup>                                       | <i>C. nobilis</i> , <i>C. mirabilis</i> , <i>C. miniata</i> , <i>C. gardenii</i> , <i>C. caulescens</i>                     |
| <i>rpoB</i>      | 92                             | 6 (+ 3 <i>affinis</i> <i>Clivas</i> )              | 11 (0)                                                   | None                                                                                                                        |
| <i>rpoC1</i>     | 50                             | 6 (+ 3 <i>affinis</i> <i>Clivas</i> )              | 1 (0-1)                                                  | <i>C. nobilis</i>                                                                                                           |
| <i>trnH-psbA</i> | 17                             | 6                                                  | 4 (9-11)                                                 | <i>C. nobilis</i>                                                                                                           |
| <i>trnL-F</i>    | 37                             | 6 (+ 3 <i>affinis</i> <i>Clivas</i> )              | 7 (4)                                                    | <i>C. caulescens</i> / <i>×nimbicola</i> , <i>C. miniata</i> (excluding 2 samples), <i>C. nobilis</i> , <i>C. mirabilis</i> |
| <i>rbcLa</i>     | 57                             | 6                                                  | 4 (1)                                                    | <i>C. mirabilis</i> , <i>C. nobilis</i>                                                                                     |

\* *C. miniata* OTU includes as single *C. gardenii* taxon

<sup>#</sup> At cut-off value of 1 different *C. mirabilis* populations are separated in MOTUs

The *matK* sequencing dataset distinguish *C. mirabilis*, *C. nobilis* and *C. caulescens* (the hybrid *C. ×nimbicola* is indistinguishable) from the other three species at a cut-off value of 2. When the *rbcLa* dataset is analysed in jMOTU, only *C. mirabilis* and *C. nobilis* have distinct units at a cut-off value from 1 to 3. The rest of the species have a similar pattern as the grouping in the BI cladogram. Therefore, even at a cut-off value of zero, a group of *C. gardenii* specimens fall into the same grouping of *C. caulescens* (or *C. ×nimbicola*). By increasing the cut-off value, more samples belonging to different species are clumped together.

To conclude, to identify an unknown sample, the *trnL-F* region can be analysed in jMOTU using a cut-off value of 4.0. The second effective region is *matK*, distinguishing between *C. nobilis*, *C. mirabilis* and *C. caulescens* (or *C. ×nimbicola*) at a cut-off value of 2.0.

This type of analysis can effectively be used when the query sample belongs to any one

of the properly delimited species, i.e. *C. mirabilis*, *C. nobilis* or *C. caulescens* / *C. xnimbicola* and should be equally effective as an identification tool as the tree-based method.

#### 4.3.3.3 Character-based analysis

Generating sequences in a lab that is not equipped to handle high throughput sequencing analysis can be time-consuming. If an unknown *Clivia* sample needs to be identified, sequencing of both the *matK* and the *trnL-F* regions are recommended as described in the previous section. There may be instances where an individual needs a quick identification, e.g. where seeds or seedlings have been sold as *C. mirabilis* is suspected to have a *C. nobilis* origin. In these instances, faster and reliable methods that are still barcode-based may be needed to make a quick identification.

SNP based analysis requires a SNP that is unique for a *Clivia* species to positively identify an unknown sample. The aligned sequences of all the gene regions were screened in Geneious R6 for polymorphisms (SNPs and INDELS) that can be used as markers for the different species. Only unique polymorphisms were considered (Table 4.8)

All the species except *C. gardenii* and *C. robusta* have unique polymorphisms that can be used as species-specific markers. Three of the polymorphisms in *C. mirabilis* are the product of short tandem repeats (STR) in *trnL-F*, *atpH-I* and *trnH-psbA*.

To conclude, any one of the unique SNP markers identified in this study can be used to identify a query sequence by aligning the sequence with the database (from this study) and identifying the samples based on similarities with any of these unique polymorphisms. To validate the results, more than one polymorphism from a few sites can be used for identification. The combined polymorphisms of, for example, the *matK* and *trnL-F* regions provide an average of 3.5 polymorphisms per species and eight polymorphisms for the genus *Cryptostephanus*. The more SNP markers are used for validation, the smaller the probability will be that a species have a combination of markers by chance alone. This type of identification should be a reliable and rapid identification method to use. Furthermore, the results from this character-based analyses can be used in other, more rapid barcode based identification techniques, as discussed in section 4.3.5.

**Table 4.8** A summary of the polymorphisms in the sequencing datasets. The position of the polymorphism is determined by the position on the aligned sequences. It is recommended to use this alignment as reference for future identifications. *Cryptostephanus* was used as reference sequence to determine the polymorphisms.

| Species                                                                                    | Gene region                 | Change                                        | Position    | Polymorphism Type |
|--------------------------------------------------------------------------------------------|-----------------------------|-----------------------------------------------|-------------|-------------------|
| <i>C. aff. caulescens</i> (Spies 8575); <i>C. × nimbicola</i> , <i>C. caulescens</i> (all) | <i>matK</i> & <i>trnL-F</i> | C/T                                           | 71          | SNP               |
| <i>C. aff. caulescens</i> (Spies 8575); <i>C. × nimbicola</i> , <i>C. caulescens</i> (all) | <i>matK</i> & <i>trnL-F</i> | G/A                                           | 533         | SNP               |
| <i>C. aff. caulescens</i> (Spies 8575); <i>C. × nimbicola</i> , <i>C. caulescens</i> (all) | <i>matK</i> & <i>trnL-F</i> | A/G                                           | 785         | SNP               |
| <i>C. × nimbicola</i> , <i>C. caulescens</i> (all)                                         | <i>matK</i> & <i>trnL-F</i> | G/T                                           | 742         | SNP               |
| <i>C. miniata</i> (all); <i>C. aff. gardenii</i> (Spies 8523)                              | <i>matK</i> & <i>trnL-F</i> | A/C                                           | 430         | SNP               |
| <i>C. miniata</i> (all); <i>C. aff. gardenii</i> (Spies 8523)                              | <i>matK</i> & <i>trnL-F</i> | C/A                                           | 1 054       | SNP               |
| <i>C. miniata</i> (all); <i>C. aff. gardenii</i> (Spies 8523)                              | <i>matK</i> & <i>trnL-F</i> | T/A                                           | 1 100       | SNP               |
| <i>C. miniata</i> (all); <i>C. aff. gardenii</i> (Spies 8523)                              | <i>ITS2</i>                 | T/C                                           | 149         | SNP               |
| <i>C. miniata</i> (20/22 samples); <i>C. aff. gardenii</i> (Spies 8523)                    | <i>rpoB</i>                 | C/T                                           | 55          | SNP               |
| <i>C. mirabilis</i> (all)                                                                  | <i>matK</i> & <i>trnL-F</i> | T/C                                           | 558         | SNP               |
| <i>C. mirabilis</i> (all)                                                                  | <i>matK</i> & <i>trnL-F</i> | -/TATT                                        | 1 018-1 022 | STR               |
| <i>C. mirabilis</i> (all)                                                                  | <i>matK</i> & <i>trnL-F</i> | T/C                                           | 1 247       | SNP               |
| <i>C. mirabilis</i> (all)                                                                  | <i>atpH-I</i>               | -/TACA                                        | 358-361     | STR               |
| <i>C. mirabilis</i> (all)                                                                  | <i>psbA-trnH</i>            | -/TATC                                        | 497-501     | STR               |
| <i>C. mirabilis</i> (all)                                                                  | <i>psbA-trnH</i>            | -/AGTTTTAGCTCAACATATATGTATGG<br>AAGATGAAGGGGA | 438-476     | Indel             |
| <i>C. mirabilis</i> (all)                                                                  | <i>rpl16</i>                | T/A                                           | 88          | SNP               |
| <i>C. mirabilis</i> (all)                                                                  | <i>rpl16</i>                | G/T                                           | 392         | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>matK</i> & <i>trnL-F</i> | T/C                                           | 235         | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>matK</i> & <i>trnL-F</i> | G/A                                           | 563         | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>matK</i> & <i>trnL-F</i> | A/G                                           | 583         | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>matK</i> & <i>trnL-F</i> | G/C                                           | 1 101       | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>psbA-trnH</i>            | -/AGAT                                        | 517-521     | STR               |
| <i>C. nobilis</i> (all)                                                                    | <i>psbA-trnH</i>            | G/A                                           | 531         | SNP               |
| <i>C. nobilis</i> (all)                                                                    | <i>rpl16</i>                | A/C                                           | 43          | SNP               |

|                         |                             |                                                                                                                                                                     |         |       |
|-------------------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------|
| <i>C. nobilis</i> (all) | <i>rpl16</i>                | G/T                                                                                                                                                                 | 150     | SNP   |
| <i>C. nobilis</i> (all) | <i>rpl16</i>                | A/G                                                                                                                                                                 | 847     | SNP   |
| <i>C. nobilis</i> (all) | <i>rpoC1</i>                | T/C                                                                                                                                                                 | 19      | SNP   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | T/C                                                                                                                                                                 | 136     | SNP   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | G/A                                                                                                                                                                 | 659     | SNP   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | GCA/-                                                                                                                                                               | 732-734 | Indel |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | ATATATGAAAAATTCAGAGTTATTGTGGATCTA<br>TTCCAATCGAAGTTAAAGGAAGAATCGAATAT<br>TCAGTGATCAAATCATTCAATCCAGAGTTTGAT<br>AGACCTTTTTT-<br>GAAAAAAAAAATGATTAATCGGAC/-<br>-/TCAAT | 736-870 | Indel |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | -/TCAAT                                                                                                                                                             | 901-906 | STR   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | C/A                                                                                                                                                                 | 1 007   | SNP   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | A/C                                                                                                                                                                 | 1 123   | SNP   |
| <i>Cryptostephanus</i>  | <i>matK</i> & <i>trnL-F</i> | T                                                                                                                                                                   | 1 284   | SNP   |

#### 4.3.3.4 Comparing the tree, distance and character-based analyses

The tree and character-based methods are equally reliable tools in the DNA-barcoding analysis in *Clivia* (Table 4.9). The computational time for tree-based method can be long and in some instances use large amounts of computer memory which may not always be available.

**Table 4.9** A comparison between the three methods used for analysis in the DNA-barcoding of *Clivia* species. When a method can be used to positively identify an unknown sample, it is indicated with a “yes”, and when the contrary is the case, it is notes as a “no”.

| Species                                     | Tree-based analysis | Distance-based | Character-based |
|---------------------------------------------|---------------------|----------------|-----------------|
| <i>C. caulescens</i> / <i>C. xnimbicola</i> | Yes                 | Yes            | Yes             |
| <i>C. gardenii</i>                          | No                  | No             | No              |
| <i>C. miniata</i>                           | Yes                 | Yes            | Yes             |
| <i>C. mirabilis</i>                         | Yes                 | Yes            | Yes             |
| <i>C. nobilis</i>                           | Yes                 | Yes            | Yes             |
| <i>C. robusta</i>                           | No                  | No             | No              |

In contrast, the character-based method relies only on the interpretation of a sequence alignment, which is less time consuming and do not rely on extensive computer analyses. For both these methods, sequences need to be aligned. In *Clivia*, all the sequences of all the regions aligned readily with some minor manual corrections being made. These two methods can therefore both be implemented for identifying unknown *Clivia* samples for all the species, except *C. gardenii* and *C. robusta*. In all analysis, the hybrid species *C. xnimbicola* could not be distinguished from the parental species *C. caulescens*. Caution should thus be exercised when analysing any unknown sample from a collection or geographical area where species co-exist. Hybridization and introgression events that were involved in the historical development of a plant may lead to false positive or false negative identifications.

Operational taxonomic units (OTUs) could be created for *C. caulescens* / *C. xnimbicola*, *C. mirabilis* and *C. nobilis* using the distance-based method. *Clivia miniata* was not monophyletically grouped into its own OTU mainly because the data contains *affinis* samples. Options in the computer program jMOTU cannot be manipulated manually and monophyletic *C. miniata* groupings with an *affinis* sample included in that grouping were not recognised as an OTU.

Recently diverged species may not have enough variation at a single locus (Puillandre *et al.*, 2011). This is evident in *C. gardenii*, *C. robusta* and the *affinis* species (named the *C.*

*gardenii* complex). This complex has incomplete lineage sorting and, through the molecular evidence, the delimitation of the species seems to be incorrect. Even though a two to three locus barcode is suggested, it seems to be an insufficient number to use for this group. Puillandre *et al.* (2011) suggest that additional non-genetic data such as morphology and ecological data should be considered in such instances. Adding extra data may be helpful, but it is suggested that the delimitation of *C. gardenii* and *C. robusta* should first be reconsidered before this group can be used in DNA-barcoding analyses.

Do the results from the pre-screening correlates with the findings from the rest of the data analyses? After the pre-screening stage it was predicted that the *ITS2* and *trnH-psbA* regions have the potential for barcodes, followed by *matK* and *trnL-F*. These four regions all have variable sites > 2%. It is unfortunate that the sequencing success of *ITS2* and *trnH-psbA* was too low to include a significant number of samples. It could have been concluded that *ITS2* indeed has the potential for a barcoding region, and it was confirmed that *trnL-F* and *matK* is effective DNA-barcoding regions in *Clivia*.

#### 4.3.4 Combining datasets

DNA-barcoding loci should be combined into a two- to three loci barcode (Kress *et al.*, 2005; Chase *et al.*, 2007; Kress & Erickson, 2007; Fazekas *et al.*, 2008; Hollingsworth *et al.*, 2011; Li, F.W. *et al.*, 2011; Wang *et al.*, 2011). In the current study the *trnL-F* and *matK* regions have the highest distinguishing power and will be combined.

The regions performing the best based on number of monophyletic groupings and bootstrap support are the *ITS2* region, *rpl16*, *matK* and *trnL-F*. The first two regions were excluded in further analysis since the sequences obtained in this study does not well represent the sampling. It is recommended that the PCR reactions for these regions are optimised and that more samples should be investigated.

It was decided to combine *matK* and *trnL-F* in a matrix. These regions seem to complement each other, e.g. *matK* groups *C. mirabilis* with 89 bootstrap support (and no groupings were found in the *trnL-F* cladograms), whereas *trnL-F* supports a monophyletic grouping of *C. miniata* (with no support for such grouping in *matK*). By combining the



matrixes of these two regions, monophyletic groupings for most of the species should be obtained.

The combined matrix of *matK* and *trnL-F* is 1288 bp long with a sample size of 33. All the species and some *affinus* (*aff.*) species are represented in the dataset. This dataset has 61.6% identical sites and a pairwise % identity of 96.8%. Previous tree analysis on individual datasets of *matK* and *trnL-F* yielded the best bootstrap support for monophyletic groupings with character-based tree drawing methods (Table 4.5). A BI cladogram of the combined matrix was constructed in Geneious R6 (Figure 4.4). Monophyletic groupings with high posterior probabilities were obtained for *C. mirabilis*, *C. nobilis*, *C. caulescens* and *C. miniata* (Figure 4.4). The *C. gardenii* complex is divided into three monophyletic groupings with high posterior probabilities for each. Grouping 1 consist of *Spies 8418* (Greytown), *Spies 8405* (Entumeni Nature Reserve), *Spies 8440* (Port Shepstone), *Spies 8325, 8403, 8576, 9000*, grouping 2 of *Spies 8415, 8442, 8444, 8593*, and group 3 of *Spies 8374, 8462*. There is no geographical pattern or specific species associated with each grouping, e.g. the *C. gardenii* specimens are divided into different monophyletic groups, and similarly is specimens from the same locality such as Port Shepstone grouped into different monophyletic groups.

The BI cladogram of the combined dataset of *trnL-F* and *matK* (Figure 4.4) was used to delimit the *Clivia* species (Table 4.6) with the Species Delimitation plugin. *Clivia miniata* has the highest intraspecific distance and is equally distant (0.005) to all three *C. gardenii* sister groups in the cladogram. All the groups have a P ID (Strict) >0.6, except *C. mirabilis* (0.52) and *C. gardenii* group 3 (0.43). Thus, for most of the species the probability will be more than 60% that an unknown specimen of *C. miniata*, *C. nobilis* or *C. caulescens* / *×nimbicola* will form a monophyletic grouping and will be correctly identified. The probability of correctly identifying a *C. mirabilis* species with these two gene regions based on the BI cladogram is much lower, and alternative regions as already discussed should be used for *C. mirabilis* barcoding. *Clivia gardenii* has three monophyletic groupings and the probability that an unknown samples belonging to this species will fall within any of these three clades are 70% for group 1, 67% for group 2 and 43% for group 3.

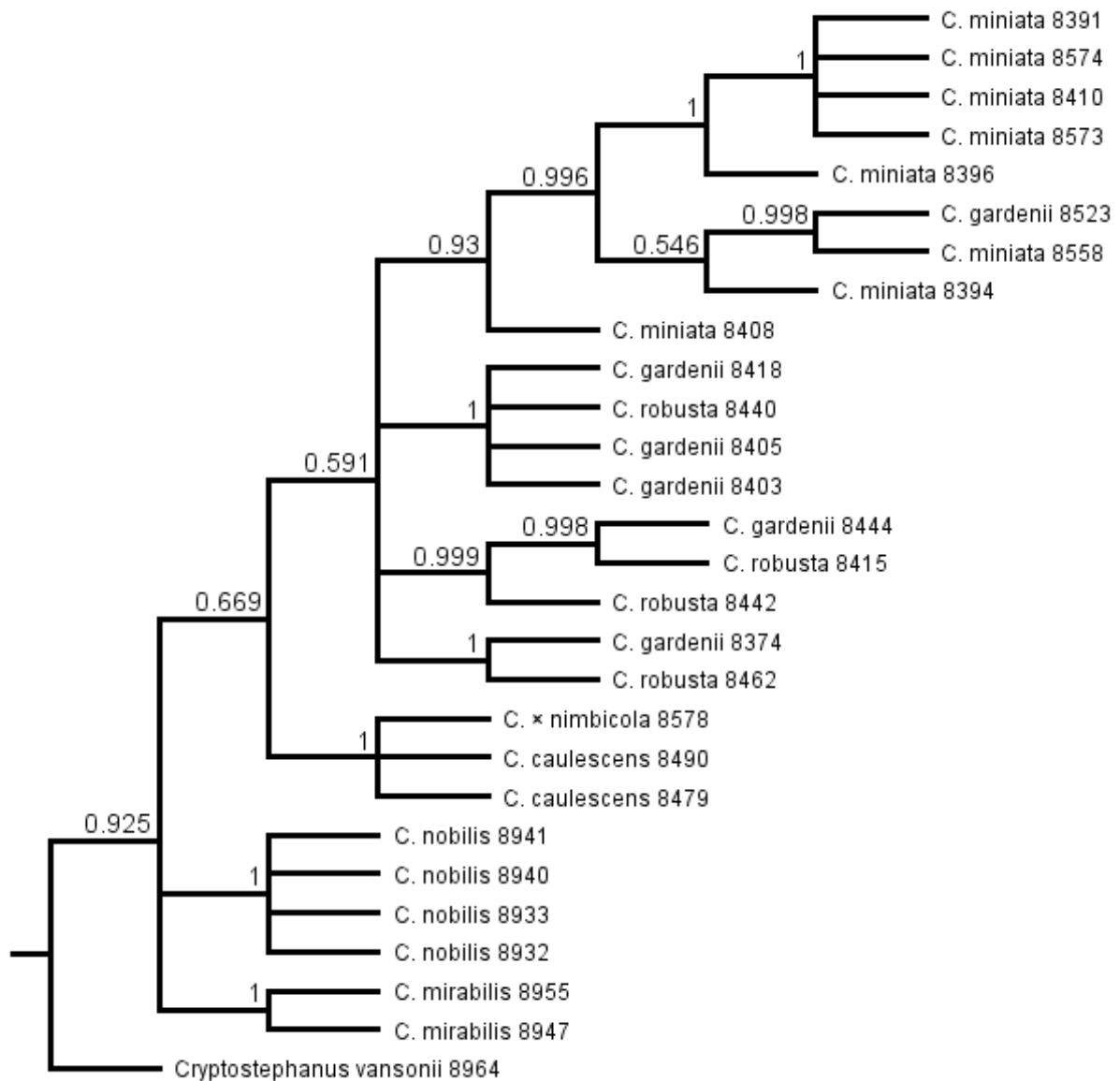


**Figure 4.4** The Bayesian Inference (BI) cladogram of the two-loci barcode *trnL-F + matK*. The values on the branches are the posterior probabilities.

P ID (Liberal) has significantly higher probabilities of grouping an unknown sample. The distance-based probabilities for all the monophyletic groupings are the lowest [the value in the middle under P ID (Liberal) in Table 4.6]. The probability of falling in a grouping using BLAST and tree based methods are much higher. One would like to argue that the liberal identification is less reliable than the strict identification since the placement of the unknown sample sister to a group, will still be identified as belonging to that group. When a strict analysis is followed, the software will regard a sample having a sister relationship to a group, as a different species. Ross *et al.* (2008) argues that using the liberal method will not pose

problematic if the amount of genetic variability within and between species is well established and when there is a clear differentiation between the species.

With the tree-based analysis the statement was made that the genera *Clivia*, *Cryptostephanus* and *Gethyllis* can be identified based on sequences of the *ITS2* region. To test this statement statistically, the BI cladogram of the *ITS2* dataset was analysed with the Species Delimitation plugin (Table 4.6). Additional GenBank sequences for *Gethyllis*, *Apodolirion* and *Cryptostephanus* were added to the sequences generated in this study. The close relatives, *Gethyllis* and *Apodolirion* are monophyletic and these two genera cannot be separated from each other with the *ITS2* gene region. Tree-based analysis under the Strict and Liberal P ID criteria are highly sufficient in identifying a *Clivia*, *Cryptostephanus vansonii* or *Gethyllis* / *Apodolirion* specimen. The P ID (Strict) probabilities using the tree-based identification range from 0.84 – 0.96, and for the P ID (Liberal) from 0.99 – 1.0, thus statistically proving the effective identification using tree-based analysis.

It has been discussed (Chapter 2) that the barcoding gap should be included in the analysis of a barcoding study. This gap relies on the mean inter- and intraspecific distances and some critics (Meier *et al.*, 2008) made the suggestion to use the smallest distances instead of the mean distances. Analysis was limited to the functions in MEGA 5.05 and Microsoft Excel 2010.

The combined *matK* and *trnL-F* dataset was used to determine the mean interspecific distances, the mean intraspecific distances and their standard deviations. These distances were calculated in MEGA 5.05 with the distance tool (see Chapter 2), exported as text (Appendix E) and the barcoding gap was graphically visualised (Figure 4.5). The mean distances between all the species are greater than the mean distances within species, suggesting that there is a clear barcoding gap for all the *Clivia* species in the *matK* and *trnL-F* regions. According to Meier *et al.* (2008), the use of mean values can lead to false interpretations of the data. The standard deviations for the distances within and between species were calculated and when taking this variation into account (represented by the vertical lines at each point in Figure 4.5), there seems to be an overlap in the inter- and intraspecific distances in *C. miniata*, *C. robusta* and *C. gardenii*. *Clivia caulescens*, *C. mirabilis* and *C. nobilis* have no overlap between the interspecific and intraspecific variation (*C.*

*xnimbicola* was not determined; only one sample was included in the calculations). Ross *et al.* (2008) used simulations to test the efficacy of BLAST, distance-based and tree-based identifications. When distance methods are used for identification, the distances can overlap by 60% up to 80% and still result in accurate identification.

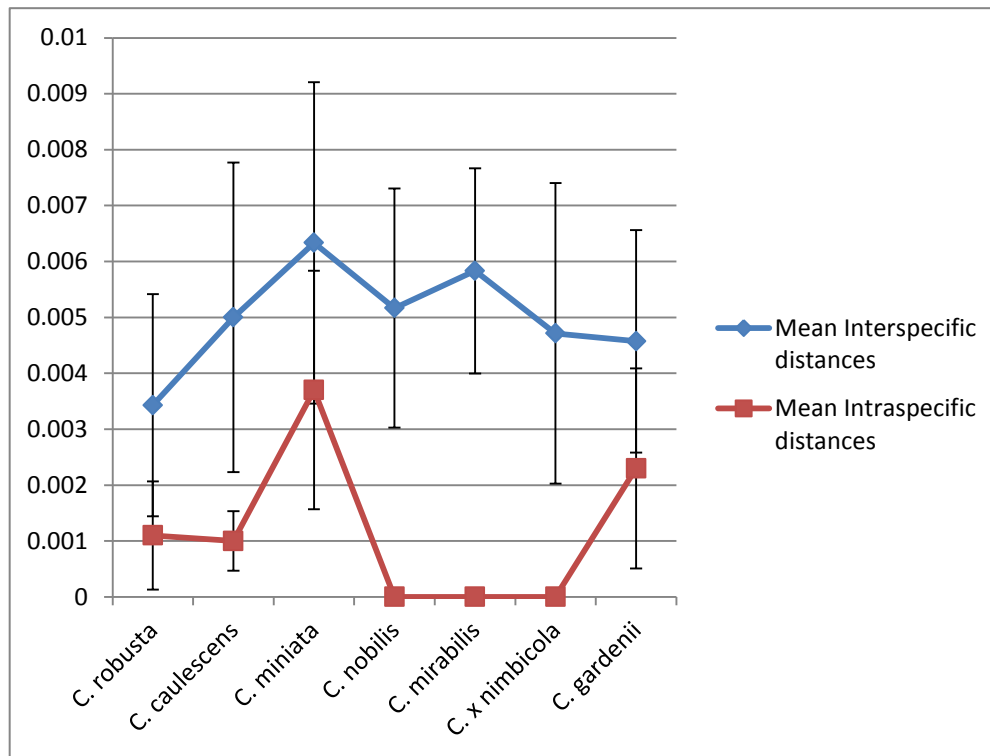


**Figure 4.5** The barcoding gap of the combined *matK* and *trnL-F* gene regions represented by the mean distances between species (top line) and the mean distances within species (bottom line). The vertical lines represent standard deviations. All the *affinis* species were included in the species it most likely represent and may influence the distances and standard deviations.

Even though an overlap of <10% result in an increase in the rate of successful identification, Ross *et al.* (2008) maintains the opinion that the overlapping distances is a poor predictor of identification success. The reason is that distance-based identification is based on a comparison of the query sequence to the nearest match, thus the barcoding gap will apply only to the query sequence and the nearest heterospecific and conspecific sequences.

**Geneious R6 analysis:** The MrBayes plugin in Geneious R6 was used to construct trees from which the patristic distances could be calculated. The patristic distances were visualised with a heatmap (Figure 4.6). When calculating these distances, the ‘character state evolution within lineages’ are taken into account (Stuessy & König, 2008). This heatmap supports the findings in the cladograms that there are two major lineages that separate the older species

that is basal in the cladogram (i.e. *C. mirabilis*, *C. caulescens* and *C. nobilis*), from the more recently developed species (*C. miniata*, *C. gardenii* and *C. robusta*). The same three subgroups in the *C. gardenii*-complex that were present in the cladograms, are also present in the heatmap of the patristic distances.

#### 4.3.5 Additional applications of barcoding in *Clivia*

The *rpL16* region was able to make a clear distinction between the two geographical sites where *C. mirabilis* specimens were sampled from. Using the tree-based analysis or SNP analysis, the region where *C. mirabilis* samples originated from may therefore be identified with a high degree of certainty.

Similar, the *trnL-F* sequences have a SNP (at position 180) that occur only in *C. gardenii* / *C. robusta* species from a 240 km stretch in central KwaZulu Natal in the area between Port Shepstone in the South, Entumeni in the North, and as far west as Greytown. The application of this finding is that the origin of plants that are confiscated from traditional healers or *Muti* traders may be linked to this area.

#### 4.3.6 Recommendations for future studies

Two techniques for rapid *Clivia* species identification based on DNA-barcodes have been tested *in silico*. Both these techniques will eliminate the need for extensive comparisons to databases and various analyses and statistical testing.

**Barcode based PCR-RFLP** This is a method that combines DNA-barcoding with restriction fragment analysis (Darling & Blum, 2007), and has successfully been successfully used on DNA-barcoding regions to identify heterozygote alleles in *rpoC1* and *rpoB* (Pawluczyk *et al.*, 2011), and to identify the seeds of the weedy species of *Sporobolus* (Shrestha *et al.*, 2010). It involves PCR amplification followed by restriction enzyme digestion and visualisation on an agarose or polyacrylamide gel. The need for bi-directional sequencing is eliminated with this technique.

The program Geneious R6 was used to search for restriction sites that will digest the PCR product on a unique marker SNP. Of all the regions and commercially available enzymes

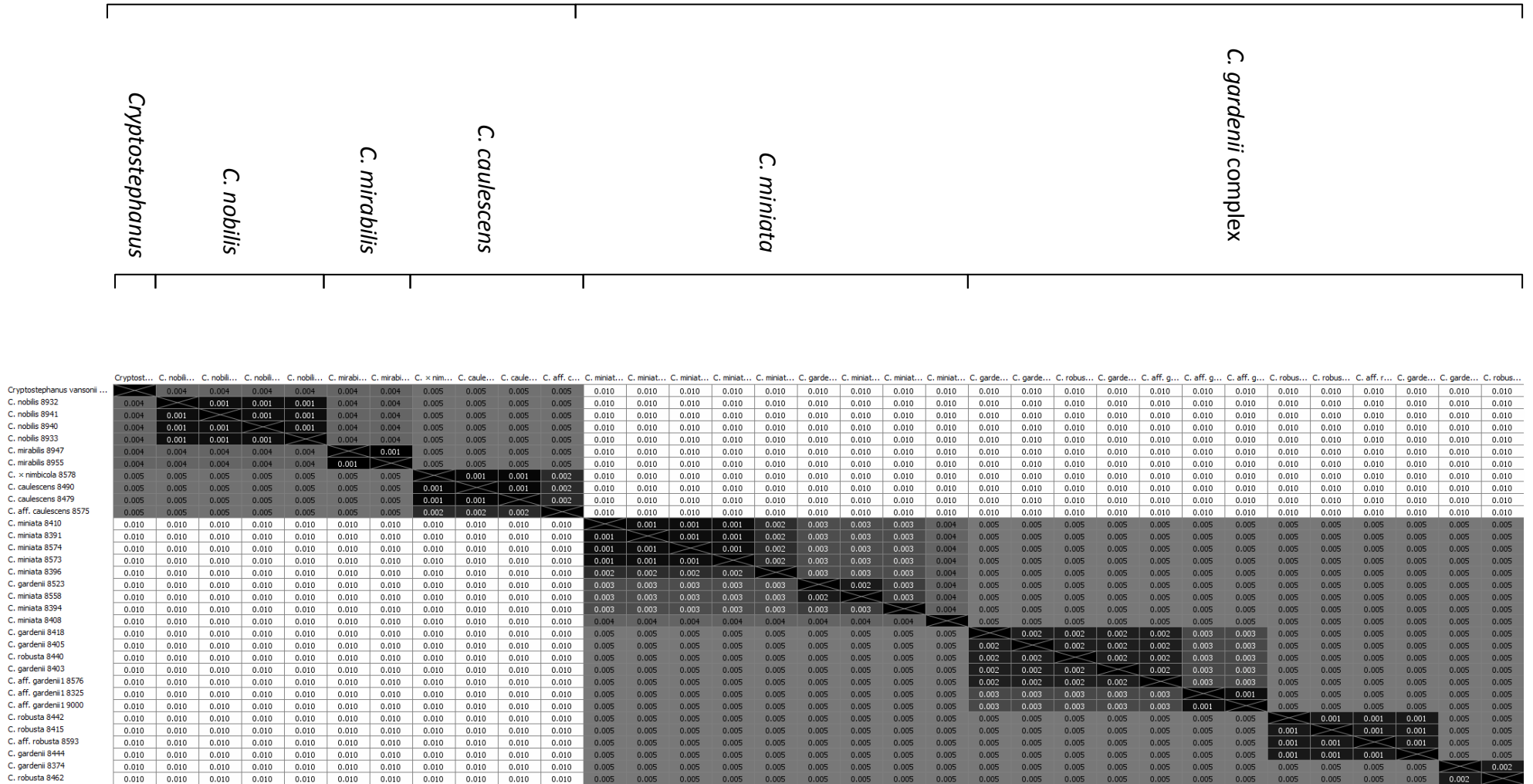


**Figure 4.6** Heatmap drawn for the combined datasets of *matK* and *trnL-F* based on the patristic distances obtained from the BI. Calculations were made based on the sum of the branch lengths. According to this heatmap there are two major groupings in *Clivia* i.e. “old” species that are the ancestral species *C. mirabilis*, *C. nobilis* and *C. caulescens* that groups with the outgroup genus *Cryptostephanus*. The “new” species may be the more recently diverged species, i.e. *C. miniata*, *C. gardenii* and *C. robusta*.

that were tested, *HinfI* was the only enzyme that virtually digested the *rpL16* region in unique banding patterns for *C. gardenii*, *C. miniata*, *C. mirabilis* site 1, *C. mirabilis* site 2 and *C. nobilis*. The virtual gel (Figure 4.7) simulated by the Geneious software represent what the results from digestion with *HinfI* should be, and this gel can serve as a reference in future studies when the validity and applicability of this technique is tested.

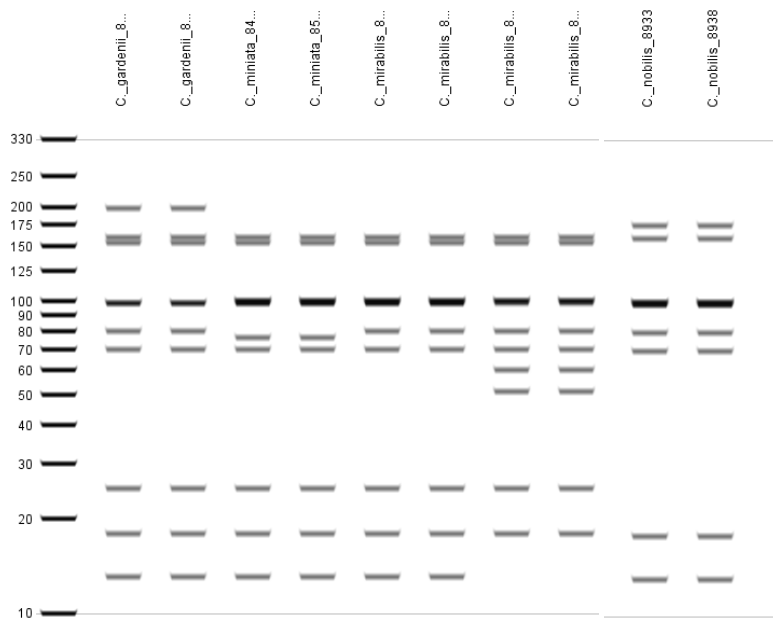


**Figure 4.7** The virtual enzyme digestion of the *rpL16* gene region with *HinfI* to simulate the unique banding patterns for *C. gardenii* (lanes one and two), *C. miniata* (lanes three and four), *C. mirabilis* site 1 (lanes five and six), *C. mirabilis* site 2 (lanes seven and eight) and *C. nobilis* (lanes nine and ten). The virtual gel was simulated with Geneious R6.

**SNaPshot® Multiplex System for SNP Genotyping (Applied Biosystems)** An unlabelled oligonucleotide primer that anneals to one nucleotide from a SNP marker is designed (Table 4.10). During amplification only one primer is used in the reaction and a single fluorescently dyed ddNTP is incorporated followed by termination of the reaction. The colour of the ddNTP that has been incorporated is analysed with a Genetic analyser such as the ABI 3130 and according to the colour it will be determined if the SNP is present.

All the gene regions investigated in this study have been screened for SNP markers. The criteria was that each selected SNP should be unique only for a single species and that there should be at least a 20 bp conserved region prior to the SNP for the primer to anneal.

Primers have been designed with Primer3 version 4.0.0<sup>19</sup> (Table 4.10). From the primers that were designed, primers for future studies were selected that complies with certain basic criteria. The primer should not have a four or longer mononucleotide repeat, especially comprising of C's or G's. For better annealing, the CG content should range between 45-60% and the length of the primer should be increased (>18) when the CG content is <50%. Best results are obtained when the melting temperatures of the primers are between 52°C and 58°C. This temperature can be lower or higher, preferably not less than 50°C and not more than 65°C. Primers that form hairpins at a temperature >50°C should be avoided (Abd-El salam, 2003).

**Table 4.10** A selection of the primers that can be used in future SNP studies to quickly identify species using the *SNaPshot® Multiplex System for SNP Genotyping (Applied Biosystems)*. The temperatures for the primers and GC content have been determined with Geneious R6. The sequence binding site is the sequence on the template that precedes the unique SNP that will be read by the genetic analyser.

| Species                    | Unique SNP for identification | Region        | Primer name | %GC  | Hairpin T <sub>m</sub> | Self Dimer T <sub>m</sub> | Sequence of primer binding site | T <sub>m</sub> |
|----------------------------|-------------------------------|---------------|-------------|------|------------------------|---------------------------|---------------------------------|----------------|
| <i>C. caulescens</i>       | T                             | <i>trnL-F</i> | 706 F       | 28   | 42.2                   | 12.1                      | CCGAATCCATATATATGCA<br>ATATAT   | 52.6           |
| <i>C. miniata</i>          | A                             | <i>trnL-F</i> | 1,063 F     | 29.2 | None                   | None                      | TCATTCATTCTACTCTTTCA<br>CAAA    | 54.5           |
| <i>C. mirabilis</i>        | G                             | <i>matK</i>   | 452 F       | 36   | None                   | None                      | CTGGGTTATCTTTCAAGTCT<br>ACTAA   | 56.1           |
| <i>C. mirabilis</i>        | G                             | <i>rpL16</i>  | 824 F       | 40.9 | 37.2                   | None                      | CCAGATTTTCGTAAGCAACA<br>TAG     | 55.3           |
| <i>C. mirabilis</i>        | C                             | <i>trnL-F</i> | 1,212 F     | 32   | 38.9                   | None                      | TATCCTTACATTACAAAAG<br>AAAGTC   | 55.3           |
| <i>C. mirabilis</i> Site 1 | G                             | <i>rpL16</i>  | 418 F       | 30   | None                   | None                      | ACCAAGAACAAATTGAGAT<br>T        | 50.7           |
| <i>C. nobilis</i>          | A                             | <i>matK</i>   | 538 F       | 32   | None                   | None                      | TAAGAAATTTGATACCATA<br>GTCCCA   | 55.8           |
| <i>C. nobilis</i>          | C                             | <i>trnL-F</i> | 1,064 F     | 28   | None                   | None                      | TCATTCATTCTACTCTTTCA<br>CAAAT   | 55             |

Twenty-five primers from all the gene regions together were selected for pre-screening, ten possible primers sites for *C. nobilis*, ten for *C. mirabilis*, three for *C. miniata* and two for *C. caulescens*. All the primer-sites in the *ITS2* region were high in CG content (72-82%) and were prone to forming self-dimers and hairpins. The *matK* and *trnL-F* regions provide sufficient SNP's and annealing sites to ensure that the above mentioned species will have at least a single SNP for identification purposes (Table 4.10). A SNP has been identified in the *rpL16* gene region that can be used to identify *C. mirabilis* samples originating from the

<sup>19</sup><http://primer3.wi.mit.edu/>



number one collection site.

## 4.4 CONCLUSIONS

Extracting pure DNA from leaves with the CTAB extraction method is only effective when using young leaves, since the leaves of *Clivia* produce larger quantities of secondary compounds as it matures. With constant new developments and improvements of extraction kits and robust direct PCR kits, obtaining pure DNA and positive amplification of any part of the plants should become more feasible. In the future it should thus be possible to obtain pure DNA from any part of the *Clivia* plant for further DNA-barcoding analysis

The tree-based or character-based analyses are both effective tools to use in the identification process of the *Clivia* species. The tree-based analysis is more time consuming and large datasets may pose computational problems. There also are controversies over which tree building method to use, i.e. character-based or distance-based tree building methods. The support for the monophyletic groupings usually differs between the NJ, UPGMA, ML, MP and BI cladograms. In this study, the distance-based and character-based tree building methods provide the same degree of support. Each method resulted in four monophyletic groupings with the highest support, and in four of the monophyletic groupings both of the methods had very similar support (see Table 4.5).

Drawing five different trees may be very time consuming. It may be a good practise to construct one distance-based cladogram (NJ or UPGMA) and one character-based cladogram (MP, ML or BI) when relaying in the tree-based method for identification of *Clivia* species. Character-based identification relies only on a proper alignment and then identifying the state of a nucleotide at the position in question. This type of identification should be very cost-effective.

Distance-based analysis with jMOTU is very quick when the researcher is familiar with the software. Alternatively, a more familiar tree-based or character-based identification will be easier to use. This program is, however, only effective when properly delimited *Clivia* species are investigated, thus *C. mirabilis*, *C. nobilis* and *C. caulescens*.

DNA-barcoding of the nrDNA *ITS2* region can be used as an effective tool to identify an unknown sample as belonging to the genus *Clivia*, *Cryptostephanus* or *Gethyllis*. The leaf morphology of *Gethyllis* is very unique. Identifying an unknown sample as belonging to the genus *Gethyllis* should not be a problem, even to an inexperienced person. *Cryptostephanus* species are very difficult to obtain, and since the leaf morphology is to a degree similar to a small *Clivia* plant, it would be very easy to sell a non-flowering *Clivia* plant as a *Cryptostephanus*. In contrast to the findings of other studies (Kress & Erickson, 2007), *ITS2* cannot be rejected as a potential barcode region in *Clivia*.

*Clivia mirabilis*, *C. nobilis*, *C. miniata* and *C. caulescens* / *C. xnimbicola* can be identified with DNA-barcoding. The best barcoding regions regarding discriminatory power are the *trnL-F* chloroplast region and the *matK* chloroplast gene.

For species-level identification, the combined sequence matrix of the two chloroplast regions *trnL-F* and *matK*, and using tree-based analysis (BI in particular) is most effective in identifying *C. mirabilis*, *C. nobilis* and *C. miniata* specimens if we can assume that hybridization has not played a role in the development of a plant under investigation.

Hybridization events in *Clivia* may lead to false positive or false negative identifications. This is evident when samples from mixed populations are included in the analysis. Identification of an unknown sample resulting in a *C. miniata*, *C. gardenii* or *C. robusta* identification should need further analysis on additional data (e.g. morphology, distribution) to confirm the results. All the *C. xnimbicola* natural hybrids included in the current study shared the chloroplast DNA of *C. caulescens*. DNA tested from any of these two species will cluster in the same monophyletic group, thus a distinction between these two species is impossible with these barcoding regions. Alternative methods should be developed to effectively distinguish and identify hybrid species.

Unknown *Clivia* samples from seed, rhizomes, leaves or flowers (any part containing chloroplast DNA) should be identifiable by sequencing only the two chloroplast regions *matK* and *trnL-F*. The geographical origin of plants confiscated from traditional healers may be identified. Because of the challenges some databases have (such as incomplete data), identification of these unknown samples can (at the time of writing this thesis) only be made

to the *Clivia* database of the Department of Genetics. Matches will in future be made on BOLD or BDP.

## Part C: *Lachenalia*

### *Chapter 5*

#### Cytogenetic and phylogenetic review of the genus *Lachenalia*



## ABSTRACT

The genus *Lachenalia* (family Asparagaceae), endemic to southern Africa, is a diverse genus, with many species featuring in the red data list of southern Africa. The extensive morphological variation within some species complicates species delimitation and has led to taxonomic confusion. The genus is utilised in a breeding programme at the ARC-Vegetable and Ornamental Plant Institute (Roodeplaat) where cytogenetic and phylogenetic information is important for the development of breeding strategies. Chromosome numbers of 89 species have been recorded in the literature, with  $2n = 10$  to 56 and  $n = 5$  to 28. B-chromosomes have also been described in some species. Basic chromosome numbers include  $x = 5, 6, 7, 8, 9$ , (probably 10), 11, (probably 12), 13 and (probably 15). Polyploidy was reported in 19 taxa (23%), and is most common in the  $x = 7$  group. Molecular cytogenetic studies using 5S rDNA, 18S rDNA probes and DAPI staining (from published data), as well as molecular systematic studies using *trnL-F* (from unpublished data) and *ITS1-2* (from published data) were used to assess the phylogeny of the genus. All these studies indicated that species with the same basic chromosome number are closely related. The one deviation is that it appears as if there are two separate groups within the  $x = 7$  group. The cytogenetic and molecular studies are further supported by breeding studies, where improved results are generally obtained from crosses within a phylogenetic group or between closely related groups. This review of the literature reveals how different studies obtain similar results regarding the phylogenetic relationships within the genus and how these results can be utilized to improve breeding strategies. It also accentuates that further multidisciplinary studies are needed to solve the evolutionary history of the complex genus *Lachenalia*.

## PREFACE

This chapter was published in “*Floriculture and Ornamental Biotechnology*” by invitation of the editors. The cytogenetic and phylogenetic studies on *Lachenalia* are reviewed and the effect that these two aspects have on the cross-ability in the genus, is evaluated.

My contributions to the paper were the writing of *Phylogenetic studies*, *Basic chromosome numbers and cladograms*, *Evolution and relatedness of different basic chromosome numbers* and *Existence of hybrid species*. Some of the data generated during my

M.Sc study on *Lachenalia*, were combined with sequences available on NCBI. These data were re-analysed to provide a new perspective on the phylogeny and evolution of chromosome numbers in the genus. I also contributed to some of the chromosome counts in this review article.

For the purpose of the thesis, the chapter ends with a clear statement of the research question and the sub-questions that were addressed in the current study.

## 5.1 INTRODUCTION

The genus *Lachenalia* Jacq. f. ex Murray, previously a member of the family Hyacinthaceae (Manning *et al.*, 2004; Duncan & Edwards, 2006, 2007), but since 2009 reclassified under the family Asparagaceae Juss. (Angiosperm Phylogeny Group, 2009), is endemic to southern Africa. The genus now also includes the former genus *Polyxena* (Manning *et al.*, 2004). *Lachenalia* is a horticultural diverse genus, with a distribution range extending from the south-western coast of Namibia, southward throughout the Northern-, Western -and Eastern Cape provinces of South Africa (Duncan, 1998). One species extends as far inland as the south-western part of the Free State Province (Duncan, 1996). Of the 133 species and subspecies described (Duncan 2012), 10% are endangered, 17% are vulnerable, 2% are considered to be near threatened, 6% are critically rare, 9% are rare and 2% are declining (SANBI, 2009).

The genus is geophytic, deciduous and is usually winter-growing. The centre of diversity is in the Worcester grid (3319) in the Western Cape province of South Africa, with species diversity decreasing toward the eastern and northern parts of the range (Duncan *et al.*, 2005). Although *Lachenalia* species such as *L. bifolia* and *L. obscura* are widely distributed, a substantial number of species (e.g. *L. moniliformis*, *L. mathewsii*) have a restricted distribution, contributing to the vulnerability of these species (Duncan, 1998).

*Lachenalia* occurs in a wide range of habitats, ranging from arid to high-rainfall areas. *Lachenalia punctata* for example always grows in deep, pure sand often very close to the ocean, whilst a species like *L. campanulata* on the other hand is found in heavy soil at altitudes exceeding 2000 metres (Duncan, 1998). Between these two extremes, there are a

multitude of other habitats, including humus-rich soil on granite, mineral-rich soil, barren stony flats, limestone outcrops and seasonally inundated, heavy clays (Duncan, 1998).

The morphological diversity within the genus is well known (Figure 5.1). Variation occur in several morphological characters, such as plant size, leaf number and posture, flower-size, -colour and -orientation, and flowering period (Figure 5.2). The extensive morphological variation within some species complicates species delimitation and has led to considerable taxonomic confusion (Duncan, 1992). Several attempts have thus been made to establish some sub-generic classification within this complex genus, starting with the work by Baker (1897), who divided the genus into five sub-genera based on morphology. The first cytogenetic work by (Moffett, 1936), however, already indicated that true relationships cut across the groups of Baker (1897) and this has been confirmed by various studies (Crosby, 1986; Spies, 2004; Hamatani *et al.*, 2009; amongst others).



**Figure 5.1** Morphological variation in *Lachenalia* in the greenhouse.

Due to the extensive morphological diversity in colour and appearance, collectors have recognized the horticultural potential of the genus for centuries (Duncan, 1988; Du Plessis & Duncan, 1989; Kleynhans, 2009, 2011; Reinten *et al.*, 2011). The huge phenotypic variation was also the most important reason for the initiation of a breeding programme at the



Agricultural Research Council in South Africa. This led to the production of various hybrids and the introduction of new products to the international pot plant market (Figure 5.3) (Kleynhans, 2006).



**Figure 5.2** Morphological variation in different *Lachenalia* species. (A) *L. aloides*; (B) *L. carnosa*; (C) *L. splendida*; (D) *L. bifolia*; (E) *L. longibracteata*; (F) *L. violacea*; (G) *L. contaminata*; (H) *L. pusilla*.

The variability of the genus in terms of morphology and cytogenetics, however, led to specific challenges for the breeding of new cultivars. Incompatibility and other isolation barriers exist (Kleynhans & Hancke, 2002). A large number of inter-species crosses are unsuccessful (Kleynhans *et al.*, 2009) and future breeding progress is therefore dependent on information about the genetic variation in the genus. Results generated from cytogenetic and



phylogenetic research has value for the breeding programme at the ARC-Vegetable and Ornamental Plant Institute (Roodeplaat) (Kleynhans *et al.*, 2009) and can furthermore assist in the classification and delimitation of species (Crosby, 1986; Spies *et al.*, 2002).

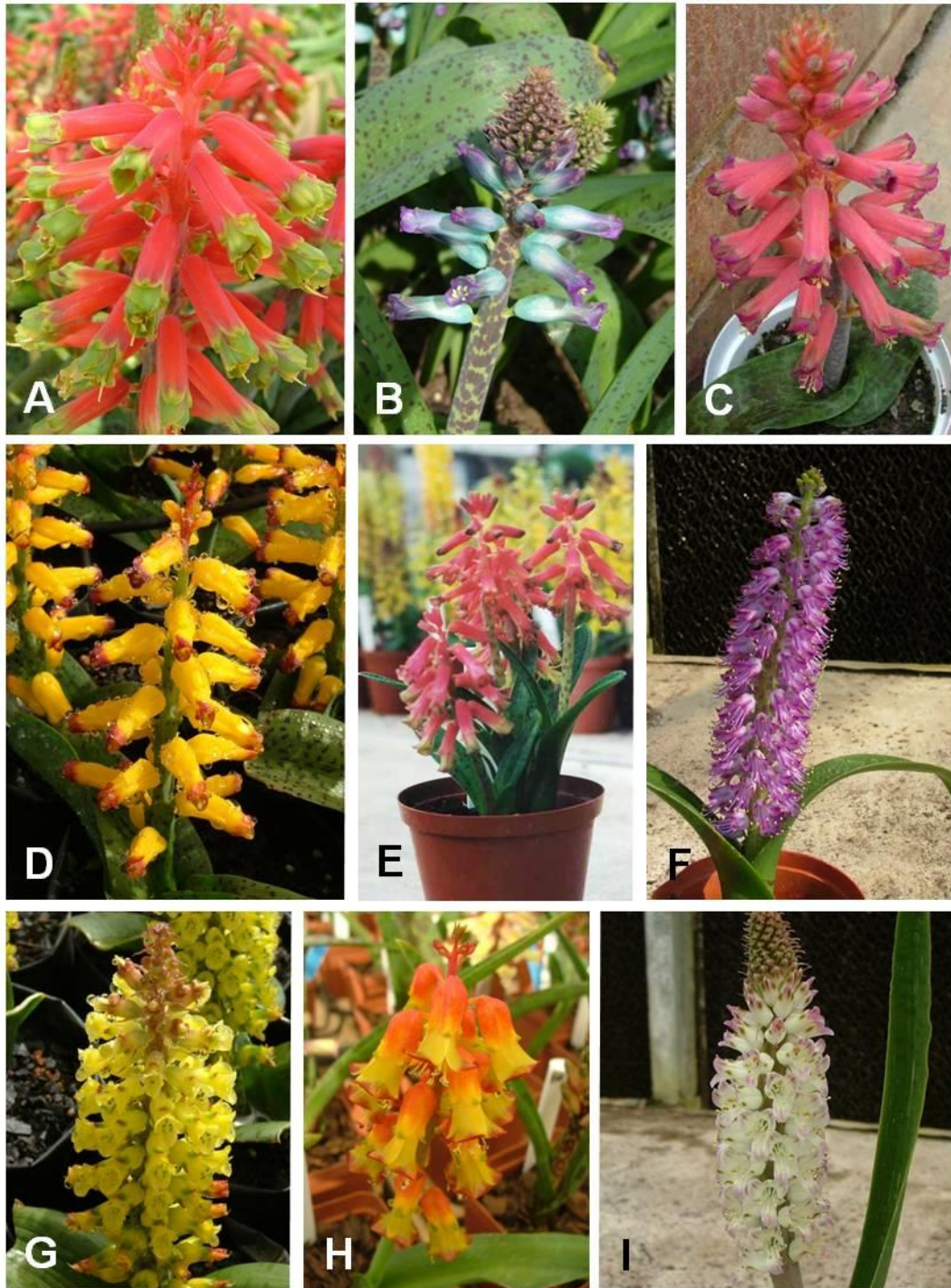


**Figure 5.3** Different *Lachenalia* cultivars developed at ARC - Roodeplaat VOPI. (A) 'Rosabeth'; (B) 'Aqua Lady'; (C) 'Cherise'; (D) 'Namakwa'; (E) *L. bifolia* x *L. punctata*; (F) *L. pallida* x *L. splendida*; (G) 'Romaud'; (H) 'Rainbow Bells'; (I) *L. bachmanii* x *L. carnosa*.

This paper reviews the current information available on cytogenetics and phylogeny for the genus *Lachenalia* and correlates this information to breeding results on cross-ability with the aim to draw some conclusions on relationships among the different species within the genus.

## 5.2 CYTOGENETIC STUDIES

### 5.2.1 Chromosome counts

*Lachenalia* is unusually variable in chromosome number with the presence of different basic chromosome numbers (Moffett, 1936; Crosby, 1986; Johnson & Brandham, 1997), polyploidy (Kleynhans & Spies, 1999) and B-chromosomes (Hancke & Liebenberg, 1990; Johnson & Brandham, 1997). The first cytogenetic studies on the genus came from Moffett (1936). Available data on chromosome numbers steadily increased over many years with information coming from various authors (Table 5.1). Currently the chromosome numbers of 89 species have been recorded in the literature. Somatic chromosome numbers vary from 10 to 56 and corresponding gametic numbers from 5 to 28.

The cytogenetics is further complicated by disagreeing chromosome number reports for a number of species (Table 5.1). Deviating chromosome counts can first of all be explained by suspected wrong identification of species. In the species *L. orchioides*, the variation could most probably be ascribed to accessions being wrongly identified. Crosby (1986) reported that both *L. fistulosa* and *L. pallida* were received under the name of *L. orchioides*. Schlechter also identified an accession of *L. pallida* as *L. orchioides* (Barker, 1983). *Lachenalia contaminata* has both  $2n = 14$  and  $2n = 16$  reported in literature (Table 5.1). (Gouws, 1965) was the first to report both these numbers. This author, however, described these two numbers in one specific bulb of *L. contaminata* exhibiting cells with both  $2n = 14$  and  $2n = 16$ . In this case the  $2n = 16$  could be B-chromosomes that were not identified. Most other chromosome counts of this species, except two by Spies *et al.* (2008, 2009), are  $2n = 16$ . In this species the variation is not a case of mistaken identity and further investigation is needed to explain the variation.

**Table 5.1** List of *Lachenalia* species with the somatic- and gametic chromosome numbers reported in literature. Number in brackets (#) indicates number of accessions for which the specific somatic or meiotic number was reported. All numbers were reported in the table under the current accepted botanical name. Aneuploidy and other abnormalities or specific detail around polyploidy are indicated with superscripts.

| Species                                                 | Somatic no. (#)            | Gametic no. (#) | Reference                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------------------|----------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>L. alba</i> W.F. Barker ex G. D. Duncan              | 18 (1), 20 (3), 20/40 (1)  |                 | Johnson & Brandham (1997)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <i>L. algoensis</i> Schönland                           | 14 (4)                     | 7 (1)           | Crosby (1986); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008, 2009)<br>Ornduff & Watters (1978)<br>Hancke (1991)                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <i>L. aloides</i> (L.f.) Engl.                          | 14 (32)+0-1B               | 7 (6)           | Moffett (1936); Therman (1956); De Wet (1957); Mogford (1978); Crosby (1986); Hancke & Liebenberg (1990); Hancke (1991); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998, 2004, 2007); Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2009); Spies <i>et al.</i> (2009)<br>Hancke & Liebenberg (1998); Moffett (1936)<br>Crosby (1986)<br>Moffett (1936); Crosby (1986)<br>Crosby (1986); Hancke & Liebenberg (1990); Hamatani <i>et al.</i> (1998); Spies <i>et al.</i> (2009); Hamatani <i>et al.</i> (2010)<br>Ornduff & Watters (1978) |
| <i>L. ameliae</i> W.F. Barker                           | 18 (2)                     | 14 (1)          | Johnson & Brandham (1997)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <i>L. anguinea</i> Sweet                                | 30 (1)+2B                  |                 | Johnson & Brandham (1997)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <i>L. arbuthnothiae</i> W.F. Barker                     | 14 (6)                     | 7 (1)           | Crosby (1986); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Spies <i>et al.</i> (2008, 2009)<br>Spies <i>et al.</i> (2009)<br>Spies <i>et al.</i> (2009)                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>L. attenuata</i> W.F. Barker ex G.D. Duncan          | 14 (1)                     |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <i>L. bachmannii</i> Baker                              | 16 (5)                     |                 | De Wet (1957); Crosby (1986); Johnson & Brandham (1997); Hamatani <i>et al.</i> (2004)                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <i>L. barkeriana</i> U. Müller-Doblies <i>et al.</i>    | 14 (3)                     |                 | Müller-Doblies <i>et al.</i> (1987)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <i>L. bifolia</i> (Burm. f.) W.F. Barker ex G.D. Duncan | 16 (2)<br>14 (1)<br>28 (7) |                 | Nordenstam (1982); Müller-Doblies <i>et al.</i> (1987)<br>Crosby (1986)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>L. bolusii</i> W.F. Barker                           | 18 (1)                     | 14 (1)          | Kleynhans & Spies (1999); Spies <i>et al.</i> (2009)<br>Ornduff & Watters (1978)                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <i>L. bowkeri</i> Baker                                 | 16 (1)                     |                 | Moffett (1936); Crosby (1986); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Kleynhans & Spies (1999); Spies <i>et al.</i> (2008)                                                                                                                                                                                                                                                                                                                                                                                                           |
| <i>L. capensis</i> W.F. Barker                          | 16 (1)<br>28 (2)           |                 | Kleynhans & Spies (1999)<br>Crosby (1986); Johnson & Brandham (1997); Kleynhans & Spies (1999)<br>Spies <i>et al.</i> (2009)                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <i>L. carnosa</i> Baker                                 | 16 (26)                    |                 | Dold & Phillipson (1998)<br>Hamatani <i>et al.</i> (1998)<br>Johnson & Brandham (1997); Spies <i>et al.</i> (2008)<br>Crosby (1986); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Du Preez <i>et al.</i> (2002); Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2009); Spies <i>et al.</i> (2009)                                                                                                                                                                                                                                     |
| <i>L. cernua</i> G.D. Duncan                            | 28 (1)                     | 8 (1)+0-2B      | Spies <i>et al.</i> (2009)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <i>L. comptonii</i> W.F. Barker                         | 20 (5)                     |                 | Spies <i>et al.</i> (2008)<br>Crosby (1986); Johnson & Brandham (1997); Spies <i>et al.</i> (2009)                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <i>L. concordiana</i> Schltr. ex W.F. Barker            | c26 (1)<br>14 (1)          | 10 (1)          | Spies (2004)<br>Crosby (1986)<br>Spies <i>et al.</i> (2008)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <i>L. congesta</i> W.F. Barker                          | 26, 28 (1)                 |                 | Johnson & Brandham (1997)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <i>L. contaminata</i> Aiton                             | 14 (3)<br>16 (11)+1B       |                 | Gouws (1965); Spies <i>et al.</i> (2008, 2009)<br>De Wet (1957); Gouws (1965); Crosby (1986); Hancke (1991); Johnson & Brandham (1997); Hamatani <i>et al.</i> (2004)                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>L. convallarioides</i> Baker                         | 32 (1)                     | 8 (2)           | Ornduff & Watters (1978)<br>Johnson & Brandham (1997)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>L. doleritica</i> G.D. Duncan                        | 30 (1)<br>18 (2)           |                 | Johnson & Brandham (1997)<br>Spies <i>et al.</i> (2008, 2009)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

<sup>20</sup> Aneuploid

<sup>21</sup> Autotriploid

<sup>22</sup> Segmental allopolyploid

| Species                                                             | Somatic no. (#) | Gametic no. (#) | Reference                                                                                                                                |
|---------------------------------------------------------------------|-----------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------------|
| <i>L. duncanii</i> W.F. Barker                                      | 18 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. elegans</i> W.F. Barker                                       | 14 (6)          |                 | Moffett (1936); Johnson & Brandham (1997); Spies <i>et al.</i> (2009)                                                                    |
|                                                                     | 28 (12)         |                 | Moffett (1936); Crosby (1986); Johnson & Brandham (1997); Spies <i>et al.</i> (2009)                                                     |
|                                                                     |                 | 14 (9)          | Ornduff & Watters (1978); Spies <i>et al.</i> (2009)                                                                                     |
|                                                                     | 42 (4)          |                 | Johnson & Brandham (1997); Duncan (2001)                                                                                                 |
|                                                                     |                 | 21 (2)          | Spies <i>et al.</i> (2009)                                                                                                               |
|                                                                     | 56 (1)          |                 | De Wet (1957)                                                                                                                            |
|                                                                     |                 | 28 (2)          | Ornduff & Watters (1978)                                                                                                                 |
| <i>L. ensifolia</i> (Thunb.) J.C. Manning and Goldblatt             | 24 (3)          |                 | Johnson & Brandham (1997)                                                                                                                |
|                                                                     | 26 (2)          |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007)                                                                                 |
| <i>L. fistulosa</i> Baker                                           | 14 (8)          |                 | Johnson & Brandham (1997); Spies <i>et al.</i> (2002); Hamatani <i>et al.</i> (2004); Spies <i>et al.</i> (2009)                         |
|                                                                     |                 | 7 (2)           | Ornduff & Watters (1978)                                                                                                                 |
|                                                                     | 28 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. framesii</i> W.F. Barker                                      | 16 (3)          |                 | Du Preez <i>et al.</i> (2002); Spies <i>et al.</i> (2008)                                                                                |
| <i>L. giessii</i> W.F. Barker                                       | 32 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. haarlemensis</i> Fourc.                                       | 18 (2)          |                 | Johnson & Brandham (1997)                                                                                                                |
| <i>L. hirta</i> (Thunb.) Thunb.                                     |                 | 9 (1)           | Ornduff & Watters (1978)                                                                                                                 |
|                                                                     | 22 (6)          |                 | Johnson & Brandham (1997); Van Rooyen <i>et al.</i> (2002); Hamatani <i>et al.</i> (2004); Spies <i>et al.</i> (2009)                    |
|                                                                     |                 | 11 (2)          | Ornduff & Watters (1978)                                                                                                                 |
|                                                                     | 24 (3)          |                 | De Wet (1957); Hancke (1991); Johnson & Brandham (1997)                                                                                  |
| <i>L. inconspicua</i> G.D. Duncan                                   | 18 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. isopetala</i> Jacq.                                           | 30 (2)          |                 | Johnson & Brandham (1997)                                                                                                                |
|                                                                     | 40 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. juncifolia</i> Baker                                          | 22 (9)          |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008, 2009); Hamatani <i>et al.</i> (2010)                |
|                                                                     |                 | 11 (1)          | Ornduff & Watters (1978)                                                                                                                 |
| <i>L. karooica</i> W.F. Barker ex G.D. Duncan                       | 16 (1)          |                 | Duncan (1996)                                                                                                                            |
| <i>L. klinghardtiana</i> Dinter                                     | 14 (2)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. kliprandensis</i> W.F. Barker                                 | 16 (1)          |                 | Johnson & Brandham (1997)                                                                                                                |
| <i>L. lactosa</i> G.D. Duncan                                       | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. latimeriae</i> W.F. Barker                                    | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
|                                                                     | 18 (2)          |                 | Hamatani <i>et al.</i> (2007, 2010)                                                                                                      |
| <i>L. leomontana</i> W.F. Barker                                    | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. liliflora</i> Jacq.                                           | 16 (7)          |                 | Moffett (1936); De Wet (1957); Hancke (1991); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998, 2009); Spies <i>et al.</i> (2009) |
|                                                                     |                 | 8 (1)           | Moffett (1936)                                                                                                                           |
| <i>L. longibracteata</i> E. Phillips                                | 14 (4)          |                 | Crosby (1986); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2009)                                  |
|                                                                     |                 | 7 (2)           | Ornduff & Watters (1978)                                                                                                                 |
| <i>L. longituba</i> (A.M. van der Merwe) J.C. Manning and Goldblatt | 28 (2)          |                 | Hamatani <i>et al.</i> (2007, 2010)                                                                                                      |
| <i>L. macgregorium</i> W.F. Barker                                  | 22 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. margaretae</i> W.F. Barker                                    | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. marginata</i> W.F. Barker                                     | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
|                                                                     | 28 (3)          |                 | Johnson & Brandham (1997)                                                                                                                |
|                                                                     | 29 (1)          |                 | Johnson & Brandham (1997)                                                                                                                |
| <i>L. marginata</i> subsp. <i>neglecta</i> Schltr. ex G.D. Duncan   | 10 (1)          |                 | Duncan (1996)                                                                                                                            |
| <i>L. marlothii</i> W.F. Barker ex G.D. Duncan                      | 14 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. martinae</i> W.F. Barker                                      | 26 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |
| <i>L. mathewsii</i> W.F. Barker                                     | 14 (4)          |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Spies <i>et al.</i> (2002, 2008, 2009)                                         |
| <i>L. maximiliani</i> Schltr. ex W.F. Barker                        | 16 (1)          |                 | Spies <i>et al.</i> (2009)                                                                                                               |
| <i>L. mediana</i> Jacq.                                             | 14 (1)          |                 | Johnson & Brandham (1997)                                                                                                                |
|                                                                     | 18 (2)          | 9 (2)           | Spies <i>et al.</i> (2009)                                                                                                               |
|                                                                     | 26 (2)          |                 | Crosby (1986); Spies <i>et al.</i> (2008)                                                                                                |
|                                                                     |                 | 13 (1)          | Spies <i>et al.</i> (2009)                                                                                                               |
| <i>L. minima</i> W.F. Barker                                        | 18 (1)          |                 | Spies <i>et al.</i> (2008)                                                                                                               |

| Species                                                       | Somatic no. (#)      | Gametic no. (#) | Reference                                                                                                                                                                                                        |
|---------------------------------------------------------------|----------------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>L. moniliformis</i> W.F. Barker                            | 22 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. mutabilis</i> Lodd. ex Sweet                            | 10 (6)               |                 | Johnson & Brandham (1997)                                                                                                                                                                                        |
|                                                               |                      | 5 (2)           | Ornduff & Watters (1978)                                                                                                                                                                                         |
|                                                               | 12 (6)               |                 | Spies <i>et al.</i> (2000, 2009)                                                                                                                                                                                 |
|                                                               |                      | 6 (2)           | Spies <i>et al.</i> (2002, 2009)                                                                                                                                                                                 |
|                                                               | 14 (20)              |                 | De Wet (1957); Crosby (1986); Hancke & Liebenberg (1990); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Spies <i>et al.</i> (2000, 2009)                                                             |
|                                                               |                      | 7 (5)           | Hancke & Liebenberg (1998); Spies <i>et al.</i> (2002, 2009)                                                                                                                                                     |
|                                                               | 24 (1)               |                 | Spies <i>et al.</i> (2000)                                                                                                                                                                                       |
|                                                               | 56 (1)               |                 | De Wet (1957)                                                                                                                                                                                                    |
| <i>L. namaquensis</i> Schltr. ex W.F. Barker                  | 16 (11)              |                 | Crosby (1986); Johnson & Brandham (1997); Du Preez <i>et al.</i> (2002); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2009); Spies <i>et al.</i> (2009)                    |
|                                                               |                      | 8 (2)           | Spies <i>et al.</i> (2009)                                                                                                                                                                                       |
| <i>L. namibiensis</i> W.F. Barker                             | 22 (2)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. neilii</i> W.F. Barker ex G.D. Duncan                   | 18 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. nervosa</i> Ker Gawll                                   | 16 (2)               |                 | Moffett (1936); Spies <i>et al.</i> (2008)                                                                                                                                                                       |
|                                                               |                      | 8 (1)           | Moffett (1936)                                                                                                                                                                                                   |
|                                                               | 24 (2)               |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007)                                                                                                                                                         |
| <i>L. obscura</i> Schltr. ex G.D. Duncan                      | 18 (2)+1B,           |                 | Johnson & Brandham (1997)                                                                                                                                                                                        |
|                                                               | 36 (2)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. orchoides</i> (L.) Aiton                                | 14 (20)              |                 | Crosby (1986); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008, 2009)                                                                                                                                   |
|                                                               |                      | 7 (19)          | Moffett (1936); Ornduff & Watters (1978); Spies <i>et al.</i> (2009)                                                                                                                                             |
|                                                               | 16 (5)               |                 | Moffett (1936); De Wet (1957); Hancke (1991)                                                                                                                                                                     |
|                                                               |                      | 8 (1)           | Moffett (1936)                                                                                                                                                                                                   |
|                                                               | 17 (1) <sup>23</sup> |                 | Moffett (1936)                                                                                                                                                                                                   |
|                                                               | 18 (1)               |                 | Riley (1962)                                                                                                                                                                                                     |
|                                                               | 28 (13)              |                 | Moffett (1936); Crosby (1986); De Wet (1957); Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2010)                                                |
|                                                               |                      | 14 (2)          | Moffett (1936); Ornduff & Watters (1978)                                                                                                                                                                         |
|                                                               | 24 (1)               |                 | Hancke & Liebenberg (1990)                                                                                                                                                                                       |
|                                                               | 29 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                                                                        |
| <i>L. orthopetala</i> Jacq.                                   | 16 (5)               |                 | Crosby (1986); Johnson & Brandham (1997); Spies <i>et al.</i> (2008, 2009)                                                                                                                                       |
| <i>L. pallida</i> Aiton                                       | 16 (52)              |                 | Moffett (1936); (Crosby, 1986); Johnson & Brandham (1997); (Hamatani <i>et al.</i> , 1998, 2004); Spies <i>et al.</i> (2008, 2009)                                                                               |
|                                                               | 16 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
|                                                               | 16 (24)              |                 | Moffett (1936); Crosby (1986); De Wet (1957); Gouws (1965); Hancke (1991); Johnson & Brandham (1997); Spies <i>et al.</i> (2000, 2008); Du Preez <i>et al.</i> (2002); Hamatani <i>et al.</i> (1998, 2004, 2009) |
|                                                               | 32 (2) <sup>24</sup> |                 | Crosby (1986); Spies <i>et al.</i> (2000)                                                                                                                                                                        |
|                                                               |                      | 8 (7)           | Moffett (1936); Ornduff & Watters (1978)                                                                                                                                                                         |
| <i>L. patula</i> Jacq.                                        | 16 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                                                                        |
| <i>L. paucifolia</i> (W.F. Barker) J.C. Manning and Goldblatt | 26 (3)               |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007, 2010)                                                                                                                                                   |
| <i>L. peersii</i> Marloth ex W.F. Barker                      | 14 (3)               |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2004); Spies <i>et al.</i> (2009)                                                                                                                             |
| <i>L. physocaulos</i> W.F. Barker                             | 14 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. polyphylla</i> Baker                                    | 22 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                                                                       |
| <i>L. punctata</i> Jacq.                                      | 14 (6)               |                 | Moffett (1936); Crosby (1986); Hamatani <i>et al.</i> (1998, 2009); Spies <i>et al.</i> (2009)                                                                                                                   |
|                                                               |                      | 7 (1)           | Moffett (1936)                                                                                                                                                                                                   |
|                                                               | 28 (1)               |                 | Crosby (1986)                                                                                                                                                                                                    |
| <i>L. purpureo-caerulea</i> Jacq.                             | 16 (4)               |                 | Moffett (1936); Johnson & Brandham (1997); Spies <i>et al.</i> (2009)                                                                                                                                            |
|                                                               |                      | 8 (2)           | Moffett (1936); Ornduff & Watters (1978)                                                                                                                                                                         |
| <i>L. pusilla</i> Jacq.                                       | 14 (8)               |                 | Crosby (1986); Müller-Doblies <i>et al.</i> (1987); Johnson & Brandham (1997); (Hamatani <i>et al.</i> , 1998, 2007, 2009)                                                                                       |
|                                                               | 16 (1) <sup>25</sup> |                 | Nordenstam (1982)                                                                                                                                                                                                |
|                                                               | 18 (1)               |                 | Spies <i>et al.</i> (2009)                                                                                                                                                                                       |
|                                                               | 28 (1)               |                 | Hancke (1991)                                                                                                                                                                                                    |
| <i>L. reflexa</i> Thunb.                                      | 14 (5)+0-2B          |                 | Crosby (1986); Hancke & Liebenberg (1990); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Spies <i>et al.</i> (2009)                                                                                  |

<sup>23</sup> Aneuploid<sup>24</sup> One cell in a specific specimen<sup>25</sup> Specific accessions later identified as *L. barkeriana*

| Species                                         | Somatic no. (#)      | Gametic no. (#) | Reference                                                                                                                                                               |
|-------------------------------------------------|----------------------|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                 |                      | 7 (1)           | Hancke & Liebenberg (1998)                                                                                                                                              |
|                                                 | 16 (1)               |                 | De Wet (1957)                                                                                                                                                           |
| <i>L. rosea</i> Andrews                         | 14 (6)               |                 | Moffett (1936); Crosby (1986); Hancke (1991); Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007); Spies <i>et al.</i> (2008)                                      |
|                                                 | 21 (1)               |                 | Crosby (1986)                                                                                                                                                           |
|                                                 | 28 (2)               |                 | Spies <i>et al.</i> (2009)                                                                                                                                              |
| <i>L. sessiliflora</i> Andrews                  | 14 (3)               |                 | Johnson & Brandham (1997); Hamatani <i>et al.</i> (2007, 2009)                                                                                                          |
| <i>L. splendida</i> Diels.                      | 16 (8)+2B            |                 | Crosby (1986); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998); Du Preez <i>et al.</i> (2002); Hamatani <i>et al.</i> (2009); Spies <i>et al.</i> (2009)       |
|                                                 |                      | 8 (2)           | Spies <i>et al.</i> (2009)                                                                                                                                              |
|                                                 | 18 (1) <sup>26</sup> |                 | Crosby (1986)                                                                                                                                                           |
| <i>L. stayneri</i> W.F. Barker                  | 24 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                               |
| <i>L. thomasiae</i> W.F. Barker ex G. D. Duncan | 14 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                              |
| <i>L. trichophylla</i> Baker                    | 14 (2)               |                 | Johnson & Brandham (1997)                                                                                                                                               |
|                                                 |                      | 7 (1)           | Ornduff & Watters (1978)                                                                                                                                                |
| <i>L. undulata</i> Masson ex Baker              | 20 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                               |
| <i>L. unifolia</i> Jacq.                        | 16 (1)               |                 | Hancke (1991)                                                                                                                                                           |
|                                                 | 21 (1)               |                 | De Wet (1957)                                                                                                                                                           |
|                                                 | 22 (24)              |                 | Moffett (1936); De Wet (1957); Crosby (1986); Johnson & Brandham (1997); Van Rooyen <i>et al.</i> (2002); Spies <i>et al.</i> (2009)                                    |
|                                                 |                      | 11 (16)         | Moffett (1936); Ornduff & Watters (1978); Spies <i>et al.</i> (2009)                                                                                                    |
|                                                 | 24 (2)               |                 | De Wet (1957); Hamatani <i>et al.</i> (2004)                                                                                                                            |
|                                                 | 26 (2)               |                 | Moffett (1936); De Wet (1957)                                                                                                                                           |
|                                                 | 44 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                               |
| <i>L. valeriae</i> G.D. Duncan                  | 16 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                              |
| <i>L. variegata</i> W.F. Barker                 | 14 (2)               |                 | Spies <i>et al.</i> (2008); Hamatani <i>et al.</i> (2009)                                                                                                               |
|                                                 | 12 (1)               |                 | Hamatani <i>et al.</i> (2004)                                                                                                                                           |
|                                                 | 28 (1)               |                 | Spies <i>et al.</i> (2002)                                                                                                                                              |
| <i>L. ventricosa</i> Schltr. ex W.F. Barker     | 14 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                              |
| <i>L. verticillata</i> W.F. Barker              | 16 (1)               |                 | Crosby (1986)                                                                                                                                                           |
| <i>L. violacea</i> Jacq                         | 14 (13)              |                 | Hancke (1991); Johnson & Brandham (1997); Hamatani <i>et al.</i> (1998)                                                                                                 |
|                                                 |                      | 7 (3)           | Ornduff & Watters (1978); Spies <i>et al.</i> (2009)                                                                                                                    |
|                                                 | 15 (1)               |                 | Johnson & Brandham (1997)                                                                                                                                               |
|                                                 | 16 (1)               |                 | Crosby (1986)                                                                                                                                                           |
| <i>L. viridiflora</i> W.F. Barker               | 14 (7)               |                 | Nordenstam (1982); Crosby (1986); Hancke & Liebenberg (1990); Hancke (1991); Johnson & Brandham (1997); Spies <i>et al.</i> (2002); Hamatani <i>et al.</i> (2007, 2009) |
|                                                 |                      | 7 (1)           | Hancke & Liebenberg (1998)                                                                                                                                              |
| <i>L. youngii</i> Baker                         | 16 (1)               |                 | Spies <i>et al.</i> (2008)                                                                                                                                              |
| <i>L. zebrina</i> W.F. Barker                   | 30 (2)               |                 | Johnson & Brandham (1997); Spies <i>et al.</i> (2008)                                                                                                                   |
| <i>L. zeyheri</i> Baker                         | 22 (2)               |                 | Johnson & Brandham (1997); Spies <i>et al.</i> (2002)                                                                                                                   |
|                                                 | 23 (2) <sup>27</sup> |                 | Hamatani <i>et al.</i> (1998, 2010)                                                                                                                                     |

The small size of the chromosomes (Hancke & Liebenberg, 1990; Spies *et al.*, 2000) in the genus can furthermore contribute to miscounts and possible miss-identification of B-chromosomes. The presence of B-chromosomes in *Lachenalia* was described by Hancke & Liebenberg (1990). According to these authors, B-chromosomes in *Lachenalia* do not have a specific staining pattern and are similar in size to the smallest chromosome in the normal complement. This characteristic makes them difficult to identify and therefore could explain some erroneous counts, reported in literature. B-chromosomes in *Lachenalia* do not occur in

<sup>26</sup> Possibly B-chromosomes

<sup>27</sup> Suspected B-chromosome

all cells of a specific individual and also not in all plants of a specific accession (Hancke & Liebenberg, 1990). It is thus important to investigate the chromosome number of several individuals from a specific population to have accurate chromosome counts and correctly identify the presence of B-chromosomes. Counting insufficient numbers of cells can similarly lead to miscounts due to chromosome damage occurring during slide preparation.

B-chromosomes have been reported in eight species, namely *L. aloides*, *L. anguinea*, *L. bifolia*, *L. carnosa*, *L. contaminata*, *L. obscura*, *L. reflexa* and *L. splendida* (Crosby, 1986; Hancke & Liebenberg, 1990; Johnson & Brandham, 1997; Kleynhans & Spies, 1999; Spies *et al.*, 2009). Hamatani *et al.* (1998) also reported an expected B-chromosome in a  $2n = 23$  accession of *L. zeyheri*. Another example where possible B-chromosomes have not been identified, can be found in *L. barkeriana* where both  $2n = 14$  and  $2n = 16$  were reported (Table 5.1). The  $2n = 16$  number was, however, only found in one cell (Müller-Doblies *et al.*, 1987) of an otherwise  $2n = 14$  accession and could most possibly be ascribed to extra chromosomes.

### 5.2.2 Chromosome morphology

The chromosome morphology of *Lachenalia* has been described in various reports (Moffett, 1936; De Wet, 1957; Hamatani *et al.*, 1998, 2004, 2007, 2009, 2010; Hancke & Liebenberg, 1998). Both Moffett (1936) and (Hamatani *et al.*, 1998, 2004, 2007) attempted to group the species of the genus based on chromosome length and basic chromosome number. The groupings by Moffett (1936) and Hamatani *et al.* (1998) agreed, except for the division of the first group of Moffett into two separate groups by Hamatani *et al.* (1998). Further studies by Hamatani *et al.* (2004, 2007) added four groups based on chromosome numbers and varying numbers of larger chromosomes within specific basic chromosome numbers.

Ideograms presented by De Wet (1957) do not agree with karyograms by Moffett (1936) or (Hamatani *et al.*, 1998, 2004, 2007), neither does it agree with ideograms presented by Hancke & Liebenberg (1998), Hancke *et al.* (2001) and (Hamatani *et al.*, 2009). The ideogram for *L. aloides* presented by Hancke *et al.* (2001) agrees with Mofett's division, but differs from the karyograms of Hamatani *et al.* (1998, 2004, 2007) in having six longer chromosomes and not only two long chromosomes. Ideograms for *L. aloides* and *L. splendida* constructed by Hamatani *et al.* (2009) are similar to those of Hancke *et al.* (2001).



Spies *et al.* (2000) reported that accessions of *L. mutabilis* contained 4-8 very short chromosomes. According to these authors, the number of short chromosomes can vary between different localities and even between specimens collected at the same locality. Hamatani *et al.* (2007) furthermore reported on varying karyotypes within the same species for a number of *Lachenalia* species. This reported variation and conflicting results thus indicate that karyomorphological data alone cannot be utilized successfully to construct phylogenetic relationships in the genus *Lachenalia*. Similar conclusions were reached by Hamatani *et al.* (2008), resulting in a movement towards molecular methods to determine phylogenetic relationships in the genus.

### 5.2.3 Basic chromosome numbers and polyploidy

Moffett (1936) identified four different basic chromosome numbers ( $x = 7, 8, 11$  and  $13$ ) and polyploids, including  $3x, 4x$  and  $6x$ , in the  $x = 7$  group. De Wet (1957) added a basic chromosome number of  $x = 12$  and reported on an accession with  $2n = 56$ , a possible  $8x$ . Ornduff & Watters (1978) added  $x = 6$ , in an unidentified species as well as  $x = 5$  and  $x = 9$ . Johnson & Brandham (1997) added  $x = 10$  and  $15$ .

For the purpose of this review, the 89 species in Table 5.1 were grouped according to their basic chromosome numbers. Basic chromosome numbers of  $x = 5, 10$  and  $15$  were also included as existing basic numbers for the genus and not as polyploid forms of basic group  $x = 5$ . Of the 89 species, six species (*L. mediana*, *L. latimeriae*, *L. isopetala*, *L. nervosa*, *L. congesta* and *L. capensis*) could not be placed into a specific basic chromosome number category due to varying reports in the literature, indicating different basic chromosome numbers within these species. It is possible that *L. mediana* has two different basic chromosome numbers and that  $x = 9$  is present in *L. mediana* subsp. *mediana* and  $x = 13$  is found in *L. mediana* subsp. *rogersii* (Spies *et al.*, 2008, 2009). More studies are, however, required to accurately place these six species into the correct group. Other species with varying chromosome number reports were placed into specific groups according to the most commonly reported chromosome number (Table 5.1). These include:

- basic group  $x = 8$  (*L. contaminata* as 14 out of 17 reports indicate  $2n = 16$ );



- basic group  $x = 7$  (*L. barkeriana* as 3 out of four accessions had  $2n = 14$ , *L. marginata* as 4 out of 5 reports indicate either  $2n = 14$  or tetraploids of  $x = 7$ , *L. orchioides* as majority of reports indicate  $x = 7$  and  $2n = 16$  most probably from wrongly identified species, *L. pusilla* as 8 out of 9 reports indicate  $2n = 14$ , *L. reflexa* as 5 out of 6 reports indicate  $2n = 14$  and the  $2n = 16$  could most probably be ascribed to the presence of B-chromosomes, *L. variegata* as 3 out of 4 reports indicate basic  $x = 7$  and *L. violacea* as 15 out of 17 reports indicate basic  $x = 7$ );
- basic group  $x = 10$  (*L. alba* as 4 out of 5 had  $2n = 20$  and Johnson & Brandham (1997) concluded that  $2n = 20$  forms a diploid based on  $x = 10$  rather than a tetraploid based on  $x = 5$ );
- basic group  $x = 11$  (*L. hirta* as 8 out of the 12 reports had  $2n = 22$  and *L. unifolia* as 27 out of 32 reports indicated  $2n = 22$  as the somatic chromosome number);
- basic group  $x = 12$  (*L. ensifolia* as 3 out of 5 reports indicate  $2n = 24$  but this species can also be a possible  $x = 13$  and *L. stayneri* because it formed a structural diploid based on  $x = 12$  rather than a tetraploid based on  $x = 6$  (Johnson & Brandham, 1997);
- three different basic chromosome numbers have been recorded for *L. mutabilis*. This is the only species in basic group  $x = 5$ , as well as basic group  $x = 6$ . The majority of reports however come from basic group  $x = 7$  (24 out of 38).

Of the 83 taxa that could be grouped, basic  $x = 7$  (41%) and basic  $x = 8$  (27%) were the most common, followed by basic  $x = 9$  (11%) and  $x = 11$  (10%). Basic  $x = 10$  (4%),  $x = 12$  (2%),  $x = 13$  (2%) and  $x = 15$  (4%) are only present in a small number of taxa (Table 5.1, Figure 5.4). Basic  $x = 5$  (1%) and  $x = 6$  (1%) were only present in *L. mutabilis*. Johnson & Brandham (1997) stated that  $x = 5$  reported for *L. mutabilis* were derived from plants with  $2n = 14$  via Robertsonian fusions. Based on their observations of no constant number of long and short chromosomes in *L. mutabilis*, Spies *et al.* (2000) disagreed with the conclusion of Johnson & Brandham (1997) that the  $x = 5$  *L. mutabilis* studied by them resulted from Robertsonian fusions. Spies *et al.* (2000) could not find any long chromosomes as a result of Robertsonian fusions linked to specific specimens or a specific basic number supporting the hypothesis of Johnson & Brandham (1997). Spies *et al.* (2000) thus concluded that the variation in *L.*

*mutabilis* is more likely to be the result of an aneuploid series. More studies are needed to determine the actual mode of chromosome evolution in the species *L. mutabilis*. Dysploid series also occurs in other genera such as *Prospero*:  $x = 4, 5, 6, 7$ ; *Bernardia*:  $x = 8, 9$ ; *Hyacinthella*:  $x = 9, 10, 11, 12$  and *Stellarioides*:  $x = 2, 3, 4, 5, 6, 7, 8$  and  $9$ . As in *Lachenalia* these aneuploid/dysploid series are difficult to interpret (Pfosser & Speta, 1999). Combining the chromosome counts with molecular and morphological data might aid in the interpretation of chromosomal evolution in the genus.

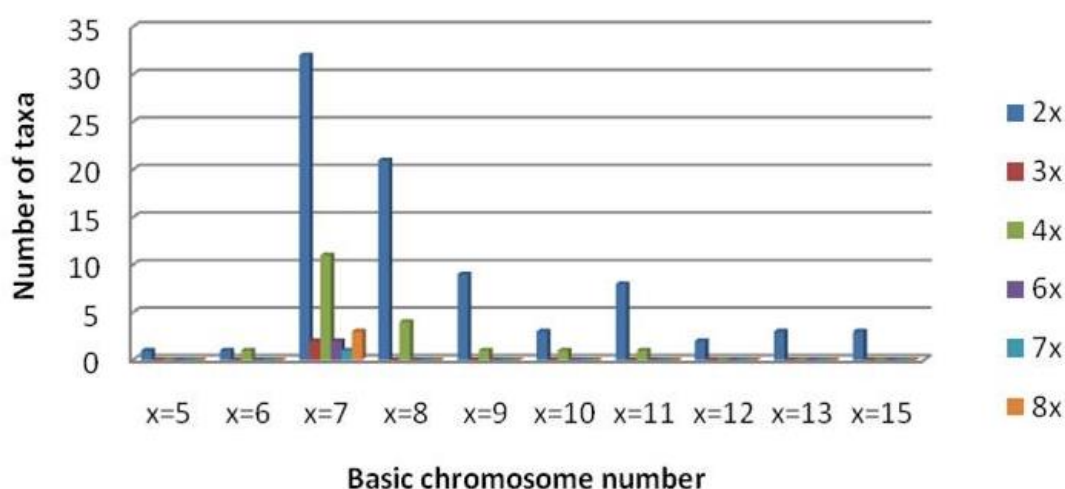


**Figure 5.4** Histogram of the number of taxa per basic chromosome number and ploidy levels reported for these basic numbers in the genus *Lachenalia*, including  $x = 10$  and  $15$ .

The presence of polyploidy was previously reported in 19 *Lachenalia* taxa (23%), excluding *L. capensis* and *L. congesta* where basic chromosome numbers could not be determined from published results. Conclusions could thus also not be drawn on polyploidy in these species (Table 5.1). Polyploidy are most common in the basic  $x = 7$  group, with 12 of the 34 species (35%) containing polyploid specimens and a few species exhibiting a range of ploidy levels from triploid to octoploid (Figure 5.4, Table 5.1). Polyploidy were also reported in basic groups  $x = 6, 8, 9, 10$  and  $11$ , but here only tetraploids were observed. Tetraploids (present in 23% of the 83 grouped taxa) are the most common followed by octoploids (4%), hexaploids (2%), triploids (2%) and heptaploids (1%).

*Lachenalia bifolia* is the species with the largest number of reported polyploid accessions including 4x, 6x, 7x and 8x accessions (Table 5.1). The heptaploid accession of *L. bifolia* originated from seed and it is thus possible that the seed could have originated from an

intra-species cross between a 6x and an 8x individual (Kleynhans & Spies, 1999). Specific ploidy levels in *L. bifolia* were better correlated to geographic distribution compared to morphology (Kleynhans & Spies, 1999).

The only other species with ploidy levels above tetraploid are *L. elegans* and one report of 8x in *L. mutabilis* (Table 5.1). The two triploid accessions in *L. aloides* and *L. rosea* could have resulted from intra-species crosses between diploid and tetraploid individuals in these species followed by vegetative propagation or through an unreduced gamete followed by vegetative propagation as suggested by Moffett (1936).

#### 5.2.4 Meiotic studies

Reports on meiotic studies within the genus are less frequent compared to reports on meiotic studies. The main reason is that meiotic divisions occur within the bulb and pollen has already been formed by the time the inflorescence appears. The bulb has to be dissected where-after the bulb is not viable. To find the correct meiotic stages, timing is crucial and many bulbs can be destroyed in the process. Moffett (1936) presented the first report on meiosis. This author found mostly normal meiosis for  $2n = 14$ , 16 and 22 species. Hancke & Liebenberg (1998) reported on the meiosis of several  $2n = 14$  species and hybrids. Species studied displayed normal meiosis with 7 bivalents. Four of the six hybrids studied also displayed normal meiosis with seven bivalents indicating a close relationship between the species *L. aloides*, *L. orchioides*, *L. viridiflora* and *L. reflexa*. Two hybrids (both between *L. aloides* and *L. mutabilis*) displayed a low percentage of trivalents and quadrivalents. Hancke & Liebenberg (1998) presented evidence of structural chromosomal changes involving three chromosomes of which the acrocentric pair of chromosomes was involved in at least one interchange. This chromosome pair also seemed to be prominent in other abnormalities observed during meiosis (Hancke & Liebenberg, 1998).

Hancke *et al.* (2001) studied the chromosome associations of one interspecific dibasic hybrid between *L. splendida* and *L. aloides* and two interspecific dibasic hybrids between *L. pallida* and *L. aloides*. Results showed that *L. aloides* is more closely related to both *L. splendida* and *L. pallida* than expected with genome affinity indexes of 0.9 and above. The results of the pairing configurations observed in these hybrids revealed homoeology between

two chromosomes of the  $x = 7$  karyotype and three chromosomes of the  $x = 8$  karyotype. This could indicate that the  $x = 7$  plants differ from the  $x = 8$  plants by at least two exchanges of chromosome material and involves also the loss of one centromere from the  $x = 8$  karyotype. Hancke *et al.* (2001) thus suggested that the change in basic chromosome number of *Lachenalia* involves a reduction in number.

Du Preez *et al.* (2002) reported on normal meiosis with eight bivalents for the following species, as well as the hybrids between *L. carnosa* and *L. splendida*, *L. splendida* and *L. carnosa*, *L. pallida* and *L. carnosa* and *L. carnosa* and *L. framesii*. The latter study indicated that these species are closely related. Hamatani *et al.* (2009) confirmed this relationship.

### 5.3 PHYLOGENETIC STUDIES

Only a few molecular studies have been done on the genus *Lachenalia* and most of these studies concentrated on the phylogenetic position of the genus. The extensive variation in the genus, and even within an individual species, as indicated by RAPD studies (Kleynhans & Spies, 2000), complicates both the phylogeny and taxonomy. In cultivation, a number of species are easily crossed and these hybrids reproduce by means of offshoots and bulb formation. The existence of possible natural hybrid species thus further complicates the phylogenetics of the genus.

#### 5.3.1 The phylogenetic position of *Lachenalia*

The genus *Lachenalia* was included in several studies to determine the phylogenetic position and classification of the constituent species, the first being the inclusion of the genus in the family Liliaceae. *Lachenalia* was reclassified to the family Hyacinthaceae (Perry, 1985) up to 2009, where-after the family Hyacinthaceae was dissolved into other families. *Lachenalia* now belongs to the family Asparagaceae (Angiosperm Phylogeny Group, 2009).

To find the relative position of *Lachenalia* in the Asparagaceae, Pfosser & Speta (1999) used sequences of the *trnL-F* chloroplast region. From these results, the authors were able to group *Lachenalia* into the tribe Massonieae (which consists of all the South African genera investigated, such as *Drimiopsis*, *Ledebouria* and *Polyxena*). The latter study also presented the first evidence suggesting a close relationship between *Lachenalia* and *Polyxena*, with

bootstrap support of 100%. This was in contrast to the results of Müller-Doblies & Müller-Doblies (1997), which placed *Lachenalia* in the sub-tribe Lachenaliinae and *Polyxena* into Massoniinae. Pfosser & Speta (1999) suggested additional studies, since only a few representative species were included in their analysis.

A later study by Pfosser *et al.* (2003) included not only more *Lachenalia* species, but also an additional chloroplast region (*atpB*), as well as data on seed morphology. *Polyxena*, *Lachenalia* and the genus *Periboea* formed a monophyletic clade with a bootstrap support of 100%. This study thus also supported the inclusion of *Polyxena* in the genus *Lachenalia*. Within the monophyletic clade, some species of *Lachenalia* and *Polyxena* had low bootstrap support values (66% and 62%, respectively) and it was suggested that the specific delimitation may not be optimal for these clades. Another explanation was that the species are more recently derived, resulting in an insufficient number of base substitutions to resolve the taxa. The authors suggested that seed size and weight is higher in the basal genera such as *Eucomis*, *Merwillia* and *Ledebouria*, with *Veltheimia bracteata* having seeds of 0.056 g and with a length of 6.1 mm. The smallest seeds were found in the genus *Lachenalia* (*L. angelica*: 0.0003 g; 0.9 mm long). Analysis on the seed size and weight supports the hypothesis of these authors that *Lachenalia* is a recently derived genus. The seed form and structure of the micropylar swelling of the seed coat in *Lachenalia* suggested that this genus was the most advanced among the genera included in this study.

The inclusion of *Polyxena* in the genus *Lachenalia* was raised again in three separate studies (Manning *et al.*, 2004; Spies, 2004; Hamatani *et al.*, 2008) using *rbcl*, *trnL-F* and *ITS1-2* sequencing data respectively. In all these studies, *Lachenalia* and *Polyxena* formed a well-supported monophyletic group. The two genera were characterised from other genera in the family by their biseriate stamens with the two series inserted at different heights. The two genera can be distinguished from each other by the relative fusion of the perianth (Manning *et al.*, 2002). Manning *et al.* (2004) thus included *Polyxena* within *Lachenalia* based on the paraphyletic nature of the two genera.

### 5.3.2 Phylogeny within the genus

Morphological studies have focused on the entire genus, and many species have, over

time, been included and excluded and shifted around from one genus to another. The first of these was when the genus was split into several genera (Salisbury, 1866). Subsequently, the species in the genus were sub-divided into smaller groups by Baker (1897), Crosby (1986) and (Duncan, 1988, 2002). These groupings, except for that of Crosby (1986), were based on different morphological characteristics, and patterns did not correspond between studies.

Duncan *et al.* (2005) used morphological data of all the species in the genus to construct a cladogram. This author included 73 characters which comprised of 57 qualitative and 16 quantitative characters. This study concluded that *Polyxena* is paraphyletic with *Lachenalia* and forms the basal clade. Many of the *Lachenalia* species formed polytomies or unrelated groups, but there were some synapomorphies or taxa sharing some traits.

Spies (2004) produced a cladogram based on chloroplast *trnL-F* sequence data from 129 taxa, including four *Massonia* taxa as outgroup. Hamatani *et al.* (2008) investigated nuclear *ITS1-2* sequence data of 56 taxa, including two *Massonia* and one *Ornithogalum* as outgroup. Both authors identified specific clades within the genus *Lachenalia*. The topologies of the cladograms produced by these authors largely correspond.

#### 5.4 CROSS-ABILITY IN *LACHENALIA*

The Rev. John Nelson raised the first authenticated *Lachenalia* hybrid in 1878 (Moore, 1905). Since then a number of claims of interspecific hybridization were published (see Crosby (1978) for a review of early work). None of these early hybrids became available commercially. In 1965 the genus was identified as an indigenous genus with potential for development in South Africa. A breeding programme for the development of flowering pot plants was started at the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council and the first hybrids became available commercially in 1997/1998 (Kleynhans, 2006).

The extensive morphological and cytological variation in the genus *Lachenalia* result in the existence of both internal and external crossing barriers (Lubbinge, 1980; Kleynhans & Hancke, 2002; Kleynhans, 2006). External crossing barriers such as geographical separation and varying flowering periods can be overcome through the cultivation of species in

controlled environments and the successful storage of pollen for a 12 month period (Kleynhans, 2006). Internal crossing barriers include both post- and pre-fertilization barriers. Mechanical isolation (Lubbinge, 1980) is one of the first internal pre-fertilization barriers. Flower length in *Lachenalia* species can vary from 5 to 30 mm (Duncan *et al.*, 2005). Pollen from small flowered species is thus not adapted to traverse the long distance from the stigma to the ovary of large flowered species (Stebbins, 1950). The use of reciprocal crosses has been successful in overcoming this barrier (Lubbinge, 1980; Kleynhans, 2006). Other pre- and post-fertilization barriers have not been studied in detail, but the extent of these barriers become clear when the success rate of inter-species crosses are taken into account.

In a breeding programme at the ARC-Vegetable and Ornamental Plant Institute, crossing combinations of at least 10 flowers, within two different inflorescences were pollinated for each experiment to ensure that wrong conclusions were not drawn, due to specific physiological or developmental problems in the inflorescence or floret. Kleynhans *et al.* (2009) reported that only 33% of the inter-species crosses (n=1498) made over a 30 year period were successful. With additional crosses (n=382) made since 2005, this percentage dropped to only 18% (Table 5.2). Of the 82% of crosses that did not succeed, 50% was related to the absence of seed, indicating the presence of possible pre-fertilization barriers. A further 31% of the combinations produced abnormal or non-viable seed that could be ascribed to post-fertilization barriers. Lastly 1% of the crossing combinations did not succeed due to seedling death shortly after germination. The death of the latter seedlings can not necessarily be ascribed to hybrid breakdown, as seedlings can also be affected by diseases and environmental factors.

The genetic variability within the genus as described above has a direct influence on the cross-ability. With the additional data presented in this review, the comparison between cross-ability and the cytogenetic and molecular data will be discussed in the next section.

## 5.5 COMPARISON BETWEEN CROSS-ABILITY, CYTOGENETIC AND MOLECULAR DATA

The complexity in the genus *Lachenalia*, in terms of morphology, cytogenetic and genetic variation, complicates the determination of the relationship within and between

different species. There are questions on the existence and origin of the different basic chromosome numbers, as well as on the mode of speciation. Do the different basic chromosome numbers correlate with the phylogeny of the genus? Can the phylogenetic information assist in the taxonomic grouping of some difficult species and, furthermore, can phylogenetic information shed some light on the existence of possible natural hybrids? How does the phylogeny correlate with the cross-ability between species and finally what conclusions can be drawn when the different data sets are compared?

**Table 5.2** Number of inter-species crosses made among various different *Lachenalia* species over a 35 year period and the results obtained from these crossing combinations. Crosses that did not succeed were linked to three different aspects namely no seed set, abnormal seeds or seedling death. Results are linked to the basic chromosome complement of the species.

| Basic chromosome number of parents                  | No. of successful crosses | No of unsuccessful crosses      |                                   |                                    |
|-----------------------------------------------------|---------------------------|---------------------------------|-----------------------------------|------------------------------------|
|                                                     |                           | No. of crosses with no seed set | No. of crosses with abnormal seed | No. of crosses with seedling death |
| 7x7                                                 | 169 (27%)                 | 274 (44%)                       | 169 (27%)                         | 10 (2%)                            |
| 8x8                                                 | 72 (46%)                  | 44 (28%)                        | 40 (45%)                          | 1 (1%)                             |
| 11x11                                               | 2 (67%)                   |                                 | 1 (33%)                           |                                    |
| 7x8                                                 | 20 (6%)                   | 251 (79%)                       | 44 (14%)                          | 3 (1%)                             |
| 8x7                                                 | 59 (18%)                  | 111 (34%)                       | 155 (47%)                         | 6 (2%)                             |
| 7x10                                                |                           | 17 (100%)                       |                                   |                                    |
| 10x7                                                | 1 (5%)                    | 5 (25%)                         | 13 (65%)                          | 1 (5%)                             |
| 7x11                                                | 1 (2%)                    | 54 (86%)                        | 8 (13%)                           |                                    |
| 11x7                                                | 4 (6)                     | 23 (33%)                        | 39 (57%)                          | 3 (4%)                             |
| 9x8                                                 |                           |                                 | 1 (100%)                          |                                    |
| 8x10                                                |                           | 1 (33%)                         | 2 (67%)                           |                                    |
| 10x8                                                | 2 (33%)                   | 1 (17%)                         | 2 (33%)                           | 1 (17%)                            |
| 8x11                                                | 1 (3%)                    | 23 (79%)                        | 5 (17%)                           |                                    |
| 11x8                                                | 1 (3%)                    | 15 (39%)                        | 22 (58%)                          |                                    |
| 11x10                                               |                           | 1 (100%)                        |                                   |                                    |
| 15x7                                                |                           | 2 (67%)                         | 1 (33%)                           |                                    |
| Unknown basic numbers in one or both of the parents | 4 (2%)                    | 117 (59%)                       | 78 (39%)                          |                                    |
| Total                                               | 336 (18%)                 | 939 (50%)                       | 580 (31%)                         | 25 (1%)                            |

### 5.5.1 Basic chromosome numbers and cladograms

A comparison between the groupings from Crosby (1986) (based on chromosome numbers), Spies (2004) (chloroplast *trnL-F*), Duncan (2005) (morphology) and Hamatani *et al.* (2008) (nuclear *ITS1-2*) revealed that, with the exception of a few species, there is a good correlation between basic chromosome numbers and the monophyletic groups identified in the different studies. When chromosome numbers were superimposed on the cladogram of Duncan *et al.* (2005), most of the  $x = 7$  and  $x = 8$  species fall into exclusive monophyletic groups for each chromosome number. There are only two exceptions where  $x = 7$  species (*L. congesta* and *L. mathewsii*) grouped with  $x = 8$ . Species with  $x = 11$  were closely related, even though they did not form a monophyletic group. The rest of the chromosome numbers form



a polytomy. Although monophyletic groups linked to basic chromosome numbers were obtained, the morphological cladogram is poorly resolved for many of the species.

Spies (2004) studied chromosome numbers and determined the molecular phylogeny of *Lachenalia*. The latter study used *trnL-F* chloroplast DNA-sequences of 129 taxa and distinguished several well-defined groups. The first group consisted of seven species with a basic number of 11. Species with  $x = 7$  and 8 formed a monophyletic clade (the *Lachenalia* 1 group), suggesting a close relationship between these two basic numbers. Within this monophyletic clade,  $x = 8$  formed a monophyletic subclade with the exception of only one species with a basic chromosome number of  $x = 8$ , *L. verticillata*, and including *L. pusilla* ( $x = 7$ ), which was basal to this group. All species having a basic chromosome number of  $x = 7$  were distributed in different sister subclades, of which the two largest includes 25 and 10 taxa respectively. The second large group in the cladogram (the *Lachenalia* 2 group), consisted of 48 poorly resolved taxa having chromosome numbers of  $x = 6, 7, 8, 9, 10$  and 13. This group has no consistent pattern regarding chromosome numbers. These results led the author to conclude that hybridization might have played a role in speciation and that the genus might represent a hybrid swarm.

In the cladogram based on *ITS1-2* sequence data (Hamatani *et al.*, 2008), a monophyletic group for  $x = 8$  (supported with a bootstrap value of 83.3) as well as for  $x = 7$  forming a polytomy was obtained. Two species, *L. sessiliflora* and *L. pusilla*, both with a basic number of 7, grouped with the  $x = 8$  clade, but formed the base for the rest of the  $x = 8$  species. The *ITS1-2* region seemed to have more variation in the  $x = 8$  taxa than in the  $x = 7$  taxa, since the clade for  $x = 8$  was better resolved. A similar observation was made by Spies (2004) based on *trnL-F* sequences.

The good correlation between basic chromosome numbers and phylogenetic groupings could in future be used to confirm basic numbers for species. A single count of  $2n = 32$  was reported for *L. giessii* (Spies *et al.*, 2008) but based upon a close phylogenetic grouping with  $x = 11$  (Spies, 2004), it seems that this species could also be regarded as  $x = 11$  ( $2n = 33$ ) rather than  $x = 8$  ( $2n = 32$ ). In this review, it was included as a tetraploid of  $x = 8$  for the purpose of calculations, but this species should be investigated further. Similarly, *L. capensis* groups with the  $x = 7$  group (Spies, 2004), thus supporting the chromosome counts of Johnson

& Brandham (1997) and Spies *et al.* (2008) and suggesting that *L. capensis* could be a basic  $x = 7$  rather than a basic  $x = 8$  as reported by (Hamatani *et al.*, 1998). Further investigations and correct identification of species are, however, essential to solve the inconsistent reports in chromosome numbers in some species.

### 5.5.2 Basic chromosome number and cross-ability

Kleynhans *et al.* (2009) presented data showing that the success rate of crossing combinations increased when crosses were made between species containing the same basic chromosome number. The information from additional crosses made in the preceding five years were added to this data and the number of successful crosses between species with the same basic chromosome number was substantially higher than between species from different basic chromosome numbers (Table 5.2). The success rate of crossing combinations dropped to below 20% when species with different basic chromosome numbers were crossed. The only exception to this is the combination of basic  $x = 10$  crossed with basic  $x = 8$  (Table 5.2). The most successful crosses resulted from a *L. alba* x *L. pallida* combination (specific results of all crosses not shown).

The increased success rate reported between species with the same basic chromosome number were a confirmation of a report by Crosby (1986), who also indicated that species cross more readily within certain basic chromosome number groupings. Based on differences in the cross-ability and morphology, the latter author also split the basic  $x = 7$  group of species into two different groups. The existence of different groupings within the basic  $x = 7$  was confirmed by Spies (2004) as discussed above. Meiotic data presented by Hancke & Liebenberg (1998), as discussed above, also indicated differences between especially the species *L. mutabilis* and *L. aloides* as illustrated by structural chromosome changes. Kleynhans *et al.* (2009) used the three basic clades as well as the phylogenetic groupings within the basic  $x = 7$  group as reported by Spies (2004) and presented data that showed improved cross-ability when crosses were made between individual species within the same phylogenetic groupings. The cross-ability was at least 10 to 20% higher when crossing combinations were attempted within the groups, than between groups. The cross-ability data thus supported phylogenetic groupings as identified by Spies (2004).

The close relationship illustrated in the phylogenetic trees, between species with basic  $x = 8$  was also confirmed by the cross-ability data with a success rate of 46% (Table 5.2). The only success rate higher than this was reciprocal crosses between species with basic  $x = 11$ . This data, however, only included three crossing combinations in comparison to the 157 combinations within the basic  $x = 8$  group and would most probably decline with the inclusion of additional crossing combinations. The relationship among species with  $x = 8$  was further illustrated by Du Preez *et al.* (2002). In this meiotic study several hybrids between different species with  $x = 8$  were investigated and all hybrids produced eight bivalents. Hybrids resulting from these crosses are also fertile and was successfully utilized in further crossing combinations (results not shown).

### 5.5.3 Evolution and relatedness of different basic chromosome numbers

The largest number of species in *Lachenalia* are found within the basic  $x = 7$  and 8 groups. Molecular data from *ITS1-2* (Hamatani *et al.*, 2009) and *trnL-F* (Spies, 2004) sequences indicated a strong relationship between these two basic chromosome number groups and suggested that these groups might have evolved from a common ancestor. Cross-ability data confirmed a relationship between these two basic chromosome number groups, with higher success rates (18% for  $x = 8$  crossed with  $x = 7$ ) than most of the other between-group success rates (Table 5.2). The existence of genome affinity indices of 0.9 in three interspecific dibasic hybrids (Hancke *et al.*, 2001), as discussed above, also confirmed this relationship.

Karyomorphological data presented by Hamatani *et al.* (2009) using FISH and DAPI staining to determine the chromosomal evolution of the  $x = 7$  and  $x = 8$  groups, confirmed the results found from both the phylogeny and the cross-ability. The results of this study between a group of  $x = 7$  (consisting of *L. sessiliflora*, *L. aloides*, *L. flava*, *L. longibracteata*, *L. variegata*, *L. viridiflora*, *L. mutabilis*, *L. punctata*, and *L. pusilla*) and  $x = 8$  (consisting of *L. carnosae*, *L. liliflora*, *L. namaquensis*, *L. splendida* and *L. pallida*) led to the conclusion that there was little morphological chromosome variation within the  $x = 8$  group and that this group was derived from an ancestral species followed by ongoing speciation.

The  $x = 7$  group showed much more variation, with four karyotype patterns indicating several morphological alterations of chromosomes within this group. This was in contrast with the *ITS1-2* region data that seemed to have more variation in the  $x = 8$  taxa than in the  $x = 7$  taxa, since the clade for  $x = 8$  was better resolved than the polytomic  $x = 7$  clade (Hamatani *et al.*, 2009).

Hamatani *et al.* (2008; 2009) suggested several theories for the evolution of the  $x = 7$  and  $x = 8$  groups. Both groups might have evolved from a common ancestor (as indicated in sequencing data) or they could be the product of mutation or putative hybridization between species in the same geographical distribution area. Reduction in chromosome number either by losing a chromosome or by translocation might have contributed to speciation in these two groups. Hancke *et al.* (2001) speculated that  $x = 7$  evolved from  $x = 8$  through a reduction in chromosome number based on the homoeology between two chromosomes in the  $x = 7$  and three chromosomes in the  $x = 8$  species studied.

Five of the nine species in the  $x = 7$  group (*L. aloides*, *L. flava*, *L. longibracteata*, *L. variegata*, *L. viridiflora*) had very similar chromosome morphology (Hamatani *et al.*, 2009) and seemed to be closely related. The close relationship between *L. aloides* and *L. viridiflora* can be confirmed from crossing data with a success rate of between 25 and 100% depending on the reciprocal direction (data not shown) and the production of fertile  $F_1$  hybrids with seven bivalents in meiotic analysis (Hancke & Liebenberg, 1998).

According to (Hamatani *et al.*, 2009) the chromosome morphologies of *L. mutabilis* and *L. punctata* were very similar, but differed from the above group, and the authors concluded that these species probably originated from a single ancestral species. For the purpose of this review a selection of *ITS1-2* sequences representing only those species used in the FISH study (Hamatani *et al.*, 2009) were obtained from GenBank and a phylogram was constructed (Figure 5.5). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The ITS phylogram yielded similar monophyletic groupings compared to the *ITS1-2* cladogram (Hamatani *et al.*, 2009) and included both *L. mutabilis* and *L. punctata* within the  $x = 7$  clade. Both these species have a similar branch length that was much longer than the other species in the clade, which supported the similarity in chromosome morphology. This relationship cannot be confirmed

from crossing data (success rate of only 10%), nor by the data presented by Spies (2004) or Hamatani *et al.* (2008).

The remaining two species in the  $x = 7$  group that were investigated (Hamatani *et al.*, 2009), *L. sessiliflora* and *L. pusilla*, shared chromosomal characteristics with species in both the  $x = 7$  and 8 groups. The relationship to both  $x = 7$  and  $x = 8$  of *L. sessiliflora* and *L. pusilla* was confirmed by Hamatani *et al.* (2008). Hamatani *et al.* (2009) suggested that *L. pusilla* might be intermediate between the  $x = 7$  and  $x = 8$  group. None of the crosses made with *L. pusilla* as either parent were successful, neither with  $x = 7$  nor with  $x = 8$  species. The crossability data available can thus not shed any light on the position of *L. pusilla*.

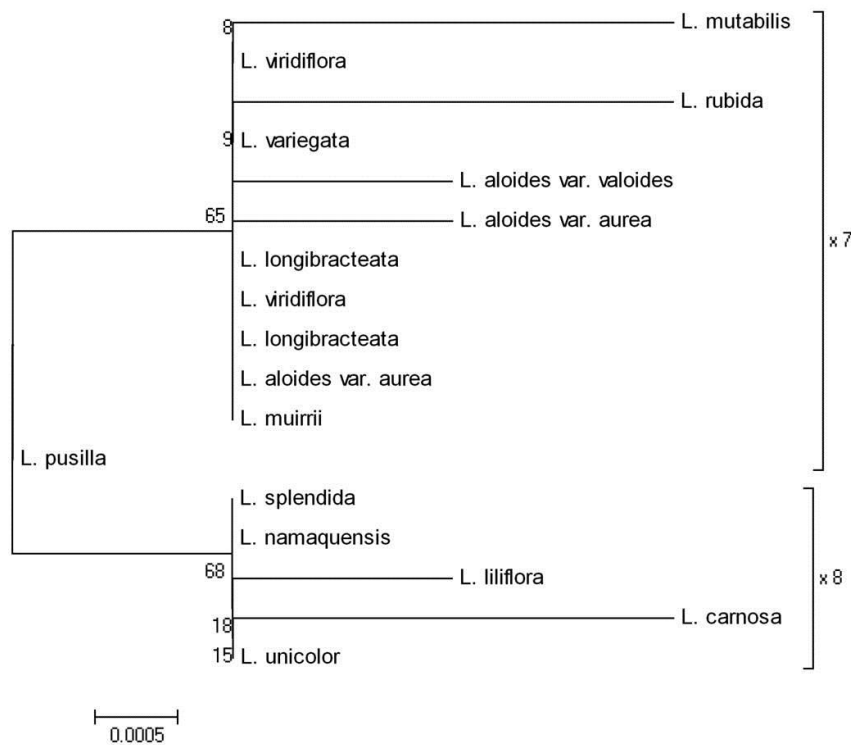


**Figure 5.5** Evolutionary relationships of 17 taxa based on the ITS1-2 region. The phylogram was constructed using the Maximum Likelihood option of MEGA 5 (Tamura *et al.*, 2011) to compare the evolutionary development of the  $x = 7$  and 8 groups. Synonyms (Duncan, 2012): *L. rubida* = *L. punctata*; *L. aloides* var. *aloides* = *L. callista*; *L. aloides* var. *aurea* = *L. flava*; *L. unicolor* = *L. pallida*.

There seem to be evolutionary relationships between some of the other basic chromosome number groups and even with other genera. For better insight in the evolution of the rest of the chromosome numbers, sequences from Spies (2004) were selected to represent a broad spectrum of chromosome numbers in the genus. Sequences were selected

based on the cladogram produced by Spies (2004), but all sequences forming a polytomy were excluded, and a new cladogram (Figure 5.6) was constructed.

Although many of the clades are not well supported, the new *trnL-F* cladogram (Figure 5.6) supports the suggestion that the genus evolved from a common ancestor. The basic numbers  $x = 7$  and 8 evolved from a common predecessor, even though many of the clades are not well supported, thus confirming the data presented above. The higher basic numbers ( $x = 9, 10, 11$  and 13) form a poorly supported monophyletic clade (bootstrap value 57). It seems as if the higher numbers evolved independently from the lower numbers in at least two separate events. The basic numbers  $x = 9$  and 10 forms a polytomy in the higher clade and seems to be the bridge from the lower to the higher numbers or *vice versa* (Figure 5.6). Because none of the  $x = 9$  or 10 taxa are well resolved, this group might be a recent group. The low level of variation in these two basic numbers indicates that evolution was recent and that these numbers have not evolved into two definite clades.

For this revision, a median-joining network (Bandelt *et al.*, 1999) was constructed with the software NETWORK 4.6.1.0 (Fluxus Technology, 2012). Two separate networks were drawn, one from the *ITS* data generated by Hamatani *et al.* (2008) (Figure 5.7), and the other from 43 *trnL-F* sequences generated by Spies (2004) (Figure 5.8). The *trnL-F* network suggests that  $x = 11$  and  $x = 8$  have evolved independently from a common ancestor, and that  $x = 9$  and 10 could have evolved from any one of these two numbers. The *ITS* network (Figure 5.7) could not confirm or disprove this, due to the lack of  $x = 10$  species and the inclusion of only a single  $x = 9$  species. Both the networks support a close relationship between the  $x = 7$  and 8 groups. The cross-ability success rate of 33% between basic  $x = 10$  and basic  $x = 8$  (Table 5.2) could be a confirmation of the possible bridge between  $x = 7$  and 8 and the higher numbers. The *ITS* network also supported the relationship between *L. mutabilis* and *L. punctata* (Figure 5.8) and the *trnL-F* network positioned *L. pusilla* in an ancestral position to  $x = 7$  and 8, thus supporting the molecular cytogenetic data.

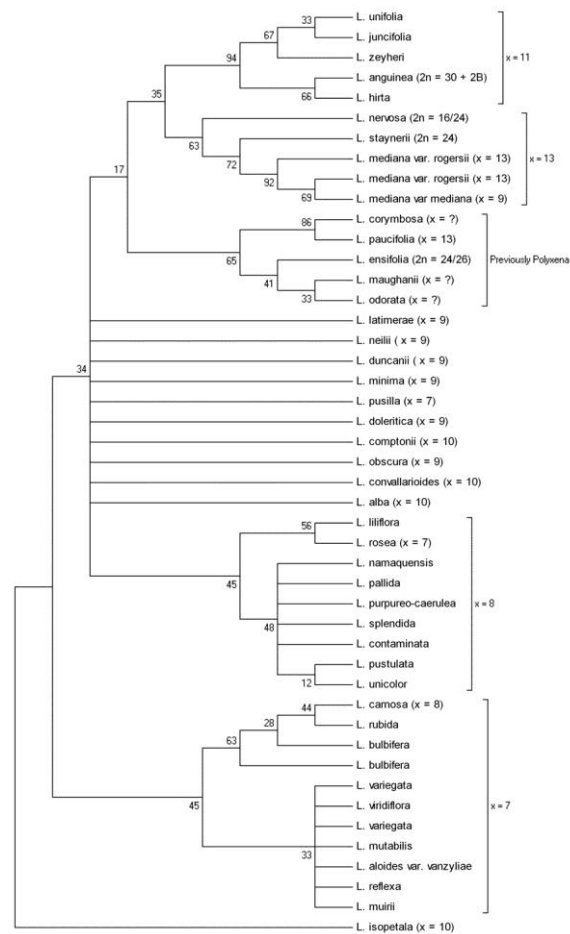


**Figure 5.6** Evolutionary relationships of 43 taxa based on the *trnL-F* region (Spies, 2004), inferred using the Maximum Likelihood option of MEGA 5 (Tamura *et al.*, 2011). Synonyms (Duncan, 2012): *L. aloides* var. *vanzyliae* = *L. vanzylliae*; *L. bulbifera* = *L. bifolia*; *L. ensifolia* = *L. ensifolia* subsp. *ensifolia*; *L. maughanii* = *L. ensifolia* subsp. *maughanii*; *L. mediana* var. *mediana* = *L. mediana* subsp. *mediana*; *L. mediana* var. *rogersii* = *L. mediana* subsp. *rogersii*; *L. pustulata* = *L. pallida*; *L. rubida* = *L. punctata*; *L. unicolor* = *L. pallida*.

Dysploidy (through the fusion of acrocentric chromosomes at the centromere to form larger metacentric to submetacentric chromosomes) has been shown to be important in the chromosomal evolution of other plant families, e.g. the *Commelinaceae* (Jones, 1976). If dysploidy is the mode of speciation in *Lachenalia*, a study on the chromosome morphology of species with higher basic chromosome numbers compared to lower basic chromosome numbers could assist in confirming the hypotheses. A study of *L. latimerae* ( $x = 9$  according to Hamatani *et al.* (2007)) indicated that this species has three large chromosomes, of which two are very similar, with the third one having a satellite (Hamatani *et al.*, 2007). The chromosome morphology thus supports the theory of dysploidy, but it must be further investigated using chromosome banding techniques. A second hypothesis is the possibility that *L. latimerae* could have originated from a hybridization event (Hamatani *et al.*, 2007)

between  $x = 7$  and  $x = 11$ , resulting in a gametic number of  $n = 18$ . If this theory is correct for other  $x = 9$  species, it would be expected that at least some of the  $x = 9$  species should group with either  $x = 7$  or  $x = 11$  in the chloroplast cladogram. All of the  $x = 9$  species fall between the  $x = 7/8$  groups and the higher numbers, but because the *trnL-F* cladogram (Figure 5.6) is not supported with high bootstrap values, neither the dysploid theory nor the hybridization theory could be proven. The *trnL-F* median-joining network (Figure 5.8) is inconclusive in this matter, since the evolutionary direction for  $x = 9$  can be from either  $x = 11$  or  $x = 7/8$  or both (thus hybridization).

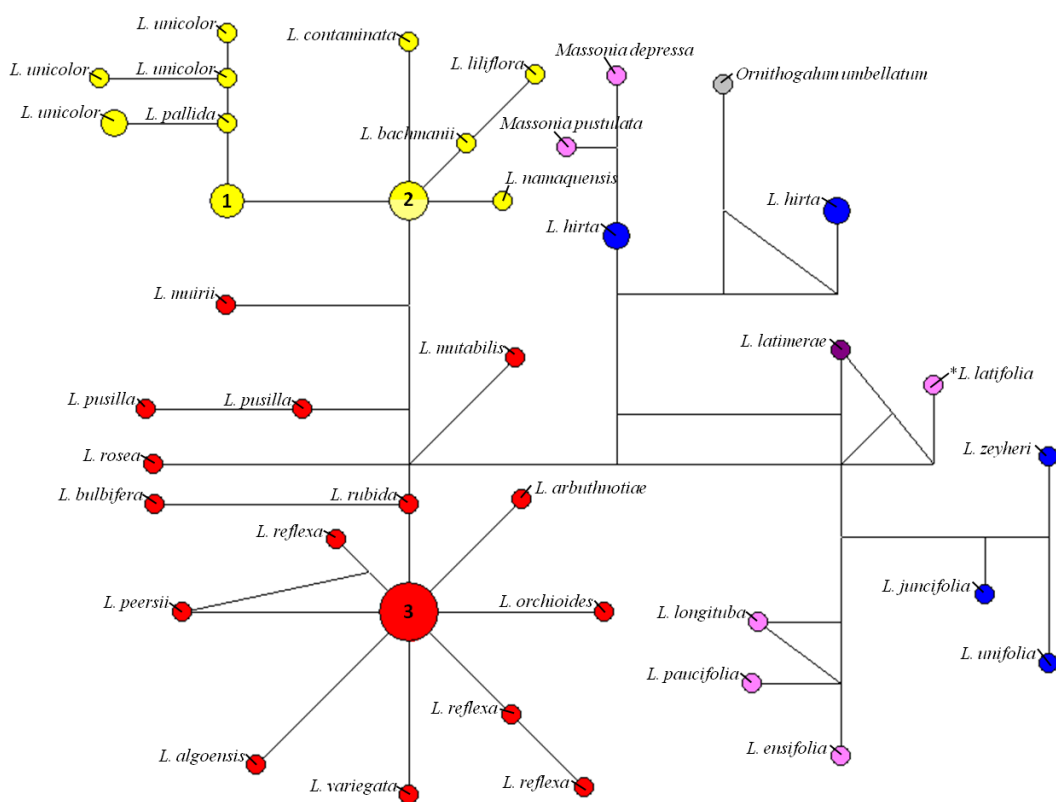


**Figure 5.7** Network of *Lachenalia* species based on ITS data using NETWORK 4.6.1.0 (Fluxus Technology, 2012). The correct current citation of *L. latifolia* (indicated with \*) is *L. nervosa*. Synonyms for species in the network (Duncan, 2012): *L. bulbifera* = *L. bifolia*; *L. ensifolia* = *L. ensifolia* subsp. *ensifolia*; *L. orchioides* = *L. orchioides* subsp. *orchioides*; *L. rubida* = *L. punctata*; *L. unicolor* = *L. pallida*. Colour codes: Red,  $x = 7$ ; Yellow,  $x = 8$ ; Blue,  $x = 11$ ; Purple,  $2n = 24/26/28$ ; Grey,  $x =$  unknown. Node 1, *L. pallida* and *L. purpureo-caerulea*; Node 2, *L. carnosae* and *L. splendida*; Node 3, *L. aloides* var. *aloides*, *L. aloides* 'Pearsonii', *L. aloides* var. *luteola*, *L. vanzyliae*, *L. quadricolor*, *L. flava*, *L. viridiflora*, *L. orchioides* subsp. *orchioides* and *L. longibracteata*.

The group  $x = 11$  is very well supported with a bootstrap value of 94 in the *trnL-F* cladogram (Figure 5.6), suggesting strong unity within this group. The close relationship



within this group is also supported by the morphological cladogram constructed by Duncan (2005), even though these species do not form a monophyletic group. The evolution of  $x = 11$  is not clear, but from the cladograms obtained in the different studies, i.e. morphological (Duncan *et al.*, 2005), *ITS*-based (Hamatani *et al.*, 2008) and *trnL-F*-based (Spies, 2004),  $x = 11$  (and  $x = 13$ ) is basal to the lower numbers and it seems that species with  $x = 11/13$  is the intermediate between the outgroup species (which have higher numbers) and the lower numbers in the genus. The network drawn from the *ITS* sequences provides evidence of the link between the higher basic numbers in *Lachenalia* and outgroup species used in this study. The outgroup for the *ITS* network (Figure 5.7) is *Massonia* and *Ornithogalum umbellatum*. The latter species has a high degree of cytogenetical variation (Czapic, 1968) with numbers of  $2n = 18-30$  and B-chromosomes reported. Hamatani *et al.* (2008) obtained the *ITS* sequences for *L. hirta* ( $x = 11$ ) by cloning the maternal and paternal genomes. One genome was cloned in some specimens and seems to have evolved from *Massonia*, while the other genome has evolved from *Ornithogalum*. This may be the reason why different specimens form two different nodes in the network.

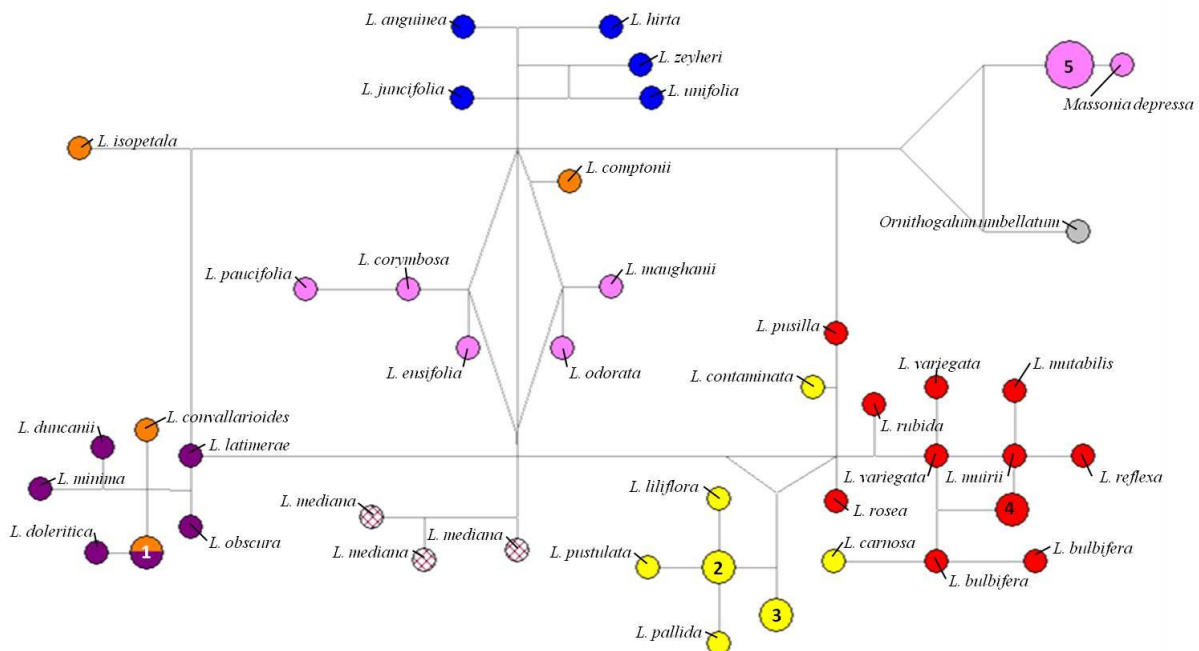


**Figure 5.8** Network of *Lachenalia* species based on *trnL-F* data using NETWORK 4.6.1.0 (Fluxus Technology, 2012). Synonyms for species in the network (Duncan, 2012): *L. bulbifera* = *L. bifolia*; *L. ensifolia* = *L. ensifolia* subsp. *ensifolia*; *L. maughanii* = *L. ensifolia* subsp. *maughanii*; *L. mediana* = *L. mediana* subsp. *mediana*; *L. pustulata* = *L. pallida*; *L. rubida* = *L. punctata*. Colour codes: Red, x = 7; Yellow, x = 8; Blue, x = 11; Light purple, 2n = 24/26/28; Dark purple, x = 9; Orange, x = 10; Diagonal crosses, x = 9 or 13; Grey, x = unknown. Node 1, *L. neilii*; *L. alba*; Node 2, *L. purpureo-caerulea*; *L. pallida*; Node 3, *L. namaquensis*; *L. splendida*; Node 4, *L. viridiflora*; *L. vanzyliae*; Node 5, *Massonia pustulata*; *M. depressa*; *M. echinata*; *M. jasminiflora*.

#### 5.5.4 Existence of different basic chromosome numbers

The evolution and even existence of certain chromosome numbers (such as  $x = 5, 6, 12, 13$  and  $15$ ) have not been investigated and confirmed to the same extent as  $x = 7, 8, 9$  and  $11$ . With basic chromosome numbers of  $5, 6, 7, 8, 9, 10, 11, 12, 13$  and  $15$  recorded, it is still speculated whether basic numbers of  $x = 5, 6, 10, 12, 13$  and  $15$  exists.

There are very few reports for  $n$  or  $x = 5$  in *Lachenalia*, and usually when  $x = 5$  has been reported for a species, it was based on only one accession. Both *L. violacea* and *L. aloides* are  $x = 7$  species, with a single  $2n = 15$  reported, indicating possible miss-counts in these species. *Lachenalia mutabilis* has chromosome counts of  $x = 5, 6$  and  $7$ . This is the only species where multiple counts have been recorded for all three these numbers. This species is morphologically distinct and wrong identification could not explain the differences in counts. All reports for  $x = 5$  for *L. mutabilis* are from the same geographical distribution area (Clanwilliam in the Western Cape Province), but there are also reports of  $x = 7$  from Clanwilliam. Other species from the Clanwilliam district include  $x = 7$  (*L. elegans* var. *sauveolens*, *L. thomasiae* and *L. violacea*);  $x = 8$  (*L. pallida*);  $x = 10$  (*L. marginata* and *L. undulata*) and  $x = 11$  (*L. hirta* and *L. unifolia*). It was suggested that the three basic numbers for *L. mutabilis* form an aneuploidy series (Spies *et al.*, 2000), but there is no proof of what attributed to the chromosome diversity in this species. Based on molecular systematics, *L. mutabilis* specimens always group with other  $x = 7$  species, regardless of their chromosome number (Spies, 2004; Hamatani *et al.*, 2008), are karyotypically similar to *L. punctata* ( $x = 7$ ) and has the highest number of  $x = 7$  counts recorded, thus supporting the theory of an aneuploid series in the species.

Johnson & Brandham (1997) studied the karyotypes of  $x = 7-13$  and  $15$ , and reported that all the species studied formed structural diploids and thus concluded that  $2n = 20$  represents a diploid based on  $x = 10$  rather than a tetraploid based on  $x = 5$ . These authors did state that  $2n = 30$  ( $x = 15$ ) could be an allotetraploid derived from taxa with  $x = 7$  and  $8$ , following hybridization and doubling of the chromosome number. Considering this theory, it would be expected that  $x = 10$  taxa have a phylogenetic grouping either with  $x = 7$  or  $x = 8$  taxa, but this has not been observed in the *trnL-F* cladogram (Spies, 2004). The fact that the cross-ability between  $x = 10$  and  $8$  is relatively high could be an indication of the validity of this

theory. The existence of the basic number  $x = 10$ , however, seem to be a reality, proven by the fact that some species have chromosome counts of  $2n = 20, 40$  (*L. alba*) and  $2n = 30, 40$  (*L. isopetala* – not grouped in this study) indicating the existence of polyploids. Considering all the evidence, it is still not clear whether  $x = 5$  exist in any species other than *L. mutabilis*.

Reports for six species with either  $x = 6$  or  $2n = 24$  were mostly based on only one accession and differed from the majority number of counts for these species. *Lachenalia nervosa* has counts of  $n = 8$  and  $2n = 24$ , indicating that this species has a basic number of  $x = 8$  and have a triploid somatic number. *Lachenalia stayneri* is also  $2n = 24$ , and the lack of meiotic studies in this species may lead to the conclusion that this species represents a tetraploid based on  $x = 6$  or also a triploid with  $x = 8$ . Therefore,  $x = 6$  should also be considered as a basic number. Based on *trnL-F* sequences, both these species indicate close relations with *L. mediana* ( $x = 9$  and  $13$ ) and do not group with  $x = 8$  (Spies, 2004). Consequently, species with  $2n = 24$  cannot be considered as “typical”  $x = 8$  species, and might even be considered as being wrong counts based on  $x = 13$ . None of the  $2n = 24$  species have their own monophyletic grouping and it seems as if  $x = 6$  does not exist except perhaps in *L. mutabilis*.

Somatic counts of  $2n = 28$  and  $56$  have been reported by several authors (Moffett, 1936; De Wet, 1957; Crosby, 1986; Hancke & Liebenberg, 1990; Johnson & Brandham, 1997; Hamatani *et al.*, 1998, 2007; Kleynhans & Spies, 1999; Spies *et al.*, 2009; 2002; 2008), but it has not been proven whether the basic chromosome number of  $x = 14$  exists. Somatic numbers of  $2n = 28$  as sole chromosome number have been reported for *L. cernua* and *L. longituba*. Both these species were included in the basic group of  $x = 7$  for the purpose of this review, but additional accessions of these species, as well as meiosis and cytomorphological data will have to be studied to determine the actual basic chromosome number.

#### 5.5.5 Existence of hybrid species

The question of natural hybridization in the genus has been raised several times. Both the morphological and *trnL-F* based cladograms had “mixed” monophyletic groups with  $x = 6, 7, 8, 9, 10$  and  $13$ . Spies (2004) concluded that hybridization might have played a role in speciation, but it was not proven.

Some species (*L. pusilla*, *L. rosea* and *L. carnosa*) do not follow the rule of grouping into monophyletic groups with similar chromosome numbers (Figure 5.6). Considering the positions of these species in the networks drawn (Figures 5.7 & 8), the first two species are intermediate to the  $x = 7$  and  $x = 8$  groups in both networks. The position of *L. carnosa* ( $x = 8$ ) fluctuate between  $x = 7$  (Figure 5.8) and  $x = 8$  (Figure 5.7). Within the *trnL-F* cladogram, *L. carnosa*, *L. punctata* and *L. bifolia* is a sister clade with the rest of the  $x = 7$  species. *Lachenalia punctata* is intermediate to  $x = 7$  and 8 in both networks. Based on karyotypic and molecular data, some species are intermediate between  $x = 7$  and 8, and can either be considered as predecessor species or as hybrid species.

*Lachenalia carnosa* ( $x = 8$ ) is an example of a possible hybrid species, grouping with either  $x = 7$  or 8, depending on the type of sequencing data (nuclear or cytoplasmic). Spies (2004) reported what seemed to be B-chromosomes in the meiotic divisions of *L. carnosa*, which may have been unidentified univalents, also observed in cultivated *Lachenalia* hybrids (Hancke & Liebenberg, 1998). Cross-ability data, however, strongly links *L. carnosa* with other members of the  $x = 8$  group, with successful crossing with at least five different  $x = 8$  species (data not shown), producing regular meiosis with 8 bivalents (Du Preez *et al.*, 2002) as well as fertile hybrids. Natural hybridization may be present in the genus *Lachenalia* but this should be investigated further.

## 5.6 CONCLUSION

This review accentuates the complex nature of the genus *Lachenalia*. Besides the extensive morphological variation that complicates the taxonomy of the genus, the genus is also exceptionally diverse in chromosome numbers. *Lachenalia* has different basic chromosome numbers ( $x = 5, 6, 7, 8, 9, 10, 11, 12, 13$  and 15 reported in literature), contains polyploidy (ranging from triploids to octoploids), and includes B-chromosomes. Chromosome counts for the 89 species reported in literature varied from  $2n = 10$  to 56 and from  $n = 5$  to 28. Polyploidy was reported in 19 taxa (23%), and is most common in the  $x = 7$  group.

The low cross-ability (only 18% successful inter-species crosses) confirms this variation and stresses the importance of investigating the variation in order to develop breeding strategies to overcome the existing barriers to crossing. Morphological and molecular

phylogenetic studies confirm the complexity of the genus, but also assisted in the process of drawing some conclusions on the relationship between different species within the genus and the possible evolutionary history of the genus.

Phylogenetic studies have assisted in clarifying the phylogenetic position of *Lachenalia* in relation to other genera (Pfosser & Speta, 1999; Pfosser *et al.*, 2003; Manning *et al.*, 2004) and placed the genus within the Asparagaceae family (Angiosperm Phylogeny Group, 2009). Morphological (Duncan *et al.*, 2005, 2012) and phylogenetic studies within the genus (Spies, 2004; Hamatani *et al.*, 2008) have supported the inclusion of *Polyxena* in *Lachenalia*, and this inclusion increased the number of recognised *Lachenalia* species to 133.

Molecular studies on the *trnL-F* as well as *ITS* gene regions revealed monophyletic groupings of species containing the same basic chromosome numbers. This indicated a strong correlation between the phylogeny and basic chromosome numbers in the genus, although there were some exceptions in the larger *trnL-F* data set (Spies, 2004). The good correlation between basic chromosome numbers and phylogenetic groupings could in future assist to confirm basic numbers for species. The improved cross-ability when crosses were made between individual species within the same phylogenetic groupings confirms the phylogeny. Phylogenetic groupings thus have to be taken into account when crossing combinations are planned, to achieve better crossing success rates in the breeding programme.

When comparing the different studies, *Lachenalia* might have evolved from a common ancestor and the two largest basic chromosome number groups,  $x = 7$  and 8 have evolved from a common predecessor. Previous studies (Spies, 2004; Kleynhans *et al.*, 2009) also indicated a close relationship between these two basic numbers, which is supported by high success rates in cross-ability between these two groups. It seems as if the higher basic numbers ( $x = 9, 10, 11$  and 13) evolved independently from the lower numbers and that basic numbers  $x = 9$  and 10 could be the bridge from the lower to the higher numbers or *vice versa* (Figure 5.6), but evidence of this is not conclusive (Figures 5.7 & 8).

Dyploidy and hybridization might be the modes of speciation in some *Lachenalia* species but this could not be proven with molecular data and further studies are required to draw conclusions. The existence of some of the basic chromosome numbers reported in the

past (such as  $x = 5, 6, 10, 12$  and  $15$ ) can be disputed. Only a few species can be linked to  $x = 5$  and  $6$  and it is possible that these two basic numbers only exist as part of an aneuploid series in the species *L. mutabilis*. Further studies on species from these disputed basic chromosome numbers is needed to confirm the existence of all the reported numbers.

This review indicates that different genetic studies on *Lachenalia* generally result in similar results and stresses the importance of assessing the variation within complex genera, to aid in decisions around breeding programme strategies. It is clear that inter-species crosses within phylogenetic groups in the genus can improve the success rate of crossing combinations, but there are still many questions that remain unanswered. Further multidisciplinary studies are needed in the genus *Lachenalia* to solve the evolutionary history of this complex genus, to answer questions around species placement and the existence of basic chromosome number groups and to overcome crossing barriers.

## 5.7 STATEMENT OF RESEARCH QUESTIONS

Based on this literature review the following research questions should be addresses in this thesis:

6. How effective can DNA-barcoding regions be applied in the genus *Lachenalia*? This question will be answered by investigating the genetic variation within several *Lachenalia* species by comparing five different gene regions (*psbA-trnH*, *trnL-F*, *matK*, *atpH-I* and *ITS2*). This will be discussed in Chapter 6.
7. What are the influences of polyploidy on DNA-sequences? The specific focus will be on SNPs and INDELS in the sequences of the “ploidy rich” *L. bifolia*. These results will be compared to diploid species to determine if differences in DNA-sequences are only limited to specific ploidy complexes (Chapter 6).
8. What is the extent of inter- and intraspecific variation in *Lachenalia*? What are the phylogenetic relationships within the genus and how does that contribute to our knowledge on the phylogeographical and cytogeographical evolution in *Lachenalia* (Chapter 6)?

Part C: *Lachenalia*

*Chapter 6*

A comparison of the efficiency of DNA-  
barcoding regions in *Lachenalia*



## ABSTRACT

*Lachenalia* is endemic to South Africa and Namibia and consists of 133 species, many of which are under threat due to developments along the western and southern coastal areas. This genus is highly variable (morphological and cytogenetical), and although a key and new classification has recently been suggested, the degree of genetic diversity in the genus has not been studied intensively. The need exists to generate a barcode, in addition to the morphological key, and this is a preliminary study to determine the range of intraspecific diversity and to establish an effective barcode system for *Lachenalia*. A selection of four focus species i.e. *L. mediana*, *L. unifolia*, *L. bifolia*, *L. punctata*, each represented by seven to sixteen samples each, has been chosen to represent different chromosome numbers and different ploidy levels in the genus. The gene regions chosen for the barcoding study were *atpH-I*, *matK*, *trnH-psbA*, *rbcL*, *rpL16*, *trnT-L*, *trnL-F* and *ITS2*. The tree-based, distance-based and character-based analyses have been used to determine the efficacy of these different gene regions in a barcoding analysis. Putative hybridization events (ancient or recent) resulted in false positives and the chloroplast and nuclear DNA should be represented in the barcode. For effective barcoding of the entire genus, at least a two to three region barcode should be used. The most promising regions to identify *Lachenalia* species are *matK*, *trnL-F*, *ITS2* and *atpH-I* (when combined with either *ITS2* or *trnL-F*). The Bayesian Inference (BI) tree-based method proved to be the most effective tool to identify an unknown *Lachenalia* specimen.

## PREFACE

This chapter has been presented at the tenth conference of the SASSB (Southern African Society for Systematic Biology) and will be submitted for publication as a barcode data release paper.

## 6.1 INTRODUCTION

### 6.1.1 The need for DNA-barcoding in *Lachenalia*

*Lachenalia* grows in the winter rainfall areas of southern Africa, where an increasing number of species are under threat due to agricultural and commercial developments which lead to the destructions of entire plant populations (Duncan, 2003, 2012). Taxonomical revisions of genera are an important aspect of conservation assessments where a minimum of



15 herbarium specimens can provide enough data to provide 95% accuracy of geographical distribution range estimates. With five or less specimens this percentage of accuracy lowers to less than 10%. Despite a recent monograph of the genus (Duncan, 2012), 25% of the *Lachenalia* species have less than five specimens deposited in herbaria and *Lachenalia* is therefore considered as the genus with the highest priority for revision in its family (von Staden *et al.*, 2013).

The flowers of the majority of the *Lachenalia* species are self-incompatible and fertilization relies mainly on pollen dispersal by wind, insects and sunbirds. The seeds are dispersed by the wind in a very small number of species from arid regions. Most of the species do not have a natural mechanism for seed dispersal, and the seeds will germinate in close proximity to the parental plant (Duncan, 2012). In such taxa the populations would, to a degree, be isolated from each other. A mutation in a chloroplast gene region will slowly be spread throughout the population. This mutation will also be limited to a single population of a species, and will not spread to other populations of the same species due to isolation by distance. Chloroplast DNA regions in species that consist of isolated populations where intraspecific gene flow is limited, will not be suitable as barcodes. The nuclear genes are distributed via pollen over a broader geographical range and will have a higher gene flow, since it is inherited by both pollen and seeds (Wang *et al.*, 2011). Monophyly of different populations of a species will be reached more rapidly for a chloroplast gene region than for a nuclear region. The disadvantage of a nuclear DNA-barcode is that interspecific pollination can lead to “fussy” borders between species and only ‘species groups’ may be identified (Petit & Excoffier, 2009; Hollingsworth *et al.*, 2011).

There seems to be two major modes of speciation involved in the evolution of *Lachenalia*, i.e. allopatric speciation and polyploidization. Allopatric speciation resulted because the large mountain ranges such as the Cederberg, Piketberg, Koue Bokkeveld, Olifantsrivierberge and Bokkeveld Mountains disrupted gene flow. High levels of morphological diversity occur in the arid regions (Worcester-Robertson Karoo, Little Karoo, Ceres Karoo, Bokkeveld Plateau, Knersvlakte and central Namaqualand), semi-arid regions (Olifantsrivier and Tulbach Valleys), as well as the coastal areas around the Cape Peninsula (Duncan, 2012). Internal and external crossing barriers (Kleynhans *et al.*, 2009) may have

contributed to further sympatric speciation of species that grow in overlapping geographic areas or in close proximity to each other.

Polyploidy occurs in 40–70% of plant species and contributes to speciation in many plants (Stebbins, 1971; Otto & Whitton, 2000; Husband, 2000; Ramsey & Schemske, 2002). Different ploidy levels have been observed in many *Lachenalia* species with various basic chromosome numbers, but especially in species with a basic chromosome number of  $x = 7$  (see Chapter 5). Four types of polyploid events could have contributed to speciation in *Lachenalia*, i.e. autopolyploidy (the chromosome numbers within a species are doubled), allopolyploidy (hybridization of unrelated taxa are followed by chromosome doubling), auto-allopolyploidy (a mixture of auto- and allopolyploidy) and segmental allopolyploidy (hybridization between related taxa) (Stebbins, 1971; Goldblatt, 1980; Grant, 1981). The latter three types are known as hybrid speciation and are much more prevalent in plants than expected (Mallet, 2007). Different diploid parental populations ensure an introduction of increased genetic variation to the polyploids (Soltis & Soltis, 2009). This sometimes leads to problems with DNA-barcoding since the hybrid polyploid species will contain similar (or the same) plastid genome than the parental diploid. In particular if the polyploidization event was relatively recent, DNA-barcoding may not be able to distinguish between the diploid lineage and polyploid progeny (Cowan & Fay, 2012).

Many *Lachenalia* species, especially those with similar chromosome numbers, cross readily in cultivation (Kleynhans *et al.*, 2009) and it is possible that some *Lachenalia* species evolved through hybrid speciation. The implication is that a species derived from hybrid speciation, whether it is recent or ancient, may give conflicting result between the chloroplast and nuclear DNA regions. Because the chloroplast is mostly maternally inherited in Angiosperms (Hollingsworth *et al.*, 1999), an unknown sample will relate to the maternal contributor when using a chloroplast DNA-barcode. Conversely, the *nrITS* gene family can produce one of three different patterns in hybrid species or in polyploids. First, an occurrence of homogenization to the maternal or paternal type may be observed, meaning that only the maternal or paternal copies will occur in the offspring and it may differ between lineages (Wendel *et al.*, 1995). Second, both the maternal and paternal copies may be maintained in the offspring (Álvarez & Wendel, 2003) and this creates the need for cloning to obtain pure

sequences (Hamatani *et al.*, 2008). Lastly, there may be a formation of a chimeric mixture (Álvarez & Wendel, 2003). Depending on the *nrITS* inheritance pattern, there may be congruence between the chloroplast and nuclear DNA-barcode regions in *Lachenalia*. The previously mentioned three factors may give an underestimation of allopolyploid speciation and a low-copy nuclear gene region such as the *LEAFY* second intron may be a better region to use for barcoding in genera where allopolyploid speciation are prevalent (Kim *et al.*, 2008). The genus *Lachenalia* comprises of 133 species and 139 taxa (Duncan, 2012). With a high number of species in a genus, there is a high probability that there was at least one recent speciation event and genetic data alone may be insufficient to delimit species (Puillandre *et al.*, 2011).

The time of divergence between species, how closely species are related, as well as the mode of speciation will have an effect on the DNA-barcoding and the type of analysis to use. It can be problematic to generate barcodes for closely related and recently diverged species (von Cräutlein *et al.*, 2011). Species resulting from hybrid speciation will contain more than one copy (different copies) of the nuclear DNA. Different copies will result in polymorphisms in the DNA-barcodes and may lead to amplification and sequencing problems. Because chloroplast markers are maternally inherited, these allopolyploid species may not be discernible with plastid DNA-barcodes, unless DNA regions with a high mutation rate are used (Heinze, 2007). Hybridization or hybrid speciation can lead to ‘plastid capture’, where the progeny contains the nuclear genome of the paternal species and the plastid of the maternal species, but, through backcrossing, it morphologically resembles the paternal species. This will result in wrong identification of an unknown specimen (Cowan & Fay, 2012).

Nock *et al.* (2011) suggested that the entire chloroplast genome should be used as a single-locus plant barcode for identification of plants. The use of the entire chloroplast will not only improve delimitation of species, but it should also have wide application in plant biotechnology and aid in the implementation of intellectual property rights of plant breeders. It would be ideal to implement whole chloroplast genome studies on *Lachenalia* and other genera, but financial- and time constraints do not currently allow for such an elaborate study.

Very few *Lachenalia* sequences are available on NCBI, GenBank (Table 6.1), resulting in a deficiency of publicly available *Lachenalia* sequence data. The gene regions chosen for the

barcoding study on *Lachenalia* are the chloroplast regions *atpH-I*, *matK*, *trnH-psbA*, *rbcLa*, *rpL16*, *trnT-L*, *trnL-F* and the nuclear region *ITS2*.

**Table 6.1** Availability of different barcoding markers of the genera *Lachenalia* and *Polyxena* (now a subgenus of *Lachenalia*) (accessed June 2013)

| Marker           | Genomic source | Type                              | Data on GenBank      |                      |                                                          |
|------------------|----------------|-----------------------------------|----------------------|----------------------|----------------------------------------------------------|
|                  |                |                                   | Number of accessions | Data for both genera | *Number of <i>Lachenalia</i> ( <i>Polyxena</i> ) species |
| <i>nrITS1</i>    | Nuclear        | Transcribed spacers and 5.8S gene | 54                   | Yes                  | 51 (3)                                                   |
| <i>nrITS2</i>    | Nuclear        | Transcribed spacer                | 0                    | 0                    | 0                                                        |
| <i>matK</i>      | Plastid        | Protein coding                    | 3                    | Yes                  | 3 (1)                                                    |
| <i>rbcLa</i>     | Plastid        | Protein coding                    | 4                    | No                   | 4                                                        |
| <i>trnH-psbA</i> | Plastid        | Intergenic spacer                 | 0                    | 0                    | 0                                                        |
| <i>trnL-F</i>    | Plastid        | Intron and intergenic spacer      | 12                   | Yes                  | 12 (1)                                                   |
| <i>trnT-L</i>    | Plastid        | Intergenic spacer                 | 0                    | 0                    | 0                                                        |
| <i>atpH-atpI</i> | Plastid        | Intergenic spacer                 | 0                    | 0                    | 0                                                        |
| <i>rpL16</i>     | Plastid        | Intron                            | 0                    | 0                    | 0                                                        |

\*Based on the names listed in GenBank

### 6.1.2 Aims and objectives

The aims of this study were to evaluate various DNA regions as putative DNA-barcoding regions in *Lachenalia*, through the following objectives:

1. The putative new regions for barcoding *Lachenalia* (*atpH-I*, *ITS2*, *trnH-psbA*, *rbcLa*, *rpL16* and *trnL-F*) will be evaluated by analysing levels of inter- and intraspecific variation in a selection *Lachenalia* species.
2. The data sets of all the regions will be aligned and then their efficacy will be tested by performing tree-based, distance-based and character-based analyses.
3. Datasets will be combined to determine the most effective two-locus barcode.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Materials

In many barcoding studies, including this study, it is unrealistic to include the suggested minimum of five representatives per species as suggested by Ross *et al.* (2008). This implies a total of 665 samples per gene region in order to represent the entire genus *Lachenalia*. Availability of material, time and financial constraints did not allow this number of samples. The alternative would be to sample singletons of all the species, which is unrealistic for a

barcoding study. Therefore, a core selection of *Lachenalia* species was made to represent the genus. The main focus was selecting species based on availability of material. This material included a species that consist of a polyploid complex (*L. bifolia*), *L. punctata* which is a close relative of *L. bifolia*, a species with a high basic chromosome number (*L. unifolia*) and species with different chromosome numbers reported (*L. mediana*). *Lachenalia schlechteri* has changed species status (*L. schlechteri* from 1904-1989), to variety status (*L. unifolia* var. *schlechteri*; from 1989-2012) and back to species status (*L. schlechteri*; 2012-present). When commencing with this study, *L. unifolia* var. *schlechteri* samples were included. The status later changed to species (Duncan, 2012). Thus, initially the *L. schlechteri* accessions in this study were believed to be a variety of *L. unifolia* and were therefore included. The samples for each species represent a large distribution range to consider the effect of geographical speciation on the barcodes.

Materials for this study were provided in the form of *Lachenalia* bulbs (supplied by the ARC-Roodeplaat and Summerfield's Indigenous Bulbs and Seed) and leaf material (supplied by SANBI) collected in a saturated NaCl-CTAB solution.

### 6.2.2 Methods

The *Lachenalia* leaves were dried in silica. The direct PCR approach was used where a 0.5 mm punch was removed from the leaf, added to the PCR mixture and the DNA amplified in a thermo cycler. This was followed with bi-directional sequencing, sequence editing, alignment (Appendix F) and analyses (refer to Chapter 2 for a complete description of the methods used in this study).

In addition to the focus species, a few other taxa (marked with different symbols in Table 6.2), were included in the tree, distance and character-based comparisons.

**Table 6.2** A list of the *Lachenalia* samples (focus species as well as additional *Lachenalia* species) used in the study with the collection/accession numbers, localities and approximate GPS coordinates. **Bold** collection numbers in brackets correspond with the collection numbers of Kleynhans & Spies (1999). *Italic* collection numbers in brackets correspond with the collection numbers of Mr. G. Duncan (SANBI)

| Species           | Gene regions sequenced                     | Collection / NCBI Accession number /               | Locality                                                              | Latitude | Longitude |
|-------------------|--------------------------------------------|----------------------------------------------------|-----------------------------------------------------------------------|----------|-----------|
| <i>L. aloides</i> | #ITS2                                      | <i>Spies 7059</i> <sup>2</sup>                     | Riebeeck-west, Western Cape                                           | -33.3645 | 18.85525  |
|                   | <i>trnL-F</i>                              | <i>Spies 7698</i> (282) <sup>1</sup>               | Malmesbury, Western Cape                                              | -33.4529 | 18.71515  |
|                   | <i>matK</i>                                | <i>JX090373.1</i>                                  |                                                                       |          |           |
| <i>L. bifolia</i> | <i>trnL-F</i>                              | <i>Spies 6932</i>                                  | Unknown                                                               |          |           |
|                   | <i>atpH, ITS2, matK, trnH-psbA</i>         | <i>Spies 7064</i> <sup>2</sup> ( <b>E1</b> )       | Pearly beach, Western Cape                                            | -34.6569 | 19.48814  |
|                   | <i>atpH, ITS2, matK, trnH-psbA, trnL-F</i> | <i>Spies 7322</i> <sup>2</sup> ( <b>C4</b> )       | Zeekoevlei, Western Cape                                              | -34.0599 | 18.51504  |
|                   | <i>trnL-F</i>                              | <i>Spies 7720</i> (90)                             | Saldanha, Western Cape                                                | -33.4535 | 18.70794  |
|                   | <i>atpH, ITS2, matK</i>                    | <i>Spies 8893</i> <sup>2</sup> ( <b>A1</b> )       | Leipoldtville district, Western Cape                                  | -32.2238 | 18.47957  |
|                   | <i>matK</i>                                | <i>Spies 8895</i> <sup>2</sup> ( <b>B2</b> )       | Langebaan Nature Reserve, Western Cape                                | -33.0869 | 18.03366  |
|                   | <i>matK</i>                                | <i>Spies 8896</i>                                  | Atlantis, Western Cape                                                | -33.568  | 18.483    |
|                   | <i>atpH, ITS2, matK, trnH-psbA</i>         | <i>Spies 8898</i> <sup>2</sup> ( <b>C2</b> )       | Bloubergstrand, Western Cape                                          | -33.7956 | 18.46208  |
|                   | <i>matK, trnH-psbA</i>                     | <i>Spies 8899</i> <sup>2</sup> ( <b>D3</b> )       | Between Betty's Bay and Heroldt Porter Botanical Garden, Western Cape | -34.347  | 18.92067  |
|                   | <i>atpH, matK, trnH-psbA</i>               | <i>Spies 8900</i> <sup>2</sup> ( <b>D2</b> )       | Palmiet river mouth , Western Cape                                    | -34.344  | 18.99118  |
|                   | <i>atpH, ITS2, matK, trnH-psbA</i>         | <i>Spies 8902</i> <sup>2</sup> ( <b>E2</b> )       | At Elim-Bredasdorp-Struisbaai crossing, Western Cape                  | -34.5696 | 20.04931  |
|                   | <i>atpH, ITS2, matK, trnH-psbA, trnL-F</i> | <i>Spies 8903</i> <sup>2</sup> ( <b>E3</b> )       | Struisbaai, Western Cape                                              | -34.806  | 20.04686  |
|                   | <i>ITS2, matK, trnH-psbA</i>               | <i>Spies 8904</i> <sup>2</sup> ( <b>F1 or F3</b> ) | Grootjongensfontein beach, Western Cape                               | -34.4323 | 21.33336  |
|                   | <i>atpH, ITS2, matK, trnH-psbA</i>         | <i>Spies 8905</i> <sup>2</sup> ( <b>F2</b> )       | Stilbaai, Western Cape ( <b>Received as seed</b> )                    | -34.3624 | 21.43393  |
|                   | <i>ITS2, matK, trnH-psbA</i>               | <i>Spies 8906</i> <sup>2</sup> ( <b>F4</b> )       | Stilbaai, Western Cape                                                | -34.3624 | 21.43393  |
|                   | <i>ITS2, matK, trnH-</i>                   | <i>Spies 8907</i> <sup>2</sup> ( <b>G1</b> )       | Gouritz River Mouth, Western Cape                                     | -34.3429 | 21.88655  |

|                                             |                              |                                                               |                                                    |          |          |
|---------------------------------------------|------------------------------|---------------------------------------------------------------|----------------------------------------------------|----------|----------|
|                                             | <i>psbA</i>                  |                                                               |                                                    |          |          |
|                                             | <i>matK</i>                  | <i>Spies 8909</i>                                             | Elandsbaai                                         | -32.2988 | 18.35839 |
|                                             | <i>ITS2, matK</i>            | <i>Spies 9115</i>                                             | Unknown                                            |          |          |
|                                             | <i>ITS2, trnH-psbA</i>       | <i>Spies 9116</i>                                             | West coast, Western Cape                           | -33.1353 | 18.17093 |
| *                                           | <i>ITS2</i>                  | <i>AB304980.1</i>                                             |                                                    |          |          |
| <i>L. carnosa</i>                           | <i>trnL-F</i>                | <i>Spies 7723 (185)<sup>1</sup></i>                           | Kamieskroon                                        | -30.222  | 17.92073 |
| *                                           | <i>ITS2</i>                  | <i>AB439269.1,</i><br><i>AB439270.1,</i><br><i>AB439271.1</i> |                                                    |          |          |
|                                             | <i>matK</i>                  | <i>HM640625.1</i>                                             |                                                    |          |          |
| <i>L. flava</i>                             | <i>#ITS2, trnL-F</i>         | <i>Spies 7060<sup>2</sup></i>                                 | Bainskloof, Western Cape                           | -33.6139 | 19.10028 |
|                                             | <i>trnL-F</i>                | <i>Spies 7699 (363)<sup>1</sup></i>                           | Bainskloof, Western Cape                           | -33.6139 | 19.10028 |
| <i>L. framesi</i>                           | <i>trnH-psbA, matK</i>       | <i>Spies 8241<sup>3</sup></i>                                 | Komaggas, Northern Cape                            | -29.7942 | 17.48589 |
| <i>L. hirta</i> *                           | <i>ITS2</i>                  | <i>AB304982.1</i>                                             |                                                    |          |          |
| *                                           | <i>ITS2</i>                  | <i>AB439272.1</i>                                             |                                                    |          |          |
| *                                           | <i>ITS2</i>                  | <i>AB439273.1</i>                                             |                                                    |          |          |
| *                                           | <i>ITS2</i>                  | <i>AB439274.1</i>                                             |                                                    |          |          |
| <i>L. mediana</i>                           | <i>trnL-F</i>                | <i>Spies 7780 (298)<sup>1</sup></i>                           | Kuilsrivier, Western Cape                          | -34.0275 | 18.69784 |
|                                             | <i>trnH-psbA</i>             | <i>Spies 9027<sup>2</sup></i>                                 | Unknown                                            |          |          |
|                                             | <i>atpH, matK, trnH-psbA</i> | <i>Spies 9030<sup>2</sup></i>                                 | Darling, Western Cape                              | -33.3824 | 18.38336 |
|                                             | <i>matK, trnH-psbA</i>       | <i>Spies 9031<sup>2</sup></i>                                 | Potsdam behind Killarney race course, Western Cape | -33.8111 | 18.52684 |
|                                             | <i>atpH, matK, trnH-psbA</i> | <i>Spies 9033<sup>2</sup></i>                                 | Hill outside Darling, Western Cape                 | -33.3824 | 18.38336 |
|                                             | <i>atpH, matK, trnH-psbA</i> | <i>Spies 9034<sup>2</sup></i>                                 | Unknown                                            |          |          |
| <i>L. mediana</i><br>subsp. <i>rogersii</i> | <i>matK, trnL-F</i>          | <i>Spies 7079</i>                                             | Darling, Western Cape                              | -33.3869 | 18.39315 |
|                                             | <i>trnL-F</i>                | <i>Spies 7782</i>                                             | Porterville, Western Cape                          | -33.0029 | 18.98967 |
| <i>L. namaquensis</i>                       | <i>trnH-psbA</i>             | <i>Spies 6970<sup>2</sup></i>                                 | Springbok, Northern Cape                           | -29.6606 | 17.88294 |
| <i>L. orthopetala</i>                       | <i>trnL-F</i>                | <i>Spies 7807 (262)<sup>1</sup></i>                           | Durbanville, Western Cape                          | -33.8436 | 18.65055 |

|                    |                                    |                                      |                                        |          |          |
|--------------------|------------------------------------|--------------------------------------|----------------------------------------|----------|----------|
| <i>L. pallida</i>  | <i>trnH-psbA</i>                   | <i>Spies 8273</i> <sup>3</sup>       | Piketberg, Western Cape                | -32.8972 | 18.76597 |
|                    | <i>trnL-F</i>                      | <i>Spies 7809 (357)</i> <sup>1</sup> | Tulbagh, Western Cape                  | -33.2799 | 19.13644 |
|                    | <i>trnL-F</i>                      | <i>Spies 7823 (106)</i> <sup>1</sup> | Darling, Western Cape                  | -33.3824 | 18.38336 |
|                    | <i>trnL-F</i>                      | <i>Spies 7844 (346)</i> <sup>1</sup> | Dasklip Pass, Western Cape             | -32.9388 | 19.01422 |
|                    | <i>trnH-psbA, matK</i>             | <i>Spies 8284</i> <sup>3</sup>       | Unknown                                |          |          |
|                    | <i>trnH-psbA</i>                   | <i>Spies 8294</i> <sup>3</sup>       | Koringberg, Western Cape               | -33.0187 | 18.67415 |
| *                  | <i>ITS2</i>                        | <i>AB304998.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB304999.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB439277.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB439278.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB439279.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB439280.1</i>                    |                                        |          |          |
| *                  | <i>ITS2</i>                        | <i>AB439281.1</i>                    |                                        |          |          |
| <i>L. patula</i>   | <i>trnL-F</i>                      | <i>Spies 7363</i>                    | Unknown                                |          |          |
|                    | <i>trnL-F</i>                      | <i>Spies 7811</i>                    | Moedverloor, Northern Cape             | -30.3255 | 18.3165  |
|                    | <i>trnH-psbA</i>                   | <i>Spies 8276</i> <sup>3</sup>       | Knersvlakte, Western Cape              | -31.5872 | 18.48749 |
| <i>L. perryae</i>  | <i>trnL-F</i>                      | <i>Spies 7814</i>                    | Hex River Pass, Western Cape           | -33.3979 | 19.77018 |
|                    | <i>trnH-psbA, trnL-F</i>           | <i>Spies 9036</i> <sup>2</sup>       | Ceres, Western Cape                    | -33.3586 | 19.31728 |
|                    | <i>trnH-psbA</i>                   | <i>Spies 9039</i> <sup>2</sup>       | Matjiesfontein, Western Cape           | -33.2315 | 20.5821  |
|                    | <i>trnH-psbA</i>                   | <i>Spies 9040</i> <sup>2</sup>       | De Doorns, Western Cape                | -33.4824 | 19.66652 |
| <i>L. punctata</i> | <i>matK</i>                        | <i>Spies 7093</i> <sup>2</sup>       | Cape Town area, Western Cape           | -33.945  | 18.45554 |
|                    | <i>matK, trnL-F</i>                | <i>Spies 7344</i>                    | Unknown                                |          |          |
|                    | <i>trnL-F</i>                      | <i>Spies 7829 (91)</i> <sup>1</sup>  | Kommetjie, Western Cape                | -34.1448 | 18.33126 |
|                    | <i>atpH, ITS2, matK, trnH-psbA</i> | <i>Spies 8909</i> <sup>2</sup>       | Elandsbaai, Western Cape               | -32.3109 | 18.35006 |
|                    | <i>ITS2, trnH-psbA</i>             | <i>Spies 8910</i> <sup>2</sup>       | Jacobsbaai, Western Cape               | -32.9729 | 17.88449 |
|                    | <i>ITS2, matK, trnH-psbA</i>       | <i>Spies 8911</i> <sup>2</sup>       | Langebaan Nature Reserve, Western Cape | -33.0869 | 18.03366 |
|                    | <i>atpH, ITS2, matK, trnH-psbA</i> | <i>Spies 8914</i> <sup>2</sup>       | Hermanus, Fernkloof, Western Cape      | -34.3857 | 19.27686 |
|                    | <i>atpH, ITS2, matK,</i>           | <i>Spies 9042</i> <sup>2</sup>       | Strand, Western Cape                   | -34.1004 | 18.81746 |



|                       |                                    |                                |                                                                        |          |          |
|-----------------------|------------------------------------|--------------------------------|------------------------------------------------------------------------|----------|----------|
|                       | <i>trnH-psbA</i>                   |                                |                                                                        |          |          |
| *                     | <i>ITS2</i>                        | <i>AB305001.1</i>              |                                                                        |          |          |
| <i>L. pusilla</i>     | <i>matK</i>                        | <i>Spies 8896</i>              | Unknown                                                                |          |          |
|                       | <i>matK</i>                        | <i>Spies 8911</i>              | Piketberg, Western Cape                                                | -32.875  | 18.762   |
|                       | <i>matK</i>                        | <i> JX090375.1 </i>            |                                                                        |          |          |
| *                     | <i>ITS2</i>                        | <i>AB304996.1</i>              |                                                                        |          |          |
| *                     | <i>ITS2</i>                        | <i>AB304997.1</i>              |                                                                        |          |          |
| <i>L. quadricolor</i> | <i>#ITS2</i>                       | <i>Spies 7062<sup>2</sup></i>  | Darling, Western Cape                                                  | -33.3824 | 18.38336 |
| <i>L. reflexa</i> *   | <i>ITS2</i>                        | <i>AB439275.1</i>              |                                                                        |          |          |
| *                     | <i>ITS2</i>                        | <i>AB439276.1</i>              |                                                                        |          |          |
| *                     | <i>ITS2</i>                        | <i>AB439282.1</i>              |                                                                        |          |          |
| <i>L. schlechteri</i> | <i>atpH, ITS2, matK, trnH-psbA</i> | <i>Spies 6865<sup>2</sup></i>  | Wildepaaardehoek, Northern Cape                                        | -30.0215 | 17.47078 |
|                       | <i>matK, trnH-psbA</i>             | <i>Spies 6866<sup>2</sup></i>  | On the road between Soebatsfontein district Kamieskroon, Northern Cape | -30.2174 | 17.92348 |
|                       | <i>atpH, matK, trnH-psbA</i>       | <i>Spies 6880<sup>2</sup></i>  | Kamieskroon, Northern Cape                                             | -30.2197 | 17.92399 |
| <i>L. sp.</i>         | <i>#ITS2</i>                       | <i>Spies 6933<sup>2</sup></i>  | Cape Peninsula, Western Cape                                           | -34.2487 | 18.41503 |
|                       | • <i>matK</i>                      | <i>Spies 7080</i>              | Nieuwoudtville-Oorlogskloof road, Northern Cape                        | -31.4023 | 19.11856 |
|                       | • <i>matK</i>                      | <i>Spies 7081</i>              | Nieuwoudtville-Oorlogskloof road, Northern Cape                        | -31.4023 | 19.11856 |
|                       | • <i>matK</i>                      | <i>Spies 7082</i>              | Nieuwoudtville-Oorlogskloof road, Northern Cape                        | -31.4023 | 19.11856 |
|                       | • <i>matK, trnH-psbA, trnL-F</i>   | <i>Spies 9028<sup>25</sup></i> | Unknown                                                                |          |          |
|                       | <i>atpH, •matK, trnH-psbA</i>      | <i>Spies 9029<sup>2</sup></i>  | 6 km from Nieuwoudtville, Northern Cape                                | -31.3769 | 19.10794 |
|                       | <i>atpH, •matK, trnH-psbA</i>      | <i>Spies 9032<sup>2</sup></i>  | Nieuwoudtville-Oorlogskloof road, Northern Cape                        | -31.3769 | 19.10794 |
| <i>L. splendida</i>   | <i>trnH-psbA</i>                   | <i>Spies 8290<sup>3</sup></i>  | Elandsfontein private nature reserve, Western Cape                     | -33.0881 | 18.20137 |
| *                     | <i>ITS2</i>                        | <i>AB305002.1</i>              |                                                                        |          |          |
| <i>L. unifolia</i>    | <i>trnH-psbA</i>                   | <i>Spies 6861<sup>2</sup></i>  | Paleisheuwel, Western Cape                                             | -32.4426 | 18.72299 |
|                       | <i>atpH, matK, trnH-psbA</i>       | <i>Spies 6862<sup>2</sup></i>  | Darling, Western Cape                                                  |          |          |
|                       | <i>atpH, ITS2, matK,</i>           | <i>Spies 6863<sup>2</sup></i>  | Witsenberg, Western Cape                                               | -33.3165 | 19.23391 |

|                        |                                            |                               |                                     |          |          |
|------------------------|--------------------------------------------|-------------------------------|-------------------------------------|----------|----------|
|                        | <i>trnH-psbA</i>                           |                               |                                     |          |          |
|                        | <i>matK, trnH-psbA</i>                     | Spies 6867 <sup>2</sup>       | Darling, Western Cape               | -33.3824 | 18.38336 |
|                        | <i>atpH, ITS2, matK, trnH-psbA, trnL-F</i> | Spies 6869 <sup>2</sup>       | Unknown                             |          |          |
|                        | <i>atpH, matK, trnH-psbA, trnL-F</i>       | Spies 6870 <sup>2</sup>       | Bainskloof, Western Cape            | -33.6186 | 19.10099 |
|                        | <i>atpH, #ITS2, mat, trnH-psbA, trnL-F</i> | Spies 6871 <sup>2</sup>       | North of Gouda, Western Cape        | -33.2862 | 19.03258 |
|                        | <i>atpH, ITS2, matK, trnH-psbA, trnL-F</i> | Spies 6873 <sup>2</sup>       | Unknown                             |          |          |
|                        | <i>atpH, matK, trnH-psbA, trnL-F</i>       | Spies 6874 <sup>2</sup>       | Jonkershoek staatsbos, Western Cape | -33.9758 | 18.98363 |
|                        | <i>matK, trnH-psbA</i>                     | Spies 6879 <sup>2</sup>       | Unknown                             |          |          |
|                        | <i>atpH, trnH-psbA</i>                     | Spies 6881 <sup>2</sup>       | Unknown                             |          |          |
|                        | <i>atpH, trnH-psbA</i>                     | Spies 6902 <sup>2</sup>       | Darling, Western Cape               | -33.3824 | 18.38336 |
|                        | <i>trnL-F</i>                              | Spies 7849 (253) <sup>1</sup> | Yzerfontein, Western Cape           | -33.36   | 18.159   |
|                        | <i>atpH, trnH-psbA</i>                     | Spies 9038 <sup>2</sup>       | Unknown                             |          |          |
| <i>L. vanzyliae</i>    | <i>ITS2</i>                                | Spies 7063 <sup>2</sup>       | De Rust, Western Cape               | -33.4865 | 22.53808 |
|                        | <i>trnL-F</i>                              | Spies 7707                    | Piketberg, Western Cape             | -32.9016 | 18.77382 |
| <i>L. zeyheri</i>      | <i>#ITS</i>                                | Spies 7099 <sup>2</sup>       | Unknown                             |          |          |
| <i>M. depressa</i>     | <i>trnL-F</i>                              | Spies 7120                    | Fauresmith, Free State              | -29.765  | 25.30077 |
| *                      | <i>ITS2</i>                                | AB305007.1                    |                                     |          |          |
| <i>M. echinata</i>     | <i>trnL-F</i>                              | Spies 7871                    | Nieuwoudtville, Northern Cape       | -31.365  | 19.13987 |
| <i>M. jasminiflora</i> | <i>trnL-F</i>                              | Spies 7115                    | Fauresmith, Free State              | -29.765  | 25.30077 |
| <i>M. pustulata</i> *  | <i>ITS2</i>                                | AB305008.1                    |                                     |          |          |

\* Sequences obtained from NCBI.

# Sequences obtained from Ms. A. de Vos

• Sequences obtained from CCDB

<sup>1</sup> Leaf material supplied by Mr. Graham Duncan (SANBI)

<sup>2</sup> Leaf material supplied by Mrs. R. Kleynhans (ARC-Roodeplaat)

<sup>3</sup> Leaf material supplied by Mr. Gordon Summerfield (Summerfield's Indigenous Bulbs and Seed)

<sup>4</sup> Jodrell laboratories (Kew Botanical Gardens)

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Sample identification

The accessions used in this study have been identified by Mr. G.D. Duncan, SANBI (Kirstenbosch), Mrs. R. Kleynhans, ARC-Roodeplaat and Mr. G. Summerfield. The completion of the DNA-barcoding analyses indicated that a group of *L. mediana* accessions forms a sister group to the rest of the *L. mediana* accessions. These specimens were collected near Nieuwoudtville, which is outside the distribution range of *L. mediana* (Duncan, 2012). This group probably belongs to a different species, perhaps *L. canaliculata* and these samples are from here on referred to as *L. sp.*

### 6.3.2 Amplification and sequencing

The direct PCR approach resulted in a lower quality and quantity (Table 6.3) PCR product than conventional DNA-extraction and amplification. Therefore this method was only used when very little leaf material was available.

**Table 6.3** Summary of PCR and sequence amplification success per DNA-barcoding locus in *Lachenalia*. Statistics for each gene region after alignment and trimming were obtained from Geneious R6. Only the results generated in this study are represented in this Table.

| Gene region      | PCR statistics                                  |                 | Sequencing statistics                          |                      | Gene region statistics |                     |                |            |
|------------------|-------------------------------------------------|-----------------|------------------------------------------------|----------------------|------------------------|---------------------|----------------|------------|
|                  | Number of specimens amplified* / Total attempts | PCR Success (%) | Number of specimens sequenced / Total attempts | Sequence Success (%) | Aligned length (bp)    | Identical sites (%) | GC content (%) | INDELS (%) |
| <i>atpH-I</i>    | 40/45                                           | 88.8            | 35/40                                          | 87.5                 | 471                    | 69                  | 28.1           | 12.5       |
| <i>matK</i>      | 47/64                                           | 73.4            | 38/47                                          | 80.8                 | 633                    | 66.8                | 27             | 3.8        |
| <i>nrITS2</i>    | 42/45                                           | 93.3            | 21/42                                          | 50                   | 254                    | 89.8                | 72.7           | 0          |
| <i>trnH-psbA</i> | 53/53                                           | 100             | 53/53                                          | 100                  | 533                    | 73                  | 31.5           | 9.2        |
| <i>trnL-F</i>    | 34/46                                           | 73.9            | 21/34                                          | 61.7                 | 889                    | 66.3                | 29.4           | 12.6       |
| <i>trnT-L</i>    | 36/37                                           | 97.2            | 22/36                                          | 61.1                 | 856                    | 62.3                | 21.3           | 11.7       |
| <i>rbcl</i>      | BOLD                                            | BOLD            | BOLD (33)                                      | BOLD                 | 492                    | 99.8                | 42.8           | 0          |
| <i>rpL16</i>     | 13/13                                           | 100             | n/a                                            | n/a                  |                        |                     |                |            |

\*samples with a low quality amplification (thus with a smear) were included in the analyses

BOLD = DNA extraction, PCR amplification and sequencing performed by the Canadian Centre for DNA-barcoding and sequences submitted to BoldSystems.

n/a = low quality sequences

Even though *ITS2* successfully amplified on several attempts, all the PCR products were discarded if traces of contamination were present in the negative control. Because fungal contamination may be a problem when amplifying *ITS* (Hollingsworth *et al.*, 2011), all the *ITS2*

sequences from this study were validated by comparison with sequences in GenBank. Sequencing success was low with the *ITS2* region (only 50% success), and contributing factors may be the high GC content (72.7%) in *Lachenalia* resulting in secondary structures (Baldwin *et al.*, 1995; Álvarez & Wendel, 2003). Addition of DMSO did not have a significant improvement on the rate of success. The GC content in all the chloroplast regions is low (all are below 32%). PCR amplification success was 73.4% in *matK* and the dataset was supplemented with sequences from BOLD. The CCDB provided a full dataset for *rbcLa*.

A total of 257 successful PCR amplifications were obtained and 182 bidirectional sequences were assembled.

### 6.3.3 Assessing the analysis methods for selecting a barcode

#### 6.3.3.1 Tree-based analysis

To access the efficacy of tree-based methods in the barcoding of *Lachenalia*, five cladograms for each gene region were constructed with MEGA 5.05 and Geneious R6 (Appendix G). The cladograms represent the distance-based tree building methods [Neighbor-joining (NJ), UPGMA] and character-based tree building methods [Maximum likelihood (ML), Maximum parsimony (MP) and Bayesian Inference (BI)]. The five cladograms for each gene region were screened to determine which method provided the highest bootstrap support for the monophyletic groups (Table 6.4). Many barcoding studies make use of the neighbor-joining tree drawing method, including BOLD which is the database for the iBOL. The maximum likelihood (ML) analysis provided the majority of cladograms with a higher bootstrap support than the other cladograms. The maximum likelihood (ML) and neighbor-joining (NJ) tree drawing methods overall had low bootstrap support.

Erixon *et al.* (2003) suggested that the bootstrap support of the Maximum Likelihood may be superior to the posterior probabilities of the Bayesian Inference (BI) analysis. Nylander *et al.* (2008) suggested the use of Bayesian MCMC trees when constructing a phylogeny.

For this study the Bayesian Inference cladograms are presented in Figure 6.1 for

further discussions. The branch support for the monophyletic groupings is very high for *ITS2*, where all the clades are supported with a posterior probability >0.92 and 75% of the clades have a support >99%.

**Table 6.4** A summary of the different cladograms drawn for each gene region. The taxa to the left are the focus species. Each gene region is represented in a column. The values are the highest bootstrap support for a monophyletic grouping, with the cladogram next to it in brackets (e.g. MP, ML, NJ or UPGMA). Symbols: **x**: non-monophyletic species; **n/a**: species not represented in the dataset; <sup>1234567</sup>: paraphyletic taxa

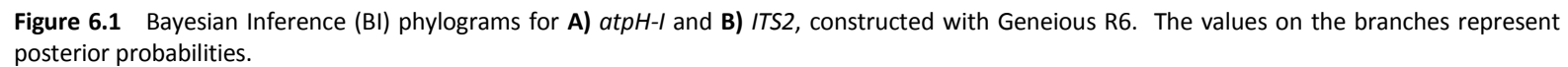
| Species            | Gene region            |                           |                      |                  |                      |
|--------------------|------------------------|---------------------------|----------------------|------------------|----------------------|
|                    | <i>atpH-I</i>          | <i>ITS2</i>               | <i>matK</i>          | <i>trnH-psbA</i> | <i>trnL-F</i>        |
| <i>L. unifolia</i> | 93 (MP) <sup>1,6</sup> | 56 (UPGMA) <sup>1,2</sup> | 96 (NJ) <sup>1</sup> | 25 (MP)          | 88 (MP) <sup>5</sup> |
| <i>L. bifolia</i>  | x                      | 53 (ML)                   | x                    | x                | x                    |
| <i>L. punctata</i> | x                      | 34 (UPGMA) <sup>3,4</sup> | 66 (MP)              | x                | 96 (NJ)              |
| <i>L. pallida</i>  | n/a                    | 90 (MP) <sup>7</sup>      | x                    | x                | x                    |
| <i>L. mediana</i>  | 39 (UPGMA)             | x                         | 95 (UPGMA)           | 64 (MP)          | 99-100 (all)         |

<sup>1</sup> *L. schlechteri* Spies 6865; <sup>2</sup> *L. zeyheri* Spies 7099; <sup>3</sup> *L. flava* Spies 7060; <sup>4</sup> *L. quadricolor* Spies 7062; <sup>5</sup> *L. wrightii* Spies 8318; <sup>6</sup> *L. unifolia* Spies 6870; <sup>7</sup> Two cloned *L. pallida* accessions (Hamatani et al., 2008)

The *trnL-F* chloroplast region has very well supported monophyletic groups for four of the eight species investigated in the dataset. Cladograms constructed for the *atpH-I* and *trnH-psbA* chloroplast regions, provided monophyletic groupings for two species in each dataset.

The *matK* gene region delivers a well-defined Bayesian Inference cladogram and from the high branch supports (0.914-1.0 posterior probabilities), it is evident that this region is a good candidate DNA-barcoding region in *Lachenalia*. However, based on preliminary results of a BI phylogram (Figure 6.2) constructed with a large number of *Lachenalia* species obtained from BOLD, the signal for *matK* alone is insufficient to give ample resolution to the tree.

Due to *Lachenalia* species being very well presented over a broad geographical range, some observations were made that can be an area of concern for the barcoding of this genus. Before the latest monograph of *Lachenalia* (Duncan, 2012) was published, the species *L. schlechteri* has been a subspecies of *L. unifolia*. These “two species” falls into a monophyletic clade with a high posterior probability of 0.9981, suggesting that the divergence between the species has been too recent to separate them. If this problem is not resolved with a multiple region barcode, identification of an unknown sample would have to be combined with a morphological identification. A further observation is that the *L. bifolia* specimens are unresolved and this is likely due to multiple polyploidization events within this group.



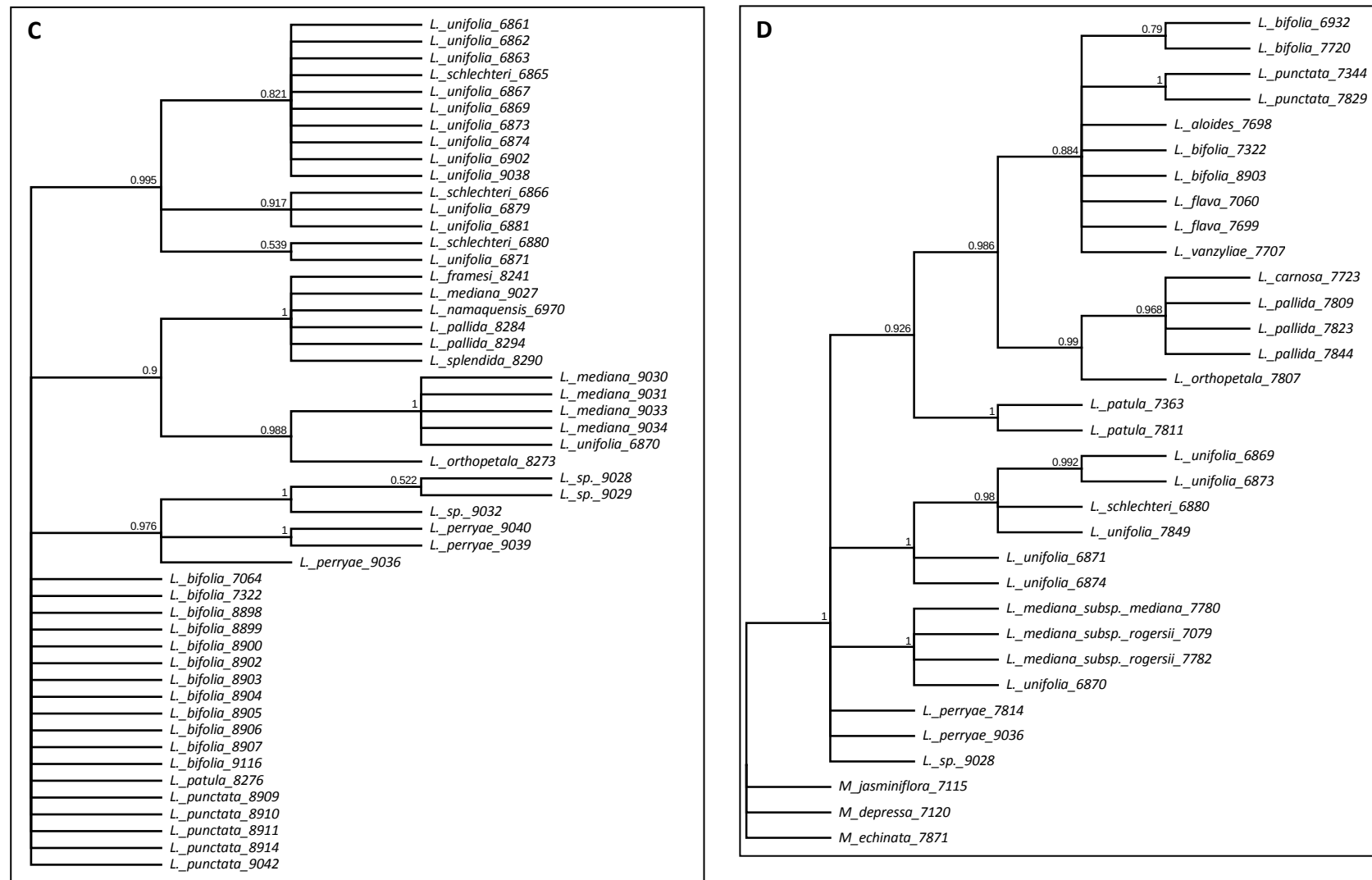


**Figure 6.1** (Continued) Bayesian Inference (BI) phylograms for **C)** *matK* and **D)** *trnL-F* constructed with Geneious R6. The values on the branches represent posterior probabilities.

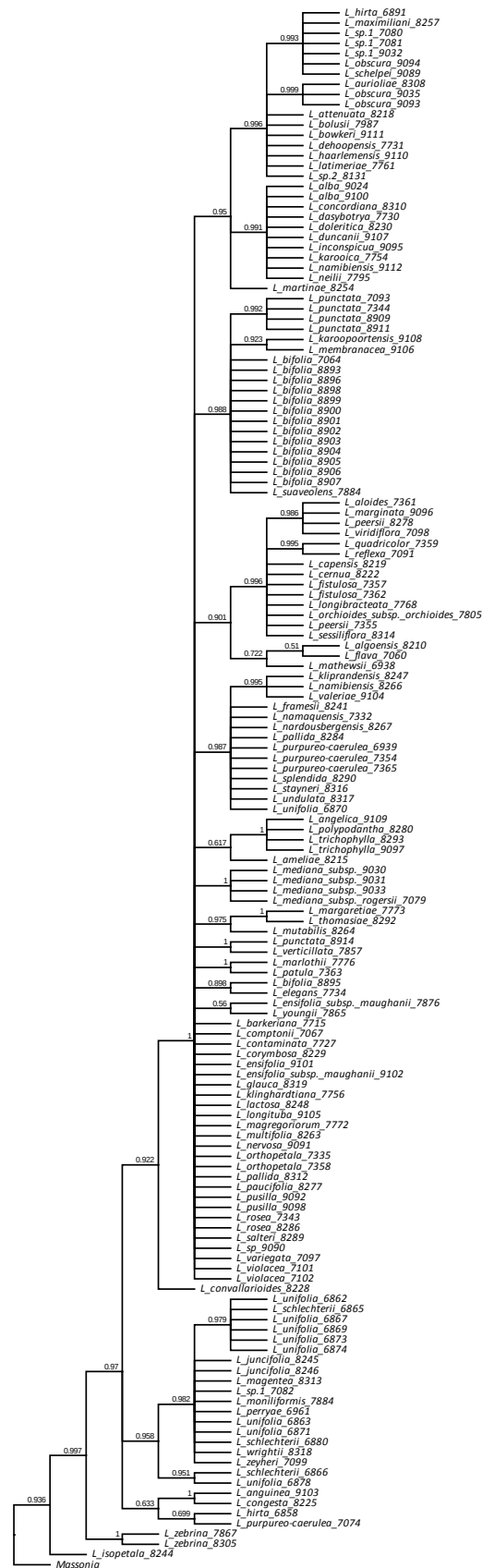


**Figure 6.2:** The BI *matK* phylogram of *Lachenalia* species from BOLD with posterior probabilities on the branches



The final observation is that the division of *L. mediana* into two separate groups is supported by a 100% posterior probability. This is probably due to mistaken identification as discussed earlier, where the northern group of *L. mediana* may be a different species, probably *L. canaliculata*. This once again proves the importance of representing a broad geographical distribution range for a species, that the proper classification of a genus is crucial, and that the correct delimitation of species in a DNA-barcoding database is of utmost importance.

Tree BI cladograms were analysed with the Species Delimitation plugin (in Geneious R6). The inter- and intraspecific variation was determined, as well as the probabilities of identifying an unknown sample. Two types of probabilities have been calculated. The strict probability [P ID (strict)] is the probability of an unknown sample being identified by its monophyletic grouping with members of its own species. In addition, the liberal probability [P ID (liberal)] determines the probability of identifying a species if the species fall sister to a monophyletic group. The only species with a relative high strict probability to be identified is *L. unifolia*, with a probability of 0.88 in the *trnH-psbA* region, 0.86 in the *atpH-I* region and 0.73 in *matK*. None of the other species have a probability [P ID (strict)] greater than 0.8. *Lachenalia bifolia* and *L. hirta* has the greatest probabilities to be identified with *ITS2* barcodes (0.79 and 0.71 respectively), and *L. punctata* has a strict probability of 0.64 to be identified with *matK* barcodes.

For a gene region to be an effective barcode, the barcoding gap should be large enough to ensure no overlap between the inter- and intraspecific distances. The intraspecific distances should be small and the interspecific distances should be large. Of all the regions, *trnL-F* has on average the lowest intraspecific distances (average is 0.0022). The relative small mean interspecific distances of this region (average is 0.00866) result in a ratio of 0.279. The lower this ratio is, the larger the barcoding gap. Thus for a region to be considered as a DNA-barcode, the ratio calculated by the species delimitation plugin should be low. This ratio is on average the highest for *ITS2* (0.37) and the lowest for *trnH-psbA* (0.2). Thus, based on the barcoding gap it seems as if the *trnH-psbA* region will provide enough interspecific variation to discriminate species (Table 6.5).

**Table 6.5** The species delimitation in *Lachenalia* using the Species Delimitation plugin for Geneious R6. Data was generated from the BI cladograms. Calculations of the intra- and interspecific distances are based on the average pairwise tree differences. P ID (Strict) and P ID (Liberal) refers to the mean probability (with a 95% confidence interval for the prediction), that a correct identification of an unknown sample will be made based on the placement of the tree. The support for each monophyletic clade is given as posterior probabilities (PP).

| Gene region      | Species                                    | Closest Species      | Intra Dist | Inter Dist - Closest | Intra / Inter | P ID (Strict)     | P ID (Liberal)    | Clade Support |
|------------------|--------------------------------------------|----------------------|------------|----------------------|---------------|-------------------|-------------------|---------------|
| <i>atpH-I</i>    | <i>L. unifolia</i> /<br><i>schlechteri</i> | <i>L. bifolia</i>    | 0.003      | 0.009                | 0.34          | 0.86 (0.79, 0.93) | 0.96 (0.91, 1.00) | 0.97          |
| <i>ITS2</i>      | <i>L. bifolia</i>                          | <i>L. reflexa</i>    | 0.014      | 0.025                | 0.55          | 0.79 (0.72, 0.86) | 0.94 (0.90, 0.98) | 0.92          |
|                  | <i>L. reflexa</i>                          | <i>L. bifolia</i>    | 0.003      | 0.025                | 0.12          | 0.53 (0.38, 0.68) | 0.91 (0.76, 1.0)  | 0.97          |
|                  | <i>L. hirta</i>                            | <i>L. reflexa</i>    | 0.008      | 0.032                | 0.24          | 0.71 (0.57, 0.85) | 0.92 (0.81, 1.0)  | 0.99          |
|                  | <i>L. pusilla</i>                          | <i>L. pallida</i>    | 0.01       | 0.021                | 0.49          | 0.34 (0.19, 0.50) | 0.68 (0.53, 0.84) | 0.99          |
|                  | <i>L. aloides</i>                          | <i>L. reflexa</i>    | 0.004      | 0.043                | 0.1           | 0.54 (0.39, 0.69) | 0.92 (0.77, 1.0)  | 0.99          |
| <i>matK</i>      | <i>L. punctata</i>                         | <i>L. unifolia</i>   | 0.001      | 0.003                | 0.44          | 0.64 (0.51, 0.76) | 0.89 (0.79, 0.99) | 0.98          |
|                  | <i>L. unifolia</i>                         | <i>L. punctata</i>   | 0.001      | 0.003                | 0.5           | 0.73 (0.65, 0.82) | 0.92 (0.86, 0.97) | 0.99          |
| <i>trnH-psbA</i> | <i>L. unifolia</i>                         | <i>L. bifolia</i>    | 0.006      | 0.018                | 0.36          | 0.88 (0.83, 0.94) | 0.97 (0.94, 0.99) | 0.995         |
|                  | <i>L. perryae</i>                          | <i>L. bifolia</i>    | 8.15E-04   | 0.018                | 0.05          | 0.57 (0.42, 0.72) | 0.95 (0.80, 1.0)  | 1             |
| <i>trnL-F</i>    | <i>L. bifolia</i>                          | <i>L. punctata</i>   | 0.003      | 0.007                | 0.4           | 0.52 (0.34, 0.70) | 0.78 (0.63, 0.93) | 0.98          |
|                  | <i>L. punctata</i>                         | <i>L. bifolia</i>    | 6.56E-04   | 0.007                | 0.1           | 0.54 (0.39, 0.69) | 0.92 (0.77, 1.0)  | 9.1E-04       |
|                  | <i>L. orchioides</i>                       | <i>L. bifolia</i>    | 0.002      | 0.007                | 0.27          | 0.46 (0.30, 0.61) | 0.82 (0.67, 0.97) | 0.96          |
|                  | <i>L. pallida</i>                          | <i>L. bifolia</i>    | 0.003      | 0.009                | 0.39          | 0.61 (0.46, 0.75) | 0.87 (0.76, 0.98) | 0.99          |
|                  | <i>L. rosea</i>                            | <i>L. bifolia</i>    | 0.001      | 0.009                | 0.15          | 0.51 (0.36, 0.66) | 0.89 (0.74, 1.0)  | 0.99          |
|                  | <i>L. patula</i>                           | <i>L. bifolia</i>    | 0.001      | 0.01                 | 0.14          | 0.52 (0.37, 0.67) | 0.89 (0.74, 1.0)  | 1.90E-04      |
|                  | <i>L. unifolia</i>                         | <i>L. juncifolia</i> | 0.002      | 0.004                | 0.54          | 0.57 (0.45, 0.70) | 0.85 (0.75, 0.95) | 0.99          |
|                  | <i>L. juncifolia</i>                       | <i>L. unifolia</i>   | 0.002      | 0.004                | 0.36          | 0.41 (0.26, 0.56) | 0.76 (0.61, 0.92) | 0.01          |
|                  | <i>L. aloides</i>                          | <i>L. bifolia</i>    | 0.002      | 0.007                | 0.24          | 0.63 (0.46, 0.81) | 0.87 (0.73, 1.0)  | 1.80E-04      |

Although the average ratio between the intra-and interspecific variation is the highest for *ITS2*, values for the other factors, such as the number of well supported clades and the number of species with a high probability to be identified, are good. These results indicated that *ITS2* is a good barcoding candidate, but on its own, it is insufficient to identify all the species. The efficiency of a two-barcode should be investigated, but due to the size of the genus, it is likely that a three or more gene barcode will be necessary.

### 6.3.3.2 Distance-based analysis

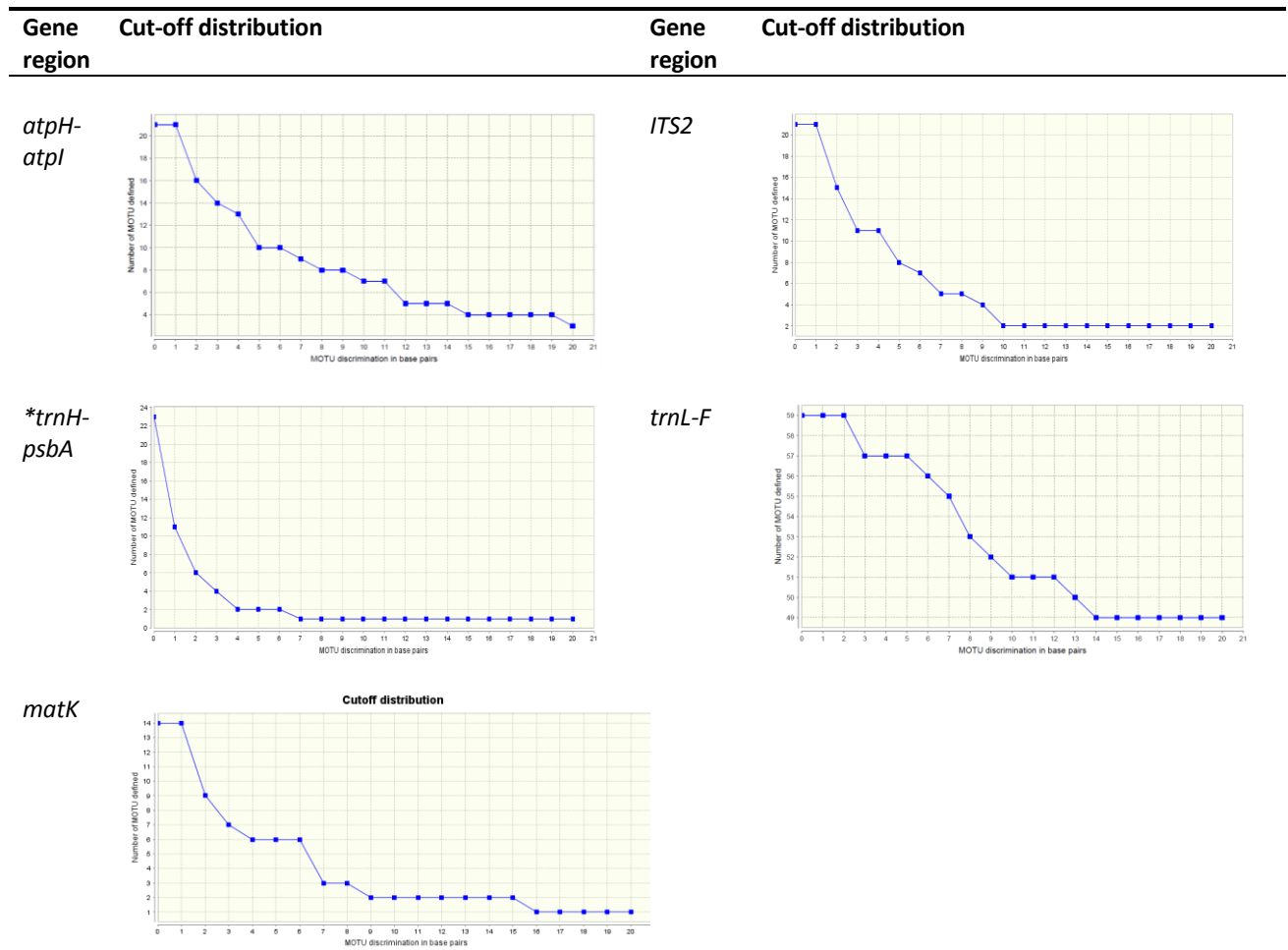
The software jMOTU implements a distance-based analysis to group the species into

molecular taxonomic units. The program was designed to be used as an identification tool in barcoding and it works on the principle that an unknown sample can be identified based on the MOTU (molecular operational taxonomic units) it clusters in. Prior to be implemented as a barcoding tool, the most reliable cut-off value for each dataset was determined. The cut-off value is the number of nucleotide changes that occur within a molecular taxonomic unit. The higher the cut-off value, the more polymorphisms there are in a taxonomic unit and the more variation there is within a group. Each sequence dataset was analysed separately in jMOTU and a graph was created with cut-off values from 0 to 20 bases (Figure 6.3). The data generated in Microsoft Excel 2010 files were screened to determine at which cut-off value the MOTU correspond best with the taxonomic OTUs.

The best cut-off value for the *atpH-atpI* region is a six base-pair cut-off (Figure 6.3). This cut-off value results in the majority of species in the dataset clustered into MOTUs corresponding with taxonomic OTUs (Appendix H). However, possible ancient hybrid speciation may result in false positive results. One such example is the clustering of a single *L. bifolia* sample (*Spies 8907*) with the *L. punctata* cluster, whereas the rest (eight *L. bifolia* specimens) form a single cluster. Geographical isolation between populations of plants belonging to the same species can result in the development of independent polymorphisms belonging to a specific geographical region and that is not unique to the species. The MOTUs may favour the geographical distribution (as is likely the case in *L. bifolia*) rather than the taxonomic classification and then may result in false negatives when making an identification using jMOTU.

The *ITS2* dataset represents 18 species, of which eight species are represented by two or more specimens. At a cut-off value of two (thus a maximum of two bp differences between sequences), the 52 sequences in the dataset clustered into 15 clusters, of which only *L. hirta* forms a clade. The *Lachenalia unifolia* specimens are scattered over three different MOTU's and *L. bifolia*, *L. punctata*, *L. reflexa*, *L. flava*, *L. quadricolor* are lumped into the same cluster. Although the morphology of these species differs, they share the same basic chromosome number of  $x = 7$ . By lowering the cut-off value to a maximum of one base-pair difference between specimens that are grouped together, the number of clusters increases to 21 (Appendix H) and *L. bifolia* and *L. reflexa* are split from the rest of the five species cluster. To

conclude, a jMOTU analysis of the *ITS2* gene region is ineffective in identifying an unknown sample of *Lachenalia*.



\*Cut-off distribution can only be calculated with unaligned sequences due to the large number of INDELS

**Figure 6.3** The cut-off distribution for each gene region. These graphs were used to determine the best cut-off value for generating MOTUs. The X-axis is the MOTU discrimination in base pairs. This is thus the maximum number of nucleotide changes in a MOTU. The Y-axis is the number of MOTUs defined.

MOTUs corresponding to morphological taxonomic units (Appendix H) could not be produced with the *trnH-psbA* region and this region is consequently unusable for species identification using jMOTU as tool (Appendix H). The program, jMOTU, is unable to sufficiently cluster species with the *trnL-F* dataset into unique molecular taxonomic groups, regardless of the cut-off value. Sixteen species are represented by two or more specimens in this dataset, and of these only three cluster into molecular units. Thus, analysis of the *trnL-F* region with jMOTU does not provide an effective tool for *Lachenalia* identification.

At a cut-off value of either one or two base pairs, the *matK* region forms clear clusters for only two closely related species, i.e. the polyploid complex of *L. bifolia* and the diploid

related species, *L. punctata*. Increasing the cut-off value does not improve the clustering pattern and rather lumps more species into molecular taxonomic units. Analysing this single gene region (*matK*) in jMOTU does not provide sufficient information to be used for identification purposes. This emphasizes and supports the need to use multiple DNA-barcoding sequences, especially in *Lachenalia*.

### 6.3.3.3 Character-based analysis

All the gene regions were investigated for polymorphisms (SNPs and INDELS) and a character-based table was generated in Geneious R6. Any polymorphisms that are shared across species were removed to keep only species-specific polymorphisms in the dataset. Thirteen *Lachenalia* species have unique species-specific polymorphisms: *L. aloides*, *L. bifolia*, *L. hirta*, *L. juncifolia*, *L. orchioides*, *L. pallida*, *L. patula*, *L. perryae*, *L. punctata*, *L. pusilla*, *L. rosea*, *L. unifolia* and *L. schlechteri*. Several of these species were represented by only two specimens. The degree of intraspecific variability may be underestimated if the species is underrepresented, thus the following discussion will only include species that were well represented.

The four species that were represented across all the loci and also have ample samples per region (>3), all have unique polymorphisms in the chloroplast regions (Table 6.6). The *atpH-I* region provides markers for the two species *L. bifolia* and *L. unifolia*. The *trnH-psbA* region provides additional markers for *L. bifolia* and *L. unifolia*. The *matK* region provides a marker for *L. punctata*. The nuclear *ITS2* region does not have species-specific polymorphisms for any of the species.

The use of character-based identification in *Lachenalia* seems to be promising and it has been proven with this data that the *atpH-I* region combined with *trnH-psbA* may provide the preferred loci for character-based identification in *Lachenalia*. An interesting observation is that the *atpH-I* region has species-specific polymorphisms for *L. unifolia* and the *L. bifolia* polyploid complex. The diploid species *L. punctata* has a species-specific polymorphism in a region that none of the other species have, namely the *matK* region.

Similar to the results in *Clivia* (Chapter 4), unique markers can be linked to

geographical distribution in certain *Lachenalia* species. The *L. bifolia* group west of the Bot River (Figure 6.4) have a unique G->T transversion at position 365 in the *atpH-I* region. The area of origin (thus west or east of the Bot River) of a *L. bifolia* specimen can thus be identified with SNP analysis.

**Table 6.6** A summary of unique polymorphisms for the focus species in the different gene regions, indicated with an X if there is at least one unique polymorphism for the taxa listed in the left column. No unique polymorphism are indicated by a (–)

|                          | <i>atpH-I</i> | <i>ITS2</i> | <i>matK</i> | <i>trnH-psbA</i> | <i>trnL-F</i> |
|--------------------------|---------------|-------------|-------------|------------------|---------------|
| <i>L. bifolia</i>        | X             | –           | –           | X                | –             |
| <i>L. bifolia</i> (west) | X             | –           | –           | –                | –             |
| <i>L. bifolia</i> (east) | –             | –           | –           | –                | –             |
| <i>L. punctata</i>       | –             | –           | X           | –                | –             |
| <i>L. unifolia</i>       | X*            | –           | –           | X                | –             |
| <i>x</i> = 7             | –             | –           | –           | X                | –             |
| <i>x</i> = 8             | –             | X           | –           | –                | –             |
| <i>x</i> = 11            | –             | –           | –           | –                | X             |

\*The single *L. schlechteri* sample shares the polymorphism with *L. unifolia*. *Lachenalia schlechteri* was a variety of *L. unifolia* before the new classification of (Duncan, 2012).

Before the reclassification of Manning *et al.* (2004), *L. corymbrosa*, *L. ensifolia*, *L. pauciflora*, *L. maughanii*, *L. longituba* and *L. odorata* belonged to the genus *Polyxena*. Duncan (2012) grouped these species into the subgenus *Polyxena*. This subgenus of *Lachenalia* shares a unique transition in position 519 in the *trnL-F* region and any species from this subgenus can be identified based on this polymorphism.

Character-based analyses may be implemented to predict chromosome numbers of a species. In this study, certain polymorphisms link species with the same basic chromosome number, almost in the same manner as the monophyletic ‘species groups’ described by Hollingsworth *et al.* (2011). In a more comprehensive study where more *Lachenalia* species were included and more samples per species, basic chromosome numbers in *Lachenalia* may be predicted with a higher confidence, using character-based analysis. For instance, species with a basic chromosome number of *x* = 11 share six polymorphisms in the *trnL-F* region, as well as polymorphisms in *trnT-L* (Appendix I). Species with a basic number of *x* = 8 share two polymorphism in *ITS2*. By identifying the marker for species with *x* = 8, a preliminary identification of this chromosome number can be performed on vegetative material collected without disturbing the plant. The traditional determination of a chromosome number relies on the collection of roots (mitosis) or excision of the inflores-

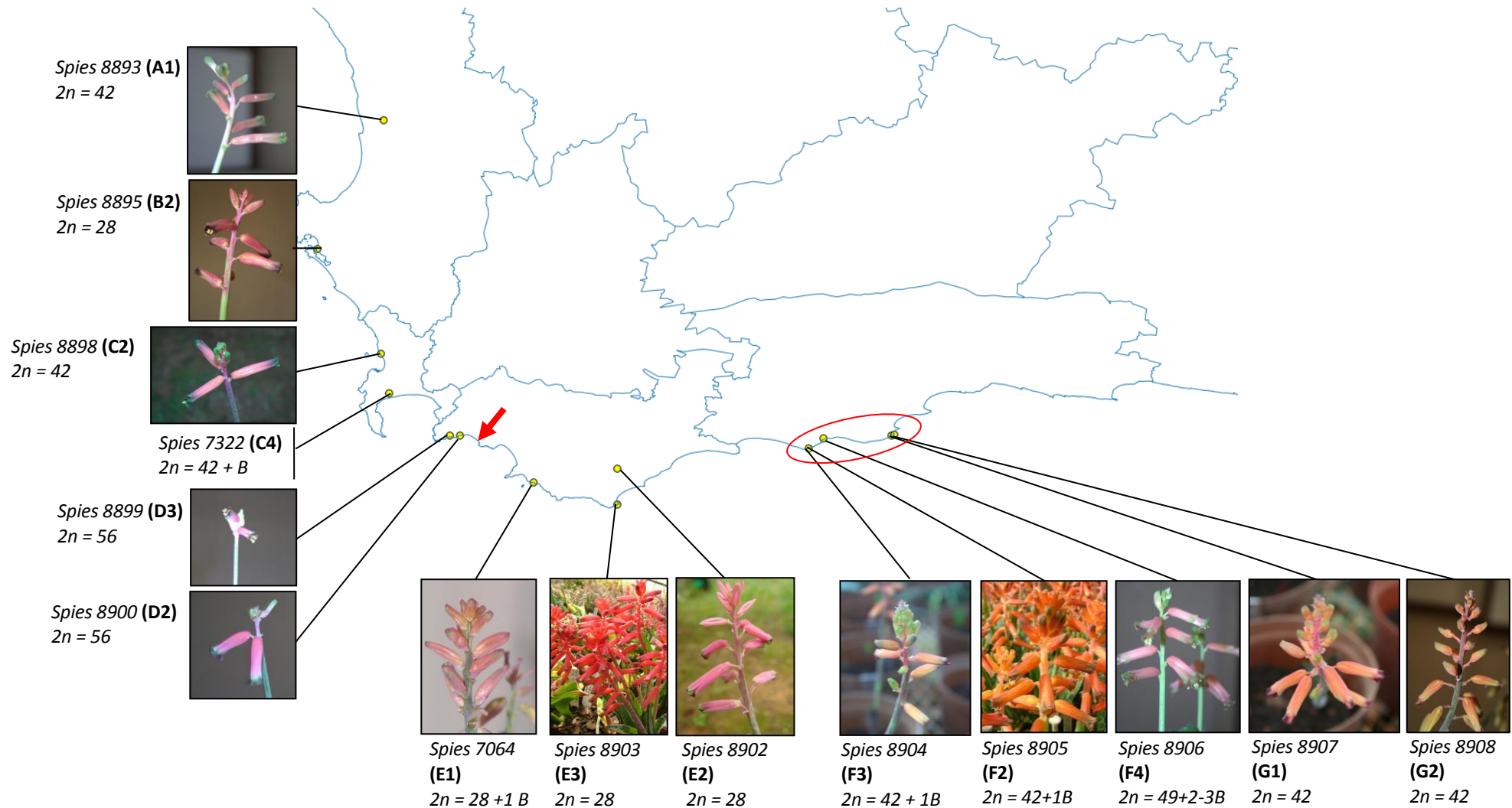


**Figure 6.4** The geographical distribution of the *L. bifolia* samples used in the study of Kleynhans & Spies (1999) with their collection numbers indicated in brackets. The same samples were used in this study. Based on a previous study (Kleynhans & Spies, 2000) it was determined that groups F and G (circled) are closely related and the flowers of these plants have an orange appearance. From this study and that of Kleynhans & Spies (2000) it was determined that F4 is a hybrid. The red arrow indicates the location of the Bot River.

cence from the bulb (meiosis) and doing squashes to determine chromosome counts. *Lachenalia* is a difficult genus to obtain a proper spread of chromosomes for analyses, thus SNP analysis may provide a quick and easy method to screen the chromosome number of a *Lachenalia* species with an unknown chromosome number.

It can be concluded that molecular characters can be used as an identification tool in the *Lachenalia* species used during this study. The disadvantage of this method is that it is not always the same one- or two gene regions that provide polymorphisms over the whole spectrum of the genus. Especially because of the size of the genus (133 species), a combination of more than two gene regions may not even be enough to provide polymorphisms for all the species. Many more species should be included in future studies to determine with the same level of confidence as Rach *et al.* (2008) “that the character-based barcode approach can offer an efficient and reliable method for accurate species and conservation unit identification”.

#### **6.3.3.4 Comparing the tree, distance and character-based analyses**

The tree and character-based (SNPs) methods are the most reliable DNA-barcoding analysis tools in *Lachenalia* (Table 6.7). Tree-based analysis could distinguish between all the focus species in the study and seems to be the most reliable tool to use in DNA-barcoding identification. The disadvantage of this method is that it can be time consuming and computers with large processors may be needed to analyse large datasets. For example: Assume that a complete dataset for *Lachenalia* with at least three specimens per taxon are available. *Lachenalia* consists of 133 species and 139 taxa. A complete DNA-barcoding dataset per locus will contain 411 sequences (specimens), and because of the size of the genus, we suspect that two datasets will not provide enough diagnostic characters to distinguish all the species. Thus, the tree-drawing method may be very slow.

Creating MOTUs in jMOTU using distance-based analysis can be very fast. The disadvantages are that MOTU cannot be created for all the focus species in this study and choosing a cut-off value is subjective.

Character-based analysis is straightforward since this approach do not rely on decisions



about cut-off values (Bergmann *et al.*, 2009), the sequences are aligned and species can be identified based on species-specific markers. It relies on the use of a single program, where the other analysis methods may rely on additional programs. This method is effective to identify the species in this study. Single polymorphisms (SNPs) may likely be insufficient for the identification of all the *Lachenalia* species, thus a combination of multi-polymorphism sites or co-implementation with other systems should be investigated in future studies. CAOS (Sarkar *et al.*, 2008) uses a character identification system that relies on the hierarchical organization of the sequences. The use of CAOS in the character-based analysis of *Lachenalia* should be further explored in future studies.

**Table 6.7** A comparison between the three methods used for analysis in the DNA-barcoding of *Lachenalia* species. When a method can be used to positively identify an unknown sample, it is indicated with a “yes”, and when the contrary is the case, it is noted as a “no”.

| Species                | Tree-based analysis | Distance-based | Character-based |
|------------------------|---------------------|----------------|-----------------|
| <i>L. bifolia</i>      | yes                 | yes            | yes             |
| <i>L. bifolia west</i> | yes                 | yes            | yes             |
| <i>L. bifolia east</i> | yes                 | yes            | no              |
| <i>L. mediana</i>      | yes                 | no             | yes             |
| <i>L. punctata</i>     | yes                 | yes            | yes             |
| <i>L. unifolia</i>     | yes                 | no             | yes             |

An interesting observation is that the type of analysis has a large impact on the identification efficacy of a gene region. To summarize the results from this study, the top four gene regions to implement for the barcoding of *Lachenalia* using the tree-based method as identification tool are *matK*, *atpH-I*, *trnL-F* and *ITS2*. Only *atpH-I* can be used with distance-based analysis in jMOTU. When using character-based identification the best gene regions are *atpH-I*, *trnH-psbA* and *matK*. The *atpH-I* region is the only region than can effectively be analysed with any of the three methods.

#### 6.3.4 DNA-barcoding analyses of concatenated data of focus species

All the species that were represented by only a small number of specimens were removed. The aim of this was to investigate the effect of concatenated data on a large number of samples per species (representing a large geographical range) (Figure 6.5). These are referred to as the focus species.

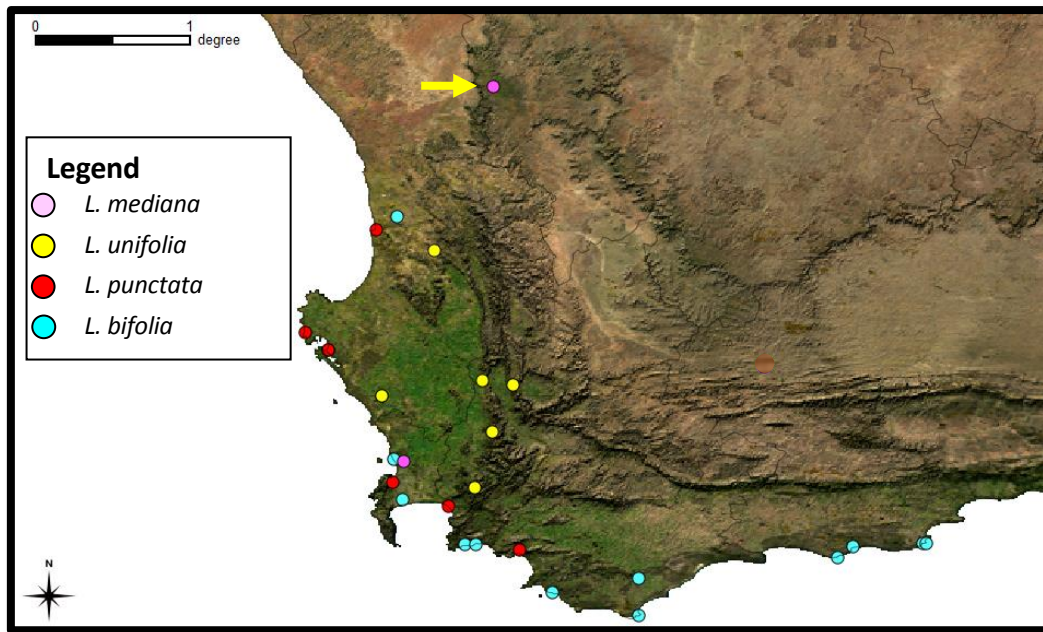


**Figure 6.5** The distribution range of the focus species in the barcoding study of *Lachenalia*. The *L. mediana* taxa from the northern (Nieuwoudtville) distribution, indicated by a yellow arrow, may have been wrongly identified since the distribution of *L. mediana* is more to the south.

The gene regions were analysed separately as well as in different combinations. The aligned lengths, percentages of variable sites and the number of monophyletic species amongst the gene regions were compared (Table 6.8). *ITS2*, *matK* and *trnL-F* have the highest number (100%) of monophyletic species groupings, followed by *atpH-I*. The lowest number of monophyletic groupings occurs in the *trnH-psbA* cladogram.

**Table 6.8** A comparison between the aligned lengths, variable sites and percentage of monophyletic species per gene region.

| Region           | Aligned length | Variable sites (%) | Unique species (%) |
|------------------|----------------|--------------------|--------------------|
| <i>atpH-I</i>    | 461            | 10 (2.17)          | 3/5 (60)           |
| <i>ITS2</i>      | 256            | 9 (3.52)           | 3/3 (100)          |
| <i>matK</i>      | 621            | 15 (2.42)          | 5/5 (100)          |
| <i>trnH-psbA</i> | 533            | 20 (3.75)          | 2/5 (40)           |
| <i>trnL-F</i>    | 863            | 31 (3.59)          | 4/4 (100)          |

After combining *atpH-I*, *ITS2*, *matK* and *trnL-F* in SequenceMatrix 1.7.8 (Table 6.9) and excluding accessions with only one dataset, the results were as follow: 15% accessions have full datasets, 38% have three datasets and 47% two datasets. According to previous studies (Kearney, 2002; Yan *et al.*, 2005; Wiens, 2006), incomplete datasets did not influence the accuracy of the phylogenetic analyses to a great extent. For the *Lachenalia* analyses, four datasets were combined in different combinations: *matK* + *atpH-I*, *matK* + *trnL-F*, *matK* + *ITS2*, *atpH-I* + *trnL-F*, *atpH-I* + *ITS2* and *trnL-F* + *ITS2*. Incomplete datasets did not have a significant

effect on the topology of the BI cladograms. Although any combination of two datasets provided 100% identification for the focus species, the best posterior probability branch support (highest support on the branches and the highest average support), were obtained in the *atpH-I* + *ITS2* combination (Table 6.10). The *matK* + *atpH-I* combination did not have the best average support, but it did have the highest number of branches with a 100% posterior probability.

*Lachenalia bifolia* and its diploid relative, *L. punctata*, is paraphyletic in two of the combined datasets (*matK* + *atpH-I* and *matK* + *trnL-F*). In the other combined datasets these two species have a sister relationship. A sister relationship is a more reliable reflection of the taxonomic relationship between these species. Any one of the concatenated sets indicated with asterisks in Table 6.10 will be the more reliable datasets for identification purposes.

The discriminatory power of the barcoding regions in *Lachenalia* is insufficient to distinguish between the *L. mediana* subspecies. The DNA-barcoding data support the close relationship between *L. unifolia* and *L. schlechteri*. These two species are indistinguishable and monophyletic in all the BI analyses. I therefore regarded them as subspecies/varieties and considered *L. unifolia* and *L. schlechteri* as the same species, namely *L. unifolia* in all the discussions.

To conclude, although *atpH-I* is ineffective as single barcode, in combination with *ITS2* they provide species that are monophyletic and in well supported clades. The other combinations that are also effective are *atpH-I* + *trnL-F* and *trnL-F* + *ITS2* with average posterior probabilities of 0.9764 and 0.9768 respectively. The alternative will be to use *matK* or *trnL-F* as single barcodes.

### 6.3.5 The effect of wrong identification, hybridization and samples from a broad geographical range on the interpretation of barcoding analyses

DNA-barcoding in *Lachenalia* can only be effective if any incongruence regarding the species delimitation on morphological and molecular levels is eliminated. Based on a revision of *Lachenalia* (Duncan, 2012), a few species (*L. pallida*, *L. unicolor* and *L. pustulata*) have been reduced to one species, namely *L. pallida*. As part of the current study, the *ITS2* sequences of

**Table 6.9** A summary of the output from SequenceMatrix after combining the sequences (character sets) of *ITS2*, *atpH-I*, *matK* and *trnL-F*. The number of INDELS are indicated in brackets. The combined dataset from SequenceMatrix was exported in nexus format and was used to construct a combined BI cladogram.

| Taxon                                         | Total length | No of character sets | <i>ITS2</i> sequence length (# INDELS) | <i>atpH-I</i> sequence length (# INDELS) | <i>matK</i> sequence length (# INDELS) | <i>trnL-F</i> sequence length (# INDELS) |
|-----------------------------------------------|--------------|----------------------|----------------------------------------|------------------------------------------|----------------------------------------|------------------------------------------|
| <i>L. bifolia</i> Spies 7064                  | 1338 bp      | 3                    | 256 (2)                                | 461 (41)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 7322                  | 2201 bp      | 4                    | 256 (2)                                | 461 (41)                                 | 621 (16)                               | 863 (82)                                 |
| <i>L. bifolia</i> Spies 8893                  | 1338 bp      | 3                    | 256 (2)                                | 461 (42)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8898                  | 1338 bp      | 3                    | 256 (2)                                | 461 (43)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8900                  | 1082 bp      | 2                    | No data                                | 461 (41)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8902                  | 1338 bp      | 3                    | 256 (2)                                | 461 (43)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8903                  | 2201 bp      | 4                    | 256 (2)                                | 461 (42)                                 | 621 (16)                               | 863 (82)                                 |
| <i>L. bifolia</i> Spies 8904                  | 877 bp       | 2                    | 256 (2)                                | No data                                  | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8905                  | 1338 bp      | 3                    | 256 (2)                                | 461 (42)                                 | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8906                  | 877 bp       | 2                    | 256 (2)                                | No data                                  | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 8907                  | 877 bp       | 2                    | 256 (2)                                | No data                                  | 621 (16)                               | No data                                  |
| <i>L. bifolia</i> Spies 9115                  | 877 bp       | 2                    | 256 (2)                                | No data                                  | 621 (16)                               | No data                                  |
| <i>L. mediana</i> Spies 9030                  | 1082 bp      | 2                    | No data                                | 461 (47)                                 | 621 (1)                                | No data                                  |
| <i>L. mediana</i> Spies 9033                  | 1082 bp      | 2                    | No data                                | 461 (48)                                 | 621 (1)                                | No data                                  |
| <i>L. mediana</i> Spies 9034                  | 1082 bp      | 2                    | No data                                | 461 (47)                                 | 621 (9)                                | No data                                  |
| <i>L. mediana</i> subsp. <i>rogersii</i> 7079 | 1484 bp      | 2                    | No data                                | No data                                  | 621 (1)                                | 863 (91)                                 |
| <i>L. punctata</i> Spies 7344                 | 1484 bp      | 2                    | No data                                | No data                                  | 621 (16)                               | 863 (97)                                 |
| <i>L. punctata</i> Spies 8909                 | 1338 bp      | 3                    | 256 (2)                                | 461 (57)                                 | 621 (16)                               | No data                                  |
| <i>L. punctata</i> Spies 8910                 | 717 bp       | 2                    | 256 (2)                                | 461 (56)                                 | No data                                | No data                                  |
| <i>L. punctata</i> Spies 8911                 | 877 bp       | 2                    | 256 (2)                                | No data                                  | 621 (16)                               | No data                                  |
| <i>L. punctata</i> Spies 8914                 | 1338 bp      | 3                    | 256 (2)                                | 461 (56)                                 | 621 (16)                               | No data                                  |
| <i>L. punctata</i> Spies 9042                 | 1338 bp      | 3                    | 256 (2)                                | 461 (56)                                 | 621 (16)                               | No data                                  |
| <i>L. schlechteri</i> Spies 6865              | 1338 bp      | 3                    | 256 (2)                                | 461 (68)                                 | 621 (1)                                | No data                                  |
| <i>L. schlechteri</i> Spies 6880              | 1945 bp      | 3                    | No data                                | 461 (64)                                 | 621 (1)                                | 863 (55)                                 |
| <i>L. sp.</i> 9028                            | 1484 bp      | 2                    | No data                                | No data                                  | 621 (4)                                | 863 (85)                                 |
| <i>L. sp.</i> 9029                            | 1082 bp      | 2                    | No data                                | 461 (53)                                 | 621 (3)                                | No data                                  |
| <i>L. sp.</i> 9032                            | 1082 bp      | 2                    | No data                                | 461 (49)                                 | 621 (1)                                | No data                                  |
| <i>L. unifolia</i> Spies 6862                 | 1082 bp      | 2                    | No data                                | 461 (68)                                 | 621 (1)                                | No data                                  |
| <i>L. unifolia</i> Spies 6863                 | 1338 bp      | 3                    | 256 (2)                                | 461 (71)                                 | 621 (1)                                | No data                                  |
| <i>L. unifolia</i> Spies 6869                 | 2201 bp      | 4                    | 256 (2)                                | 461 (68)                                 | 621 (5)                                | 863 (67)                                 |
| <i>L. unifolia</i> Spies 6870                 | 1945 bp      | 3                    | No data                                | 461 (96)                                 | 621 (16)                               | 863 (42)                                 |
| <i>L. unifolia</i> Spies 6871                 | 2201 bp      | 4                    | 256 (14)                               | 461 (64)                                 | 621 (1)                                | 863 (31)                                 |
| <i>L. unifolia</i> Spies 6873                 | 2201 bp      | 4                    | 256 (2)                                | 461 (68)                                 | 621 (1)                                | 863 (55)                                 |
| <i>L. unifolia</i> Spies 6874                 | 1945 bp      | 3                    | No data                                | 461 (64)                                 | 621 (1)                                | 863 (8)                                  |

Hamatani *et al.* (2008) were added to sequences from this study and a BI cladogram was constructed (Figure 6.6). The names of *L. unicolor* and *L. pustulata* in Hamatani *et al.* (2008)

have, in this study, been changed to *L. pallida* according to the new classification of Duncan (2012). The groupings, according to the BI cladogram, contradict the new classification of these three species into one. *Lachenalia pustulata* (new name *L. pallida*) is paraphyletic with the other *L. pallida* accessions and monophyletic with *L. carnosus* and *L. splendida*. *Lachenalia pallida* is a highly polymorphic species complex (Duncan, 2012) and it is evident that this group needs a revision (Figure 6.6). This and other incongruences between species delimitation and molecular results in *Lachenalia* will hinder the effective use of barcodes.

**Table 6.10** The branch support expressed as posterior probabilities (PP) in the Bayesian Inference (BI) cladograms drawn for different combinations of the datasets. The lowest, the highest and the average posterior probability in each cladogram are indicated in the last rows. The best values for each category are indicated in bold.

|                    | <i>matK + atpH-I</i> | <i>matK + trnL-F</i> | <i>*matK + ITS2</i> | <i>*atpH-I + trnL-F</i> | <i>*atpH-I + ITS2</i> | <i>*trnL-F + ITS2</i> |
|--------------------|----------------------|----------------------|---------------------|-------------------------|-----------------------|-----------------------|
| <i>L. unifolia</i> | 1                    | 0.871                | 0.999               | 1                       | 1                     | 1                     |
| <i>L. mediana</i>  | 1                    | 0.963                | 0.942               | 1                       | 1                     | 0.999                 |
| <i>L. sp.</i>      | 1                    | 0.933                | 0.955               | 0.999                   | 0.999                 | 0.999                 |
| <i>L. punctata</i> | 0.734                | 0.891                | 0.654               | 0.892                   | 0.894                 | 0.894                 |
| <i>L. bifolia</i>  | 0.898                | 0.53                 | 0.935               | 0.991                   | 0.995                 | 0.992                 |
| <b>Lowest PP</b>   | 0.734                | 0.53                 | 0.654               | 0.892                   | <b>0.894</b>          | <b>0.894</b>          |
| <b>Highest PP</b>  | <b>1</b>             | 0.963                | 0.999               | <b>1</b>                | <b>1</b>              | <b>1</b>              |
| <b>Average PP</b>  | 0.9264               | 0.8376               | 0.897               | 0.9764                  | <b>0.9776</b>         | 0.9768                |

\* Combinations where *L. punctata* and *L. bifolia* have a sister relationship. *Lachenalia punctata* is paraphyletic with *L. bifolia* in the other combinations

Possible hybridization or introgression results in the incongruent grouping of the *L. unifolia* specimen (*Spies 6870*). In none of the chloroplast trees did it group with *L. unifolia*, but in the majority of the trees it rather grouped with *L. mediana*. The flower morphology of this accession is different from the other *L. unifolia* accessions (Figure 6.7). The flowers are more open and have a similar blue base as the typical *L. mediana* (Figure 6.8 & 9) species (Mrs. R. Kleynhans, ARC, personal communication).

Even though *L. unifolia* and *L. mediana* grow geographically in close proximity [see distribution maps in Duncan (2012)], only *L. unifolia* grows in the Bainskloof Pass area where *L. unifolia* (*Spies 6870*) has been collected. *Lachenalia mediana* ( $2n = 18$ ) and *L. unifolia* ( $2n = 11$ ) are reproductively incompatible (Mrs. R. Kleynhans, ARC, pers. comm.) and hybridization in the greenhouse can be ruled out.

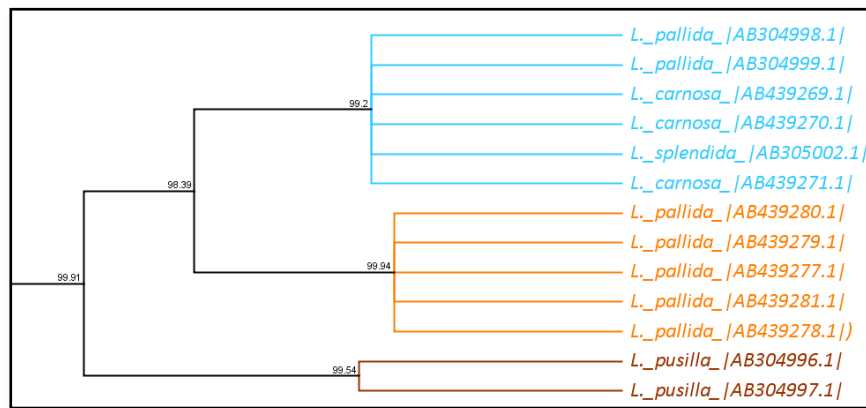


**Figure 6.6** The Bayesian Inference cladogram from sequences of the study of Hamatani *et al.* (2008) where the new name changes (Duncan, 2012) have been applied. The *L. pallida* specimens in the first monophyletic group were previously classified as *L. pustulata* and the *L. pallida* specimens in the second monophyletic group were previously classified as *L. unicolor*.



**Figure 6.7** Examples of the phenotypes represented in the cladogram (Figure 6.6) **A)** *L. carnososa*, **B)** *L. pustulata* (now *L. pallida* and occurs in the first monophyletic group in Figure 6.6), **C)** *L. splendida*, **D)** *L. unicolor*. *Lachenalia carnososa*, *L. pustulata* and *L. splendida* are monophyletic in the ITS2 cladogram. Photo's A, C, and D: Mrs. R. Kleyhans.

When analysing single barcodes in *Lachenalia*, several factors contribute to incongruence between the barcoding cladograms, including hybrid speciation (Hamatani *et al.*, 2007, 2008), polyploid speciation, recent speciation (resulting in incomplete lineage sorting) and a low mutation rate of the region in question (Hollingsworth *et al.*, 2011) or incorrect identification. One such example is *L. bifolia*. Depending on the gene region, the species are either paraphyletic or monophyletic with other species.

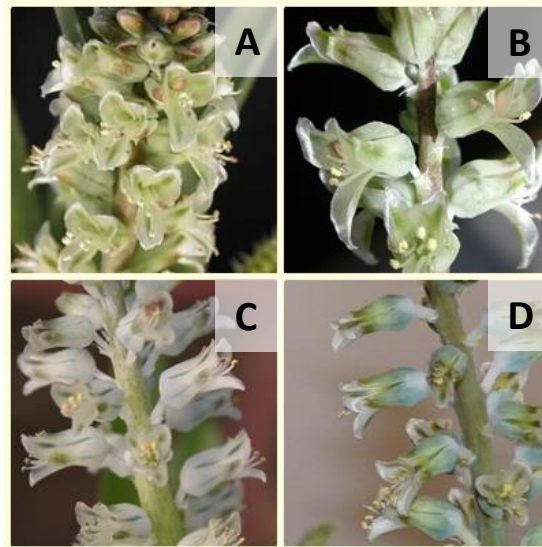


**Figure 6.8** Flowers of *L. mediana* and the unknown sister species. **A** – *L. sp. Spies 7080* (identified as *L. mediana*); **B** – *L. sp. Spies 7081* (identified as *L. mediana*); **C** – *L. mediana Spies 9031*; **D** – *L. mediana* subsp. *rogersii Spies 7079*.

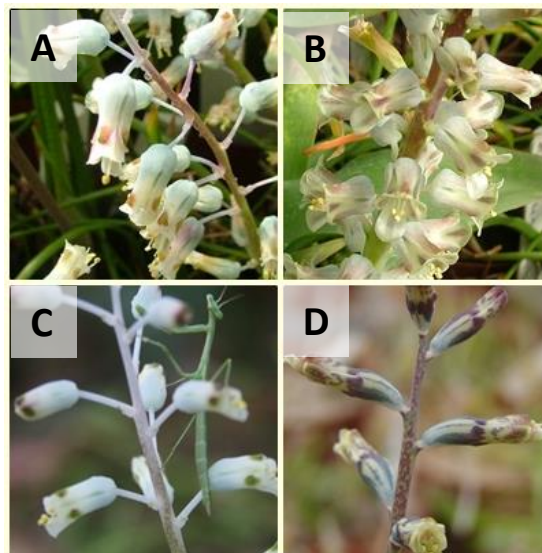


**Figure 6.9** Flowers of some specimens of *L. unifolia* (**A, B & C**) and *L. schlechterii* (**D**). Although these two species has morphological differences, they share the same chromosome number and are monophyletic in the *matK* gene region cladogram. **A** – *L. unifolia Spies 6869*; **B** – *L. unifolia Spies 6870*; **C** – *L. unifolia Spies 6869*; **D** – *L. schlechterii*.

The *L. bifolia* polyploid complex [synonym: *L. bulbifera* (Cyrilla) Engl] is an interesting example where independent origins of polyploids probably lead to the formation of the complex (Kleynhans & Spies, 2000). This appears to be an old polyploid complex (Grant, 1982), consisting of polyploids ranging from tetraploids ( $2n = 4x = 28$ ) to octoploids ( $2n = 8x = 56$ ). Comparative studies between the chromosomes, flower morphology, genetic variation



and geographical distribution of 21 specimens (Kleynhans & Spies, 1999, 2000) revealed no correlation between the flower morphologies and the ploidy levels, but rather a correlation between geographical distribution and ploidy levels. Samples of fourteen *L. bifolia* accessions from the study of Kleynhans & Spies (1999, 2000) were included in this study. Our finding supports their presumption that one accession (*Spies 8906* in this study and F4 in their study) is a hybrid. Seeds were obtained from a nursery where hybridization probably occurred between octoploids from the Palmiet River area and hexaploids from the Stilbaai area resulting in the  $2n = 49$  observed by Kleynhans & Spies (1999). The hybrid origin of this accession, as well as the closer relationship to samples from the western distribution area (Kleynhans & Spies, 2000), is confirmed by the results from this study. This accession is monophyletic with specimens in the distribution range west of the Bot River (based on the *ITS2* data), and it is related to plants from the eastern distribution area (Figure 6.5) based on the chloroplast *trnH-psbA* cladograms. Morphologically it represents the pollen parent in the western distribution area. Although a taxonomic investigation is not part of our study, it is rather prominent that the samples in the western distribution area are monophyletic according to the *atpH-I* and *ITS2* region and it has little variation in flower colour and morphology (Figure 6.5). The flower colour of the *L. bifolia* plants in the eastern distribution area is much more variable. The sequences from accessions from this area fall in an unresolved clade with other species such as *L. pusilla*, *L. orthopetala* and *L. punctata* in most of the cladograms. This suggests that *L. bifolia* in the eastern distribution probably hybridized with other species.

### 6.3.6 Future studies on *Lachenalia* and recommendations

Future studies should investigate the effect of adding more *Lachenalia* species, representing a broad geographic distribution, on the efficacy of DNA-barcoding in the genus. It is therefore recommended that the entire genus, represented by at least three samples per species, should be included in future studies. Species with a wide distribution should be represented by more samples.

Very complex taxonomic groups, for example hybrid swarms, may not have a species-specific DNA-barcode (Roy *et al.*, 2010) and adding more gene regions do not always provide



sufficient species-specific barcodes (Hollingsworth *et al.*, 2011). The use of combined datasets to improve identification of *Lachenalia* species should be investigated.

The challenge with *Lachenalia* is that some species seem to have developed through hybrid speciation that influences the groupings and consequently will have a great influence on the interpretation of any barcoding results, and this will lead to cladograms with poor resolution. The cladograms of the *trnH-psbA* region consist of species being polytomic (i.e. *L. bifolia* and *L. punctata*). The two chloroplast regions that are the most effective in distinguishing between species are *trnL-F* and *atpH-I*. Combining these regions with *ITS2* may provide sufficient data for barcoding analysis using tree-based methods.

Other studies (Li, D.-Z. *et al.*, 2011) have suggested that the combination of barcoding regions may improve the identification, but that it may not provide enough markers to distinguish between closely related species. To overcome this problem, the author suggested combining barcoding data with whole genome fingerprinting such as Amplified Fragment Length Polymorphisms (AFLPs). However, Fay *et al.* (2005) came to the conclusion that the ploidy level and DNA content per genome has an effect on the AFLP results. The AFLP traces of polyploid plants are of better quality compared to the lower AFLP quality observed in plants with in high DNA content (>15.0 pg) per genome. Therefore, for future studies a comprehensive genome size database should be established for the *Lachenalia* species to determine if AFLP analyses (combined with DNA-barcoding) can effectively be used in distinguishing between closely related *Lachenalia* species.

In addition, compensatory base changes (CBCs) can be investigated. CBCs are found in the secondary structures of the *ITS2* regions and has been successfully used in the identification of closely related taxa that are morphologically difficult to identify (Müller *et al.*, 2007; Ruhl *et al.*, 2010). The use of CBCs in the secondary structures of *ITS2* in *Lachenalia* can be explored in future studies, but it is predicted that the implementation of this technique in the identification of all the 133 *Lachenalia* species will be limited.

## 6.4 CONCLUSION

Several speciation events (hybridization, polyploidization, aneuploidy) were involved in

the evolution of *Lachenalia*, resulting in the observed morphological and chromosomal diversity.

Premating isolation mechanisms (geographical or ecological) and post-mating isolation mechanism (for example pollen tube competition and pollen-stigma incompatibility) (Soltis & Soltis, 2009) can isolate a species long enough to create a specific gene structure that is unique to that species. Species with limited gene flow due to, for example, isolation by distance or isolation due to different basic chromosome numbers, should have species-specific DNA-barcodes. In contrast, species such as the polyploid *L. bifolia* has several origins of polyploidization and do not have a unique DNA-barcode.

Many of the species with the same basic chromosome number share a common ancestor (Spies, 2004), and it is expected that there may be incomplete lineage sorting in some species resulting in non-specific DNA-barcodes. One such example is the species *L. unifolia* and *L. schlechteri* which share very similar DNA-barcodes.

The type of analysis (distance-based, tree-based or character-bases) influences the efficacy of the DNA-barcodes. The tree-based and character-based analyses have the best identification power since all the focus species of this study could be identified with these two methods. Some gene regions such as the *trnH-psbA*, and *atpH-I* have higher PCR and sequencing success, compared to other regions such as *matK* that has a lower success rate.

The *matK*, *trnL-F* and *ITS2* regions can be effectively used to identify an unknown specimen using tree-based analyses, in contrast to *trnH-psbA* which is more effective using a character-based analysis (probably due to the high number of INDELS). However, SNP analyses can be used in the analyses of *matK* and *atpH-I*. The combined *atpH-I* and *ITS2* datasets have the highest average posterior probability (0.9776). The combination of *atpH-I* + *trnL-F* and *trnL-F* + *ITS2* will effectively distinguish an unknown sample from the focus species.

Due to the large size of the genus, a two to three DNA-barcode locus is preferred over a single DNA-barcoding locus. The nuclear *ITS2* gene region has to be included as a DNA-barcode to detect hybrid species and plastid capture.

## Part D: DNA-barcode comparison

### *Chapter 7*

Comparison between *Clivia* (small genus) and  
*Lachenalia* (large genus)



## ABSTRACT

*Lachenalia* (family: Asparagaceae) is a large genus with 133 species and *Clivia* (family: Amaryllidaceae) a small genus with six species. DNA-barcoding as a means of species identification has been successfully implemented in many plant species. It has, however, been proven in several studies that the suggested core barcoding regions of the *matK* and *rbcLa* genes do not provide sufficient signal to identify plants on species level. The efficiency of the two core barcoding regions in combination with other regions (*atpH-I*, *ITS2*, *rpl16*, *rpoB*, *rpoC1*, *trnH-psbA*, *trnL-F*, *trnT-L*) were compared between the small (*Clivia*) and large (*Lachenalia*) genus. *Clivia* was represented by at least six samples per species. *Lachenalia* was represented by four focus species with seven to sixteen samples per species. The *matK* and *rbcLa* regions are only effective to identify three of the six *Clivia* species. These two regions are also insufficient for barcoding all the *Lachenalia* species. In both genera additional barcodes should be added. The most effective barcoding regions for *Clivia* are *matK* + *trnL-F* + *ITS2*, and for *Lachenalia* *trnL-F*, *ITS2* and *atpH-I*. Cautions should however be exercise for the existence of ancient hybrids, hybrid populations, hybrid complexes and incomplete lineage sorting in these genera. These factors may contribute to wrong identifications. To conclude, the combination of the core regions is ineffective in both genera. The chloroplast *trnL-F* and nuclear *ITS2*, together with one or more additional regions, should discriminate many *Clivia* and *Lachenalia* species.

## PREFACE

Previous chapters covered aspects of DNA-barcoding in the small genus *Clivia* and the large genus *Lachenalia*. This chapter will compare the DNA-barcoding data between the two genera and discuss their efficiency in each genus. Future recommendations for each genus are also discussed.

## 7.1. INTRODUCTION

*Clivia* is a small genus with six species; *Lachenalia* is large with 133 species. They are from different families, Amaryllidaceae and Asparagaceae respectively. *Clivia* has an ancient basic chromosome number ( $x = 11$ ) and *Lachenalia* has a range of basic chromosome numbers and ploidy levels (refer to Chapter 5). Both genera have species that are difficult to identify in

the vegetative state. This chapter will compare the DNA-barcoding regions in both genera to determine if barcoding can be successfully used to identify taxa from both *Clivia* and *Lachenalia*.

## 7.2. COMPARING GENE REGIONS

PCR success is in overall higher in *Lachenalia* than in *Clivia* (Table 7.1). The major factor may be the higher content of secondary metabolites in the old leaves of *Clivia* that was donated for this research. The PCR success may be improved with post PCR chloroform – phenol DNA cleanup. Sequencing success for *ITS2*, *trnT-L* and *trnL-F* is low in both genera.

**Table 7.1** A comparison between the PCR success and sequencing success of the different gene regions in the chloroplast and nucleus of *Clivia* and *Lachenalia*.

| Gene region      | PCR Success (%) |                           | Sequence Success (%) |                           |
|------------------|-----------------|---------------------------|----------------------|---------------------------|
|                  | <i>Clivia</i>   | <i>Lachenalia</i>         | <i>Clivia</i>        | <i>Lachenalia</i>         |
| <i>atpH-I</i>    | 83.1            | 88.8                      | 73.24                | 87.5                      |
| <i>ITS2</i>      | 71.8            | 93.3                      | 51.28                | 50                        |
| <i>matK</i>      | 100             | 73.4 (focus) – 100 (CCDB) | 100                  | 80.8 (focus) – 100 (CCDB) |
| <i>rbcLa</i>     | 100             | 100                       | 100                  | 100                       |
| <i>rpL16</i>     | 85.7            | 100                       | 85.71                | n/a                       |
| <i>rpoB</i>      | 100             | n/a                       | 79.59                | n/a                       |
| <i>rpoC1</i>     | 100             | n/a                       | 93.88                | n/a                       |
| <i>trnH-psbA</i> | 60.6            | 100                       | 36.36                | 100                       |
| <i>trnL-F</i>    | 75.9            | 73.9                      | 63.79                | 61.7                      |
| <i>trnT-L</i>    | 4.2             | 97.2                      | 0                    | 61.1                      |

There is a clear difference in the number of INDELS between *Clivia* and *Lachenalia* in many of the gene regions. An example is the 12.5% INDELS in *atpH-I* in *Lachenalia* compared to 2.2% in *Clivia* (Table 7.2). Also, the number of INDELS in *trnL-F* is much higher in *Lachenalia* than in *Clivia* (12.6% vs. 2.6%). *Clivia* on the other hand has a higher percentage INDELS in the *trnH-psbA* region (15.3% vs. 9.2%). INDELS in this region may result in alignment difficulties (Aldrich *et al.*, 1988; Liu *et al.*, 2011), which can be solved by using the PTIGS-IdIt system (Liu *et al.*, 2011).

## 7.3. EVALUATING THE CORE BARCODES IN A SMALL AND LARGE GENUS

The core barcoding regions for land plants are *matK* and *rbcLa*. A concatenated barcode of these two gene regions cannot distinguish all the species of neither *Clivia* nor *Lachenalia*.

Three *Clivia* species can be identified based on their monophyletic grouping, namely *C. caulescens*, *C. mirabilis* and *C. nobilis* (Figure 7.1). The other three species, *C. miniata*, *C. gardenii* and *C. robusta* are paraphyletic. This can be due to low signalling of the two gene regions, introgression, hybridization (ancient or recent) and incomplete lineage sorting. The core barcodes *matK* and *rbclA* are ineffective for the small genus *Clivia*.

**Table 7.2** Comparing the aligned lengths, identical sites (%), variable sites (%), GC content (%) and INDELS (%) between the gene regions of *Clivia* and *Lachenalia*.

| Gene region      | Aligned length (bp) |                   | Identical sites (%) |                   | Variable sites (%) |                   | GC content (%) |                   | INDELS (%)    |                   |
|------------------|---------------------|-------------------|---------------------|-------------------|--------------------|-------------------|----------------|-------------------|---------------|-------------------|
|                  | <i>Clivia</i>       | <i>Lachenalia</i> | <i>Clivia</i>       | <i>Lachenalia</i> | <i>Clivia</i>      | <i>Lachenalia</i> | <i>Clivia</i>  | <i>Lachenalia</i> | <i>Clivia</i> | <i>Lachenalia</i> |
| <i>atpH-I</i>    | 430                 | 471               | 94.9                | 69                | 1.4                | <b>2.17</b>       | 30.9           | 28.1              | 2.2           | <b>12.5</b>       |
| <i>ITS2</i>      | 262                 | 254               | 76.0                | 89.8              | <b>4.2</b>         | 3.52              | 69.5           | 72.7              | <b>1.7</b>    | 0                 |
| <i>matK</i>      | 826                 | 826               | 94.1                | 96.4              | 2.43               | 2.42              | 31.8           | 30.8              | 0             | <b>1.1</b>        |
| <i>rbclA</i>     | 548                 | 492               | 77.9                | 99.8              | 1.46               | 0.2               | 43.3           | 42.8              | 0             | 0                 |
| <i>rpl16</i>     | 886                 | n/a               | 97.5                | n/a               | 1.92               | n/a               | 33.5           | n/a               | 0.3           | n/a               |
| <i>rpoB</i>      | 387                 | n/a               | 97.2                | n/a               | 1.55               | n/a               | 39.6           | n/a               | 0             | n/a               |
| <i>rpoC1</i>     | 264                 | n/a               | 95.1                | n/a               | 1.89               | n/a               | 40.6           | n/a               | 0.7           | n/a               |
| <i>trnH-psbA</i> | 554                 | 533               | 78.6                | 73                | 2.17               | <b>3.75</b>       | 29.6           | 31.5              | <b>15.3</b>   | 9.2               |
| <i>trnL-F</i>    | 793                 | 889               | 72.3                | 66.3              | 2.2                | <b>3.59</b>       | 32             | 29.4              | 2.6           | <b>12.6</b>       |
| <i>trnT-L</i>    | n/c                 | 856               | n/c                 | 62.3              | n/c                |                   | n/c            | 21.3              | n/c           | 11.7              |

The genus *Lachenalia* is not fully represented by three or more samples for all 133 species, but already it can be concluded that the power of resolution for *matK* and *rbclA* will be too low to serve as a barcode. The combined data is represented as an unrooted tree in Figure 7.2. Some species are monophyletic, such as *L. punctata*, *L. bifolia* and *L. mediana*, some are paraphyletic, such as *L. unifolia* and *L. peersii*, and other species are unresolved in a polytomy. A large genus such as *Lachenalia* cannot be successfully barcoded with *matK* and *rbclA* to identify all 133 species.

#### 7.4. MOST EFFECTIVE REGIONS FOR THE SMALL AND LARGE GENUS

In *Clivia*, the *matK* gene is a good candidate though not as a single barcode. The *trnL-F* gene region in *Clivia* is a more effective region than *rbclA*. Therefore, a combined *matK* and *trnL-F* barcode is more effective than the *matK* / *rbclA* combination. Barcoding of the entire *Clivia* genus is, however, still impeded by hybridization and introgression in *C. miniata*, *C. gardenii* and *C. robusta*. I conclude that, even by the addition of more gene regions, the barcode efficacy will not improve until the existence of more natural hybrids have received recognition.

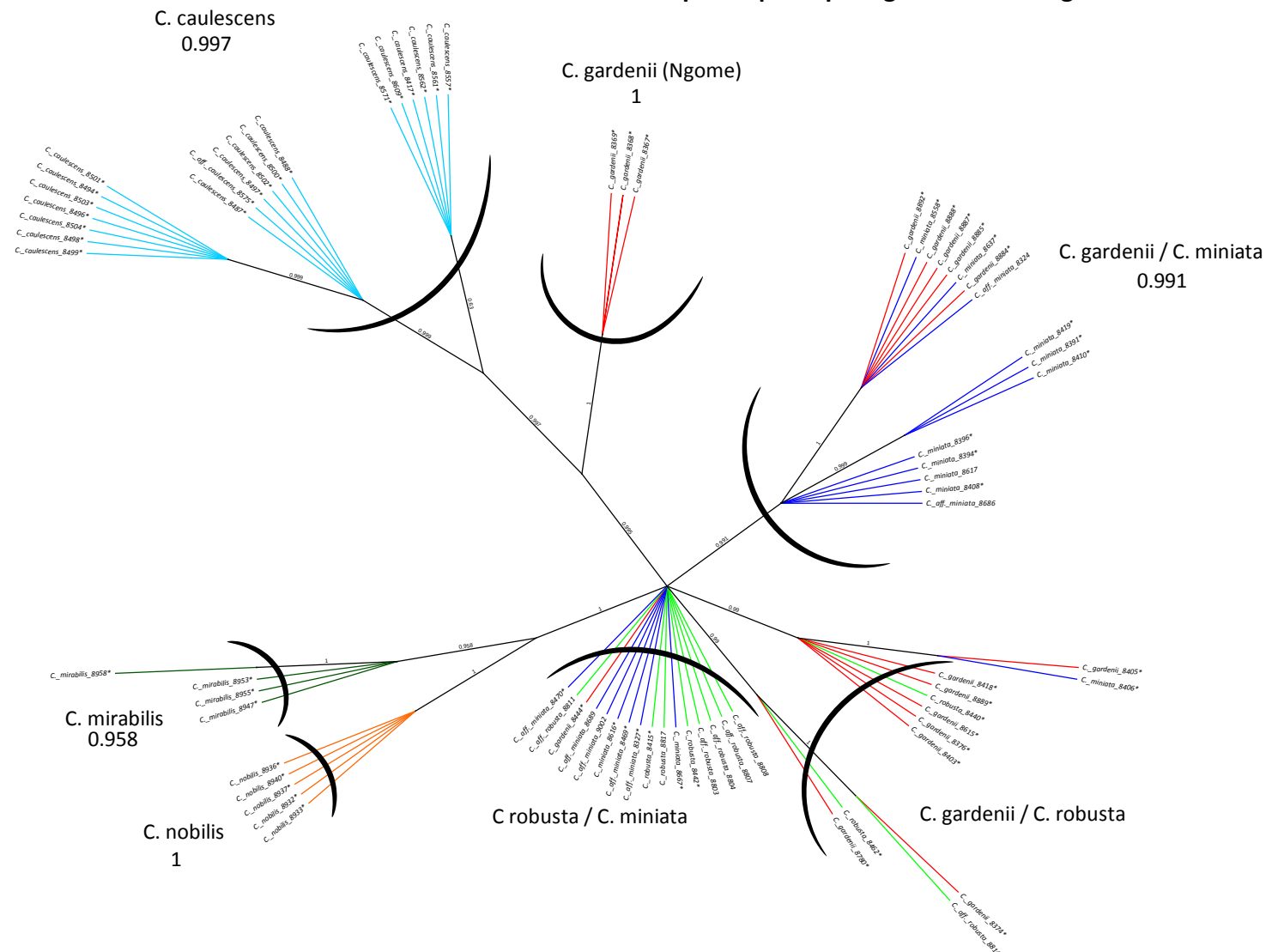


**Figure 7.1** *Clivia*: Unrooted tree of the combined *matK* and *rbcLa* matrix. Each species are represented by different coloured branches: Light blue – *C. caulescens*; Dark blue – *C. miniata*; Red – *C. gardenii*; Light green – *C. robusta*; Dark green – *C. mirabilis*; Orange – *C. nobilis*. The thick black curved lines link similar species together based on their monophyletic groupings (such as *C. robusta*/ *C. miniata*). The number under the collective names of these groupings is the posterior probabilities.

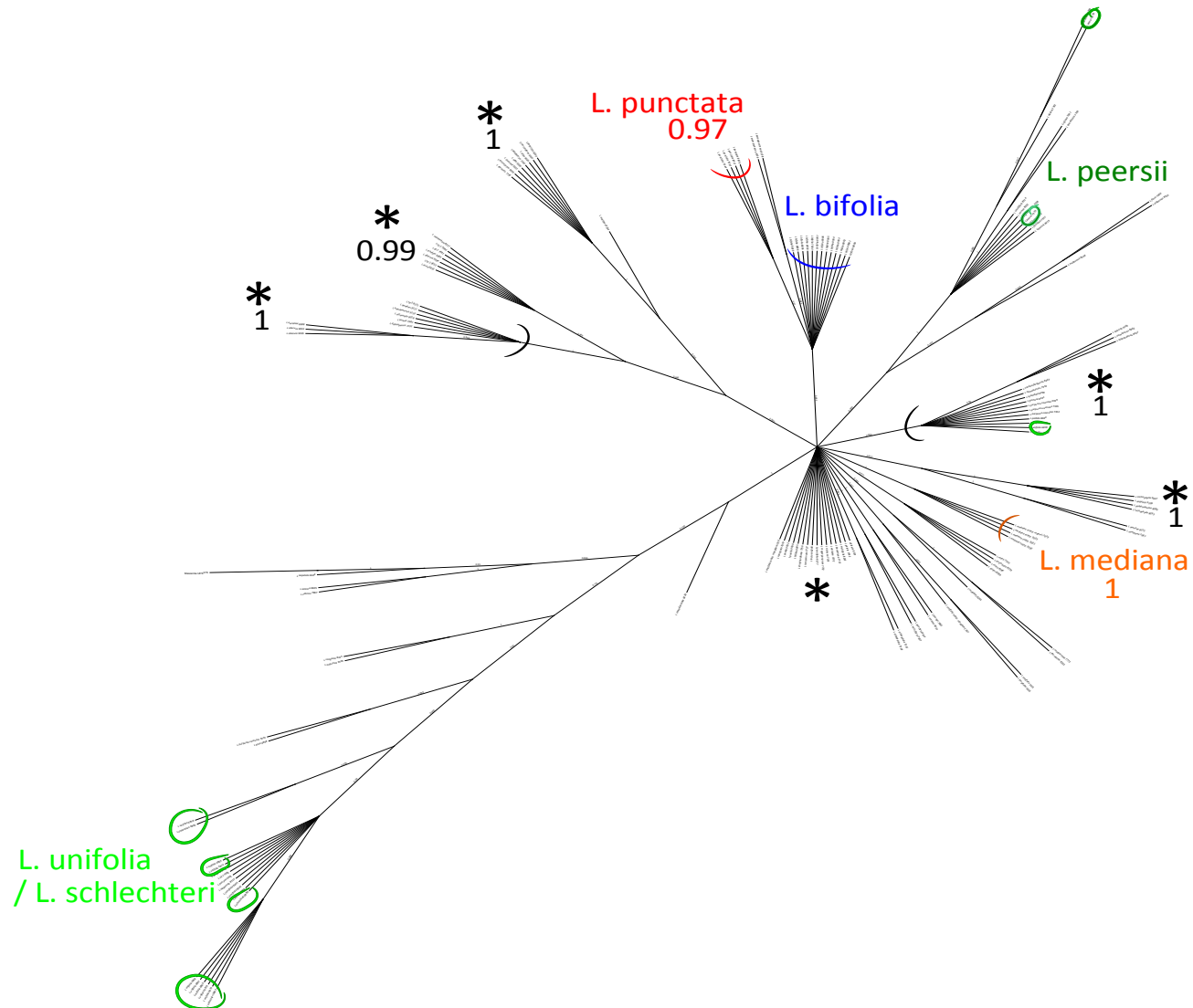


**Figure 7.2** *Lachenalia*: Unrooted tree of the combined *matK* and *rbcLa* matrix. Unresolved groupings are indicated with an asterisk (\*), resolved groupings are indicated with a coloured curved line and paraphyletic groupings are circled. The numbers represent the posterior probabilities and are indicated where available. Resolved groups: Red – *L. punctata*; Blue – *L. bifolia*; Orange – *L. mediana*. Unresolved or paraphyletic: Light green – *L. unifolia* / *L. schlechteri*; Dark green – *L. peersii*



In *Lachenalia* the two-locus barcode *atpH-I* + *trnL-F* shows potential as barcode. It proves to be an effective barcode to identify diverse *Lachenalia* species, e.g. *L. unifolia* ( $x = 11$ ) and *L. mediana* ( $x = 9$ ). The downfall of *atpH-I* is the large percentage of INDELS (12.5%) which can cause difficulty in aligning the sequences properly.

Equally effective is the *trnL-F* + *ITS2* gene regions. The sequencing of *ITS2* requires cloning in some species (Hamatani *et al.*, 2008). A barcoding region needs to amplify readily, thus *ITS2* is not ideal. The *trnL-F* region alone seems to have potential as barcode in *Lachenalia*, even though this region does not have sufficient discriminatory signal in the entire genus (Spies, 2004).

This study used many samples of a few representative species, thus focusing on the effect of large geographical distribution and large representatives per species. Because the majority of *Lachenalia* species in this study were morphologically different, we could generate barcodes for most of these species. However, closely related species may show a pattern of incomplete lineage sorting or barcodes with insufficient interspecific variation, therefore no clear barcodes. The conclusion from this study is that more species (well represented by enough samples) ought to be included in future studies. *Lachenalia* will most probably have, similar to *Clivia*, species that will be difficult to identify with DNA-barcodes.

Comparing the most effective gene regions between the two genera, the *trnL-F* and *ITS2* regions may be used universally for both *Clivia* and *Lachenalia* together with an additional region: *matK* in *Clivia* and *atpH-I* in *Lachenalia*. In both genera the addition of more regions may not improve the barcode potential due to hybrid speciation.

## 7.5. CONCLUSION

The chloroplast *trnL-F* region together with the nuclear *ITS2* region can be universally used for barcoding the small genus *Clivia* and the large genus *Lachenalia*. Both these regions will however, require an third region (or more in the case of *Lachenalia*) to be effective for identification of species.

Ancient hybridization, introgression and incomplete lineage sorting occurs in both genera. Therefore, certain species will not be effectively identified based on barcodes alone.

The barcoding may delimit the identification of such species into a specific 'species group' (such as the *C. gardenii* complex), after which additional methods such as taxonomic identification etc. (as discussed in Chapter 4) needs to be implemented for species-level identification.

There are some small differences between the DNA-barcoding of the small (*Clivia*) and the large genus (*Lachenalia*). Examples are the differences in sequencing success between regions of the different genera, differences in the number of INDELS between regions of the genera, and differences in the discriminatory power of the regions between the genera. However, the similarities between the genera are that identifications in both genera are influenced by the degree of hybridization (ancient or recent) and the time of divergence (thus incomplete lineage sorting).

I suggest that species should be clustered under different categories, and the barcoding of each category should be treated differently: **1)** "Old species" – these species were isolated long enough to have complete lineage sorting and they can be identified through conventional barcoding; **2)** Ancient hybrid species – these species may not hybridize in nature, but they may have evolved through hybrid speciation; **3)** Hybrid populations – these species may be sympatric in populations and need to be recognised as natural hybrids; **4)** "New species" – these species have evolved recently or are still in the process of evolving and will have incomplete lineage sorting in a cladogram. Categories two, three and four will need additional techniques implemented with the barcodes to identify species. Species in these groups may only be identified as belonging to a 'species group' based on barcodes alone. Therefore, only when the classification of *Clivia* and *Lachenalia* is properly resolved can barcodes be implemented for the species-level identification of these genera.

# Summary

*Clivia* (family Amaryllidaceae; tribe Haemantheae) with its six species is closest related to the genus *Cryptostephanus*. These genera share the highest chromosome numbers ( $2n = 22$  &  $24$  respectively) in the tribe and has similar  $2C$  values. Evolution in *Clivia* correlates with geographical distribution from west to east and genome size from small to large.

The tree, character and distance-based analyses are effective in the identification process of three *Clivia* species. *Clivia mirabilis*, *C. nobilis*, *C. miniata* and *C. caulescens* / *C. × nimbicola* can be identified with DNA-barcoding. The best barcoding regions regarding discriminatory power are the *trnL-F* chloroplast region, the *matK* chloroplast gene and the *ITS2* nuclear spacer region. A single DNA-barcoding locus is insufficient to be used in *Clivia* barcoding.

Hybridization events in *Clivia* may lead to false positive or false negative identifications. Analysis of an unknown sample resulting in a *C. miniata*, *C. gardenii* or *C. robusta* identification should need further analysis on additional data (e.g. morphology, distribution) to confirm the results. All the *C. × nimbicola* natural hybrids included in this study shared the chloroplast DNA of *C. caulescens*. Alternative methods should be developed to effectively distinguish and identify hybrid species.

The genus *Lachenalia* has 133 species and shows extensive morphological variation and exceptional diverse chromosome numbers ( $x = 5, 6, 7, 8, 9, 10, 11, 12, 13$  and  $15$ ). *Lachenalia* might have evolved from a common ancestor and the two largest basic chromosome number groups,  $x = 7$  and  $8$  have evolved from a common predecessor. It seems as if the higher basic numbers ( $x = 9, 10, 11$  and  $13$ ) evolved independently from the lower numbers.

Several speciation events were involved in the evolution of *Lachenalia*, resulting in the morphological and chromosomal diversity. Many of the species with the same basic chromosome numbers share a common ancestor, and it is expected that there may be incomplete lineage sorting in some species resulting in non-specific DNA-barcodes.

The tree-based and character-based analyses are effective methods to identify all the focus species (*L. unifolia*, *L. bifolia*, *L. punctata* and *L. mediana*) in this study.

The *matK*, *trnL-F* and *ITS2* regions results in the positive identification of an unknown specimen (tree-based analyses). SNP analyses can be used in the analyses of *matK* and *atpH-I*. The combination of *atpH-I* + *trnL-F* and *trnL-F* + *ITS2* will effectively distinguish an unknown sample of the focus species. Due to the large size of the genus, a two to three DNA-barcode locus is preferred over a single DNA-barcoding locus. The nuclear *ITS2* gene region has to be included as a DNA-barcode to detect hybrid species and plastid capture.

The chloroplast *trnL-F* region together with the nuclear *ITS2* region can be universally used for barcoding the small genus *Clivia* and the large genus *Lachenalia*. Both these regions require an third region (or more in the case of *Lachenalia*) to be effective for identification of species.

Ancient hybridization, introgression and incomplete lineage sorting occurs in both genera. Therefore, certain species will not be effectively identified based on barcodes alone.

There are some small differences between the DNA-barcoding of the small (*Clivia*) and the large genus (*Lachenalia*). However, the similarities between the genera are that identifications in both genera are influenced by the degree of hybridization (ancient or recent) and the time of divergence (thus incomplete lineage sorting).

The classification of *Clivia* and *Lachenalia* must be properly resolved before barcodes can be implemented for the species-level identification of these genera.

**Keywords:** Chromosome numbers, *Clivia*, DNA-barcoding, evolution, hybrid speciation, *Lachenalia*, networks, phylogeny, phylogeography

# Opsomming

*Clivia* (Familie Amaryllidaceae; Tribus Haemantheae), met sy ses spesies, is die naaste verwant aan die genus *Cryptostephanus* en deel die hoogste chromosoomgetalle ( $2n = 22$  &  $24$  onderskeidelik) in die tribus. Beide genera het soortgelyke  $2C$  waardes. Evolusie in *Clivia* stem ooreen met 'n geografiese verspreiding van wes na oos en genoom grootte van klein tot groot.

Die kladogram-, karakter- en afstandsgebaseerde ontledings is effektief in die identifiseringsproses van drie *Clivia*-spesies. *Clivia mirabilis*, *C. nobilis*, *C. miniata* en *C. caulescens*/*C. × nimbicola* kan geïdentifiseer word met DNA-strepieskodes. Die beste strepieskode (beste onderskeidingsvermoë) word met die *trnL-F* en die *matK* gebiede in die chloroplaste verkry, asook die kern se *ITS2* streek. 'n Enkele DNA-strepieskode lokus is onvoldoende om tussen *Clivia* spesies te onderskei.

Verbastering in *Clivia* kan lei tot vals positiewe of negatiewe identifikasies. Ontleding van 'n onbekende monster wat in 'n *C. miniata*, *C. gardenii* of *C. robusta* identifikasie eindig, verg verdere ontleding van die bykomende data (bv. morfologie, verspreiding) om die resultate te bevestig. Al die *C. × nimbicola* natuurlike kruisings in hierdie studie stem ooreen met die chloroplaste DNA van *C. caulescens*. Alternatiewe metodes moet ontwikkel word om baster spesies te identifiseer.

Die genus *Lachenalia* het 133 spesies en toon uitgebreide morfologiese variasie en uitsonderlik diverse chromosoomgetalle ( $x = 5, 6, 7, 8, 9, 10, 11, 12, 13$  en  $15$ ). *Lachenalia* kon ontwikkel het uit 'n gemeenskaplike voorouer en die twee grootste groepe met basiese chromosoomgetalle van  $x = 7$  en  $8$  het moontlik ontwikkel uit 'n gemeenskaplike voorvader. Dit lyk asof die hoër basiese getalle ( $x = 9, 10, 11$  en  $13$ ) onafhanklik ontwikkel het uit laer getalle.

Verskeie spesiasieprosesse was betrokke by die evolusie van *Lachenalia* en het gelei tot die morfologiese en chromosomale diversiteit. Baie van die spesies met dieselfde basiese chromosoomgetalle deel 'n gemeenskaplike voorouer, en dit word verwag dat daar dalk onvolledige lyne in sommige spesies kan voorkom wat lei tot nie-spesifieke DNA-strepieskodes.

Die kladogram- en karakter-gebaseerde ontledings is effektiewe metodes om al die fokus spesies (*L. unifolia*, *L. bifolia*, *L. punctata* en *L. mediana*) in hierdie studie te identifiseer.

Die *matK*, *trnL-F* en *ITS2* streke lewer 'n positiewe identifikasie van 'n onbekende eksemplaar (kladogram-gebaseerde ontledings). SNP ontledings kan gebruik word in die ontleding van *matK* en *atpH-I*. Die kombinasie van *atpH-I* + *trnL-F* asook *trnL-F* + *ITS2* sal effektief kan onderskei indien 'n onbekende monster van die fokus spesies bestudeer word. As gevolg van die omvang van die genus, is 'n 2-3 DNA-strepiesskode lokus verkieslik bo 'n enkele DNA-strepiesskode lokus. Die kern *ITS2* streek moet as 'n DNA-strepiesskode ingesluit word om basterspesies en introgressie waar te neem.

Die chloroplast *trnL-F* streek saam met die kern *ITS2* streek kan universeel gebruik word vir as DNA-strepiesskode in beide die klein genus *Clivia* en die groot genus *Lachenalia*. Albei streke vereis egter dat 'n derde streek (of selfs meer in die geval van *Lachenalia*) nodig is vir die identifisering van spesies.

Oerverbastering, introgressie en onvolledige geslagslyn sortering vind plaas in beide genera. Derhalwe sal sekere spesies nie effektief geïdentifiseer kan word op grond van slegs strepiesskodes nie.

Daar is 'n paar klein verskille tussen die DNA-strepiesskode van die klein (*Clivia*) en die groot genus (*Lachenalia*). Maar die ooreenkomste tussen die genera is dat die identifisering in beide genera deur die graad van verbastering (oer of onlangse) en die tyd van divergensie (dus onvolledig geslagslyn sortering) beïnvloed word.

Die klassifikasie van *Clivia* en *Lachenalia* moet behoorlik uitsorteer word voordat strepiesskodes vir spesie-vlak identifikasie van hierdie genera geïmplementeer kan word.

**Sleutelwoorde:** Chromosoomgetalle, *Clivia*, DNA-strepiesskodering, evolusie, basterspesiasie, *Lachenalia*, netwerke, filogenie, filogeografie



# References

- Abd-El Salam, K.A. 2003. Bioinformatic tools and guideline for PCR primer design. *African Journal of Biotechnology* 2: 91–95.
- Adams, K.L. & Palmer, J.D. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Molecular Phylogenetics and Evolution* 29: 380–395.
- Adendorff, J. 2008. Ultraviolet: the light fantastic. *Veld & Flora*: 22–25.
- Albu, M., Nikbakht, H., Hajibabaei, M. & Hickey, D.A. 2011. The DNA Barcode Linker. *Molecular Ecology Resources* 11: 84–88.
- Aldrich, J., Cherney, B.W., Merlin, E. & Christopherson, L. 1988. The role of insertions/deletions in the evolution of the intergenic region between psbA and trnH in the chloroplast genome. *Current Genetics* 14: 137–146.
- Alejandro, G.J.D., Meve, U., Mouly, A., Thiv, M. & Liede-Schumann, S. 2011. Molecular phylogeny and taxonomic revision of the Philippine endemic *Villaria* Rolfe (Rubiaceae). *Plant Systematics and Evolution* 296: 1–20.
- Ali, A.A., Ross, S.A., El-Moghazy, A. & El-Moghazy, S. 1983. Clivonidine, a new alkaloid from *Clivia miniata*. *Journal of Natural Products* 46: 350–352.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Álvarez, I. & Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.
- Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Armenise, L., Simeone, M.C., Piredda, R. & Schirone, B. 2012. Validation of DNA barcoding as an efficient tool for taxon identification and detection of species diversity in Italian conifers. *European Journal of Forest Research* 131: 1337–1353.
- Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R. & Potas Bircher, C. 2002. *Medicinal and magical plants of southern Africa: An annotated checklist*. National Botanical Institute, Pretoria, South Africa.
- Baker, J.G. 1897. *Lachenalia*. *Flora Capensis* pp. 421–436. Reeve & Co., London.
- Baker, D.A., Stevenson, D.W. & Little, D.P. 2012. DNA barcode identification of black cohosh herbal dietary supplements. *Journal of AOAC International* 95: 1023–1034.
- Baker, T.S., Suh, S.W. & Eisenberg, D. 1977. Structure of ribulose-1,5-bisphosphate carboxylase-oxygenase: Form III crystals. *Proceedings of the National Academy of Sciences of the United States of America* 74: 1037–1041.

- Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. 1995. The *ITS* region of nuclear ribosomal DNA: A valuable source of evidence on Angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247.
- Bandelt, H.J., Forster, P. & Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.
- Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P. & Casiraghi, M. 2010. DNA barcoding reveals fraudulent substitutions in shark seafood products: The Italian case of “palombo” (*Mustelus* spp.). *Food Research International* 43: 376–381.
- Barker, W.F. 1983. A list of the *Lachenalia* species included in Rudolf Schlechter’s collections made in 1891–1898 on his collection trips in southern Africa, with identifications added. *Journal of South African Botany* 49: 45–55.
- Barns, S.M., Delwiche, C.F., Palmer, J.D. & Pace, N.R. 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proceedings of the National Academy of Sciences of the United States of America* 93: 9188–9193.
- Barrabé, L., Buerki, S., Mouly, A., Davis, A.P., Munzinger, J. & Maggia, L. 2012. Delimitation of the genus *Margaritopsis* (Rubiaceae) in the Asian, Australasian and Pacific region, based on molecular phylogenetic inference and morphology. *Taxon* 61: 1251–1268.
- Barthet, M.M. & Hilu, K.W. 2007. Expression of *matK*: functional and evolutionary implications. *American Journal of Botany* 94: 1402–1412.
- Bay-Smith, M.G., Jäger, A.K., Krydsfeldt, K., Meerow, A.W., Stafford, G.I., Van Staden, J. & Rønsted, N. 2011. Phylogenetic selection of target species in Amaryllidaceae tribe Haemantheae for acetylcholinesterase inhibition and affinity to the serotonin reuptake transport protein. *South African Journal of Botany* 77: 175–183.
- Bennett, M.D. 1972. Nuclear DNA content and minimum generation time in herbaceous plants. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 181: 109–135.
- Bennett, M.D. 1977. The time and duration of meiosis [and discussion]. *Philosophical Transactions of the Royal Society of London, Series B - Biological Sciences* 277: 201–277.
- Bennett, A.D.T. & Cuthill, I.C. 1994. Ultraviolet vision in birds: what is its function? *Vision Research* 34: 1471–1478.
- Bennett, M.D. & Leitch, I.J. 2005. Nuclear DNA amounts in angiosperms: Progress, problems and prospects. *Annals of Botany* 95: 45–90.
- Bennett, M.D., Leitch, I.J. & Hanson, L. 1998. DNA amounts in two samples of Angiosperm weeds. *Annals of Botany* 82: 121–134.

- Bennett, M.D. & Smith, J.B. 1976. Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society of London, Series B - Biological Sciences* 274: 227–274.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Wheeler, D.L. 2008. GenBank. *Nucleic Acids Research* 36: D25–D30.
- Bergmann, T., Hadrys, H., Breves, G. & Schierwater, B. 2009. Character-based DNA barcoding: a superior tool for species classification. *Berliner und Münchener Tierärztliche Wochenschrift* 122: 446–450.
- Bertolazzi, P., Felici, G. & Weitschek, E. 2009. Learning to classify species with barcodes. *BMC Bioinformatics* 10: S7.
- Bhargava, M. & Sharma, A. 2013. DNA barcoding in plants: Evolution and applications of in silico approaches and resources. *Molecular Phylogenetics and Evolution* 67: 631–641.
- Bienert, M. 1969. *Zur Struktur und Stereochemie Einiger Lactonalkaloide aus Clivia miniata*. M.Sc., Humboldt-University, Faculty of Mathematics and Natural Sciences., Berlin.
- Blaxter, M.L. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 669–679.
- Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R. & Abebe, E. 2005. Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1935–1943.
- Bohmann, K., Monadjem, A., Lehmkuhl Noer, C., Rasmussen, M., Zeale, M.R.K., Clare, E., Jones, G., Willerslev, E. & Gilbert, M.T.P. 2011. Molecular diet analysis of two African free-tailed bats (Molossidae) using high throughput sequencing. *PLoS ONE* 6. e21441
- Boit, H.-G. & Mehliß, B. 1961. Clivimin und Clivatin, zwei neue Alkaloide aus *Clivia miniata*. *Die Naturwissenschaften* 48: 603–603.
- Booyesen, E. 2003. *Cladogram resolution with different chloroplast DNA sequences in the genus Crinum*. University of the Free State, Department of Genetics, Bloemfontein, South Africa.
- Broster, J.A. & Bourn, H. 1982. *Amagqirha: Religion, magic and medicine in Transkei*. Via Africa, Goodwood, South Africa.
- Brown, S.D.J., Collins, R.A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C.J. & Cruickshank, R.H. 2012. Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources* 12: 562–565.
- Brunton, C.F.A. & Majerus, M.E.N. 1995. Ultraviolet colours in butterflies: Intra- or inter-specific communication? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 260: 199–204.

- Bryant, A.T. 1909. *Zulu Medicine and Medicine Men*. C. Struik. London.
- Burkhardt, D. & Maier, E. 1989. The spectral sensitivity of a passerine bird is highest in the UV. *Naturwissenschaften* 76: 82–83.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA*, 106: 12794–12797
- CBOL. 2010. Barcode of Life. Identifying species with DNA barcoding. *What is CBOL?* [online]. Available from <http://www.barcodeoflife.org/content/about/what-cbol> [accessed October 2012]
- Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C., Madriñán, S., Petersen, G., Seberg, O., Jørgensen, T., Cameron, K.M., Carine, M., Pedersen, N., Hedderson, T.A.J., Conrad, F., Salazar, G.A., Richardson, J.E., Hollingsworth, M.L., Barraclough, T.G., Kelly, L. & Wilkinson, M. 2007. A proposal for a standardised protocol to barcode all land plants. *Taxon* 56: 295–299.
- Chase, M.W. & Fay, M.F. 2009. Barcoding of Plants and Fungi. *Science* 325: 682–683.
- Chase, M.W., Salamin, N., Wilkinson, M., Dunwell, J.M., Kesanakurthi, R.P., Haidar, N. & Savolainen, V. 2005. Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1889–1895.
- Chen, R.Y., Song, W.Q., Li, X.L., Li, M.X., Liang, G.L. & Chen, C.B. 2003. *Chromosome Atlas of major economic plants genome in China, Vol. 3, Chromosome Atlas of Garden Flowering Plants in China*. Science Press, Beijing.
- Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. & Leon, C. 2010. Validation of the *ITS2* region as a novel DNA barcode for identifying medicinal plant species. *PloS one* 5: e8613.
- Cho, Y., Qiu, Y.-L., Kuhlman, P. & Palmer, J.D. 1998. Explosive invasion of plant mitochondria by a group I intron. *Proceedings of the National Academy of Sciences* 95: 14244–14249.
- Choi, J.-S., Kim, J.-S., Joe, C.-O., Kim, S., Ha, K.-S. & Park, Y.-M. 1999. Improved cycle sequencing of GC-rich DNA template. *Experimental and Molecular Medicine* 31: 20–24.
- Clare, E.L., Barber, B.R., Sweeney, B.W., Hebert, P.D.N. & Fenton, M.B. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology* 20: 1772–1780.
- Coghlan, M.L., White, N.E., Parkinson, L., Haile, J., Spencer, P.B.S. & Bunce, M. 2012. Egg forensics: An appraisal of DNA sequencing to assist in species identification of illegally smuggled eggs. *Forensic Science International: Genetics* 6: 268–273.

- Conrad, F. 2008. *Molecular systematic, biogeography and dating of the tribe Haemantheae (Amaryllidaceae) and the phylogeography of Clivia*. PhD dissertation, University of Cape Town, Cape Town, South Africa.
- Conrad, F., Reeves, G. & Rourke, J.P. 2003. Phylogenetic relationships of the recently discovered species: *Clivia mirabilis*. *South African Journal of Botany* 69: 204–206.
- Conran, J.G. 2005. Ultraviolet reflectance patterns in some Agapanthaceae, Alliaceae and Amaryllidaceae and their possible significance for pollination. *Herbertia* 58: 76–90.
- Costa, P., Pot, D., Dubos, C., Frigerio, J.M., Pionneau, C., Bodenes, C., Bertocchi, E., Cervera, M.-T., Remington, D.L. & Plomion, C. 2000. A genetic map of Maritime pine based on AFLP, RAPD and protein markers. *Theoretical and Applied Genetics* 100: 39–48.
- Cowan, R.S. & Fay, M.F. 2012. Challenges in the DNA Barcoding of plant material. *Plant DNA Fingerprinting and Barcoding Methods in Molecular Biology*. (eds N.J. Sucher, J.R. Hennell & M.C. Carles), pp. 23–33. Humana Press.
- Crosby, T.S. 1978. Hybridisation in the genus *Lachenalia*. *Veld and Flora* September: 87–90.
- Crosby, T.S. 1986. The genus *Lachenalia*. *The Plantsman* 8: 129–166.
- Crouch, N.R., Mulholland, D.A., Pohl, T.L. & Ndlovu, E. 2003. The ethnobotany and chemistry of the genus *Clivia* (Amaryllidaceae). *South African Journal of Botany* 69: 144–147.
- Cullings, K. & Vogler, D.R. 1998. A 5.8S nuclear ribosomal RNA gene sequence database: applications to ecology and evolution. *Molecular ecology* 7: 919–923.
- Cunningham, A.B. 1988. *An Investigation of the Herbal Medicinal Trade in Natal/Kwazulu. Institute of Natural Resources Report No 29*. University of Natal, Pietermaritzburg, South Africa.
- Czapic, R. 1968. Chromosome numbers of *Ornithogalum umbellatum* L. from three localities in England. *Watsonia* 6: 345–349.
- Dahlgren, R.M.T., Clifford, H.T. & Yeo, P.F. 1985. *Families of the Monocotyledons: Structure, Evolution, and Taxonomy*. Springer-Verlag. London
- Dalton, D.L. & Kotze, A. 2011. DNA barcoding as a tool for species identification in three forensic wildlife cases in South Africa. *Forensic Science International* 207: e51–54.
- Damme, E.J., Smeets, K., Leuven, F. & Peumans, W.J. 1994. Molecular cloning of mannose-binding lectins from *Clivia miniata*. *Plant Molecular Biology* 24: 825–830.
- Darling, J.A. & Blum, M.J. 2007. DNA-based methods for monitoring invasive species: a review and prospectus. *Biological Invasions* 9: 751–765.
- Darwish, F.A., Bauer, R. & Hammada, H.M. 2002. High pressure liquid chromatographic (HPLC) method for determination of nobilisitine A and nobilistine B in *Clivia nobilis* grown in Egypt. *Alexandria Journal of Pharmaceutical Sciences* 16: 93–95.

- DasGupta, B., Konwar, K.M., Măndoiu, I.I. & Shvartsman, A.A. 2005. DNA-BAR: distinguisher selection for DNA barcoding. *Bioinformatics* 21: 3424–3426.
- De Groot, G.A., During, H.J., Maas, J.W., Schneider, H., Vogel, J.C. & Erkens, R.H.J. 2011. Use of *rbcl* and *trnL-F* as a Two-Locus DNA Barcode for Identification of NW-European Ferns: An Ecological Perspective. *PLoS ONE* 6: e16371.
- De Mattia, F., Bruni, I., Galimberti, A., Cattaneo, F., Casiraghi, M. & Labra, M. 2011. A comparative study of different DNA barcoding markers for the identification of some members of Lamiaceae. *Food Research International* 44: 693–702.
- DeSalle, R. 2006. What's in a character? *Journal of Biomedical Informatics* 39: 6–17.
- DeSalle, R., Egan, M.G. & Siddall, M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1905–1916.
- De Smedt, V., van Huylenbroeck, J.M. & Debergh, P.C. 1996. Influence of temperature and supplementary lighting on growth and flower initiation of *Clivia miniata* Regel. *Scientia Horticulturae* 65: 65–72.
- Desmyter, S. & Gosselin, M. 2009. COI sequence variability between Chrysomyinae of forensic interest. *Forensic Science International: Genetics* 3: 89–95.
- De Vere, N., Rich, T.C.G., Ford, C.R., Trinder, S.A., Long, C., Moore, C.W., Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S., Tatarinova, T., Garbett, H., Walker, K. & Wilkinson, M.J. 2012. DNA Barcoding the native flowering plants and conifers of Wales. *PLoS ONE* 7: e37945.
- De Wet, J.M.J. 1957. Chromosome numbers in the Scilleae. *Cytologia* 22: 145–159.
- Dexter, K.G., Pennington, T.D. & Cunningham, C.W. 2010. Using DNA to assess errors in tropical tree identifications: How often are ecologists wrong and when does it matter? *Ecological Monographs* 80: 267–286.
- Dlamini, B. 1981. *Swaziland Flora: their local names and uses*. Ministry of Agriculture and co-operatives, Mbabane, Swaziland.
- Dold, A.P. & Phillipson, P.B. 1998. A revision of *Lachenalia* (Hyacinthaceae) in the Eastern Cape, South Africa. *Bothalia* 28: 141–149.
- Doležel, J., Greilhuber, J., Lucretti, S., Meister, A., Lysák, M.A., Nardi, L. & Obermayer, R. 1998. Plant Genome Size Estimation by Flow Cytometry: Inter-laboratory Comparison. *Annals of Botany* 82: 17–26.
- Dong, L.N., Wortley, A.H., Wang, H., Li, D.Z. & Lu, L. 2011. Efficiency of DNA barcodes for species delimitation: a case in *Pterygiella* Oliv.(Orobanchaceae). *Journal of Systematics and Evolution* 49: 189–202.

- Doyle, J.J. & Dickson, E.E. 1987. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722.
- Doyle, J. & Doyle, J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Dubey, B., Meganathan, P.R. & Haque, I. 2011. DNA mini-barcoding: An approach for forensic identification of some endangered Indian snake species. *Forensic Science International: Genetics* 5: 181–184.
- Duncan, G.D. 1988. *The Lachenalia Handbook*. National Botanical Institute (NBI). Kirstenbosch
- Duncan, G.D. 1992. The genus *Lachenalia*: its distribution, conservation status and taxonomy. *Acta Horticulturae* pp. 843–845.
- Duncan, G.D. 1996. Four new species and one new subspecies of *Lachenalia* (Hyacinthaceae) from arid areas of South Africa. *Bothalia* 26: 1–9.
- Duncan, G.D. 1998. Five new species of *Lachenalia* (Hyacinthaceae) from arid areas of Namibia and South Africa. *Bothalia* 28: 131–139.
- Duncan, G. 2001. Plate 408. *Lachenalia elegans* var. *flava*. *Curtis's Botanical Magazine* 18: 18–22.
- Duncan, G.D. 2002. *Lachenalia*. *The color encyclopedia of Cape bulbs* pp. 251–264. Timber Press, Cambridge, UK.
- Duncan, G. 2003. Endangered Geophytes of the Cape Floral Kingdom. *Curtis's Botanical Magazine* 20: 245–250.
- Duncan, G.D. 2005. *Character variation and a cladistic analysis of the genus Lachenalia Jacq.f. ex Murray (Hyacinthaceae: Massonieae)*. M.Sc. thesis, University of KwaZulu-Natal.
- Duncan, G.D. 2008. *Grow Clivias*, 2nd Revised edition. National Botanical Institute, Kirstenbosch, South Africa.
- Duncan, G. 2010. 666. *Cryptostephanus densiflorus*. *Curtis's Botanical Magazine* 27: 10–22.
- Duncan, G. 2012. *The genus Lachenalia*. Royal Botanic Gardens, Kew.
- Duncan, G.D. & Edwards, T.J. 2006. Three new species of *Lachenalia* (Hyacinthaceae: Massonieae) from Western and Northern Cape, South Africa. *Bothalia* 36: 147–155.
- Duncan, G.D. & Edwards, T.J. 2007. Hyacinthaceae: A new pyrophytic *Lachenalia* species (Massonieae) from Western Cape, South Africa. *Bothalia* 37: 31–34.
- Duncan, G.D., Edwards, T.J. & Mitchell, A. 2005. Character variation and a cladistic analysis of the genus *Lachenalia* Jacq. f. ex Murray (Hyacinthaceae). *Acta Horticulturae* pp. 113–120.



- Du Plessis, N.M. & Duncan, G.D. 1989. *Bulbous plants of southern Africa*. Tafelberg Publishers, South Africa, Cape Town.
- Du Preez, J.L., Spies, J.J. & Kleynhans, R. 2002. A preliminary study of interspecific hybrids in *Lachenalia* (Hyacinthaceae). *Acta Horticulturae* 570 pp. 319–325.
- Dyer, R.A. 1943. *Clivia caulescens*. *The flowering plants of South Africa* 23: t.891.
- Ebach, M.C. & de Carvalho, M.R. 2010. Anti-intellectualism in the DNA barcoding enterprise. *Zoologia* 27: 165–178.
- Ebach, M.C. & Holdrege, C. 2005a. More taxonomy, not DNA barcoding. *BioScience* 55: 823–824.
- Ebach, M.C. & Holdrege, C. 2005b. DNA barcoding is no substitute for taxonomy. *Nature* 434: 697–697.
- Edwards, A.W. & Cavalli-Sforza, L.L. 1964. Reconstruction of evolutionary trees. *Phenetic and Phylogenetic Classification* 6: 67–76.
- Erixon, P., Svennblad, B., Britton, T. & Oxelman, B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* 52: 665–673.
- Evidente, A., Abou-Donia, A.H., Darwish, F.A., Amer, M.E., Kassem, F.F., Hammada, H.A. & Motta, A. 1999. Nobilisitine a and B, two masanane-type alkaloids from *Clivia nobilis*. *Phytochemistry* 51: 1151–1155.
- Fay, M.F., Cowan, R.S. & Leitch, I.J. 2005. The Effects of Nuclear DNA Content (C-value) on the Quality and Utility of AFLP Fingerprints. *Annals of Botany* 95: 237–246.
- Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W., Newmaster, S.G., Husband, B.C., Percy, D.M., Hajibabaei, M. & Barrett, S.C.H. 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE* 3: e2802.
- Felsenstein, J. 1989. PHYLIP - Phylogeny Interference Package (Version 3.2). *Cladistics* 5: 164–166.
- Ferri, G., Alù, M., Corradini, B. & Beduschi, G. 2009. Forensic botany: species identification of botanical trace evidence using a multigene barcoding approach. *International Journal of Legal Medicine* 123: 395–401.
- Ford, C.S., Ayres, K.L., Toomey, N., Haider, N., Van Alphen Stahl, J., Kelly, L.J., Wikström, N., Hollingsworth, P.M., Duff, R., Hoot, S.B., Cowan, R.S., Chase, M.W. & Wilkinson, M.J. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159: 1–11.
- Franke, W.W., Herth, W., VanDerWoude, W.J. & Morré, D.J. 1972. Tubular and filamentous structures in pollen tubes: Possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. *Planta* 105: 317–341.

- Fu, Y.M., Jiang, W.M. & Fu, C.X. 2011. Identification of species within *Tetrastigma* (Miq.) Planch. (Vitaceae) based on DNA barcoding techniques. *Journal of Systematics and Evolution* 49: 237–245.
- Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P. & Firoozabady, E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* 220: 1049–1051.
- Geoffroy, A., Le Gall, L. & Destombe, C. 2012. Cryptic introduction of the red alga *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) in the North Atlantic Ocean highlighted by a DNA barcoding approach. *Aquatic Botany* 100: 67–71.
- Gerstner, J. 1941. A preliminary check list of Zulu names of plants. *Bantu Studies* 15: 277–301.
- Ghosal, S., S. Saini, K. & Razdan, S. 1985. *Crinum* alkaloids: their chemistry and biology. *Phytochemistry* 24: 2141–2156.
- Gismondi, A., Rolfo, M.F., Leonardi, D., Rickards, O. & Canini, A. 2012. Identification of ancient *Olea europaea* L. and *Cornus mas* L. seeds by DNA barcoding. *Comptes Rendus Biologies* 335: 472–479.
- Goldblatt, P. 1980. Polyploidy in Angiosperms: Monocotyledons. *Polyploidy, biological relevance*. Basic life sciences. pp. 219–240. Plenum Press, New York, USA.
- Goldblatt, P. & Manning, J.C. 2000. The long-proboscid fly pollination system in southern Africa. *Annals of the Missouri Botanical Garden* 87: 146170
- Goldblatt, P. & Manning, J.C. 2002. Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Garden* 89: 281302.
- Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J.C., Barraclough, T.G. & Chase, M.W. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* 25: 341–360.
- Goloboff, P.A., Farris, J.S. & Nixon, K.C. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Gouws, J.B. 1949. Karyology of some South African Amaryllidaceae. *Plant Life* 5: 54–80.
- Gouws, J.B. 1965. Cytological studies in the genus *Lachenalia* Jacq. *Annals of the University College of the Western Cape* 2: 1–7.
- Grant, V. 1981. *Plant Speciation*, 2nd ed. Columbia University Press.
- Guo, X., Simmons, M.P., But, P.P.-H., Shaw, P.-C. & Wang, R.-J. 2011. Application of DNA barcodes in *Hedyotis* L. (Spermacoceae, Rubiaceae). *Journal of Systematics and Evolution* 49: 203–212.

- Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* 103: 968–971.
- Hajibabaei, M. & Singer, G. 2009. Googling DNA sequences on the World Wide Web. *BMC Bioinformatics* 10: S4.
- Hajibabaei, M., Singer, G., Clare, E. & Hebert, P. 2007a. Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. *BMC Biology* 5: 24.
- Hajibabaei, M., Singer, G.A., Hebert, P.D. & Hickey, D.A. 2007b. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23: 167–172.
- Hall, B.G. 2011. *Phylogenetic Trees Made Easy: a how to manual*, Fourth edition. Sinauer Associates, Inc.
- Hamatani, S., Hashimoto, K. & Kondo, K. 1998. A comparison of somatic chromosomes at metaphase in *Lachenalia* (Liliaceae). *Chromosome Science* 2: 21–25.
- Hamatani, S., Ishida, G., Hashimoto, K. & Kondo, K. 2004. A chromosome study in ten species of *Lachenalia* (Liliaceae). *Chromosome Science* 8: 55–61.
- Hamatani, S., Kondo, K., Kodaira, E. & Ogawa, H. 2007. Chromosome morphology of 12 species and one variety of *Lachenalia* and five species of closely related, allied genera (Liliaceae). *Chromosome Botany* 2: 79–86.
- Hamatani, S., Masuda, Y., Kondo, K., Kodaira, E. & Ogawa, H. 2008. Molecular phylogenetic relationships among *Lachenalia*, *Massonia* and *Polyxena* (Liliaceae) on the basis of the internal transcribed spacer (ITS) region. *Chromosome Botany* 3: 65–72.
- Hamatani, S., Tagashira, N., Ishida, G. & Kondo, K. 2009. Chromosome relationships among 13 species and one variety of *Lachenalia* (Liliaceae) with the chromosome numbers of  $2n=14$  and 16 detected by FISH using 5S rDNA and 18S rDNA probes and DAPI staining. *Chromosome Botany* 4: 57–63.
- Hamatani, S., Tagashira, N. & Kondo, K. 2010. Molecular cytogenetic analysis in seven species of *Lachenalia* (Liliaceae) with the chromosome numbers of  $2n = 18, 22, 23, 26$  and 28 by DAPI staining and FISH using 5S rDNA and 18S rDNA probes. *Chromosome Botany* 5: 55–59.
- Han, J.-P., Shi, L.-C., Chen, X.-C. & Lin, Y.-L. 2012. Comparison of four DNA barcodes in identifying certain medicinal plants of Lamiaceae. *Journal of Systematics and Evolution* 50: 227–234.
- Hancke, F.L. 1991. *'n Sitotaksonomiese ondersoek van sewe Lachenalia spesies vir gebruik in 'n Blomteeltprogram*. M.Sc. thesis, University of Pretoria.

- Hancke, F.L. & Liebenberg, H. 1990. B-chromosomes in some *Lachenalia* species and hybrids. *South African Journal of Botany* 56: 659–664.
- Hancke, F.L. & Liebenberg, H. 1998. Meiotic studies of interspecific *Lachenalia* hybrids and their parents. *South African Journal of Botany* 64: 250–255.
- Hancke, F.L., Liebenberg, H. & Janse van Rensburg, W.J. 2001. Chromosome associations of three interspecific, dibasic *Lachenalia* hybrids. *South African Journal of Botany* 67: 193–198.
- Hanner, R. 2009. Data standards for BARCODE records in INSDC (BRIs) [online]. Available from [http://barcoding.si.edu/pdf/dwg\\_data\\_standards-final.pdf](http://barcoding.si.edu/pdf/dwg_data_standards-final.pdf) [Accessed April 2013].
- Hanner, R., Becker, S., Ivanova, N.V. & Steinke, D. 2011. FISH-BOL and seafood identification: Geographically dispersed case studies reveal systemic market substitution across Canada. *Mitochondrial DNA* 22: 106–122.
- Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174.
- Hausmann, A., Haszprunar, G. & Hebert, P.D.N. 2011. DNA Barcoding the geometrid Fauna of Bavaria (Lepidoptera): Successes, surprises, and questions. *PLoS ONE* 6: e17134.
- He, Q. & Deng, Y. 1989. Study on the karyotype of *Clivia nobilis* Lindl. *Journal of Beijing Agricultural College* 3: 110–111.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. 2003a. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270: 313–321.
- Hebert, P.D.N., Ratnasingham, S. & de Waard, J.R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270: S96–S99.
- Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. 2004a. Identification of birds through DNA Barcodes. *PLoS Biol* 2: e312.
- Heinze, B. 2007. A database of PCR primers for the chloroplast genomes of higher plants. *Plant Methods* 3. doi:10.1186/1746-4811-3-4.
- Henry, R.J. 2008. *Plant Genotyping II: SNP Technology*. CABI, Egham, UK, 297p.
- Hilu, K.W., Borsch, T., Müller, K., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M.P., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A.B., Rohwer, J.G., Campbell, C.S. & Chatrou, L.W. 2003. Angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* 90: 1758–1776.
- Hollingsworth, P.M. 2011. Refining the DNA barcode for land plants. *Proceedings of the National Academy of Sciences* 108: 19451–19452.

- Hollingsworth, M.L., Bailey, J.P., Hollingsworth, P.M. & Ferris, C. 1999. Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). *Botanical Journal of the Linnean Society* 129: 139–154.
- Hollingsworth, P.M., Graham, S.W. & Little, D.P. 2011. Choosing and using a plant DNA barcode. *PloS one* 6: e19254.
- Holmes, B.H., Steinke, D. & Ward, R.D. 2009. Identification of shark and ray fins using DNA barcoding. *Fisheries Research* 95: 280–288.
- Hooker, W.J. 1856. *Clivia gardenii*. *Curtis's Botanical Magazine* 12: t.4895.
- Horn, T., Barth, A., Rühle, M., Häser, A., Jürges, G. & Nick, P. 2012. Molecular diagnostics of Lemon Myrtle (*Backhousia citriodora* versus *Leptospermum citratum*). *European Food Research and Technology* 234: 853–861.
- Hrcek, J., Miller, S.E., Quicke, D.L.J. & Smith, M.A. 2011. Molecular detection of trophic links in a complex insect host-parasitoid food web. *Molecular Ecology Resources* 11: 786–794.
- Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Applications Note* 17: 754–755.
- Husband, B.C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society: Biological sciences* 267: 217–223.
- Huson, D.H. & Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.
- Hutchings, A., Scott, A.H., Lewis, G. & Cunningham, A.B. 1996. *Zulu Medicinal Plants. An Inventory*. University of Natal Press, Pietermaritzburg, South Africa.
- Ieven, M., Vlietinck, A.J., Berghe, D.A.V., Totte, J., Dommissie, R., Esmans, E. & Alderweireldt, F. 1982. Plant antiviral agents. III. Isolation of alkaloids from *Clivia miniata* Regel (Amaryllidaceae). *Journal of Natural Products* 45: 564–573.
- Inariyama, S. 1937. Karyotype studies in Amaryllidaceae. *Scientific Report of Tokyo Bunrika Daigaku* 3: 95–113.
- Ito, M., Kawamoto, A., Kita, Y., Yukawa, T. & Kurita, S. 1999. Phylogenetic relationships of Amaryllidaceae based on *matK* sequence data. *Journal of Plant Research* 112: 207–216.
- Ivanova, N.V., deWaard, J.R., Hajibabaei, M. & Hebert, P.D.N. 2013. Protocols for high-volume DNA barcode analysis. URL [http://barcoding.si.edu/PDF/Protocols\\_for\\_High\\_Volume\\_DNA\\_Barcode\\_Analysis.pdf](http://barcoding.si.edu/PDF/Protocols_for_High_Volume_DNA_Barcode_Analysis.pdf) [accessed 23 May 2013].
- Jeanson, M.L., Labat, J.-N. & Little, D.P. 2011. DNA barcoding: a new tool for palm taxonomists? *Annals of Botany* 108: 1445–1451.

- Jefferies, P.W., Mueller, L., Abou-Donia, A.H., Seif el-Din, A.A. & Campau, D. 1988. Nobiletin, a new alkaloid from *Clivia nobilis*. *Journal of Natural Products* 51: 549–554.
- Johnson, M.A.T. & Brandham, P.E. 1997. New chromosome numbers in petaloid monocotyledons and in other miscellaneous angiosperms. *Kew Bulletin* 52: 121–128.
- Jones, K. 1976. Multiple Robertsonian fusions in the evolution of a plant genus. *Proceedings of the Kew Chromosome Conference held at the Jodrell Laboratory, the Royal Botanic Gardens, Kew, England* (eds K. Jones & P. Brandham), pp. 220–221. Elsevier, Jodrell Laboratory, the Royal Botanic Gardens, Kew.
- Jones, M., Ghoorah, A. & Blaxter, M. 2011. jMOTU and taxonator: turning DNA barcode sequences into annotated operational taxonomic units. *PloS one* 6: e19259.
- Jordan, W.C., Courtney, M.W. & Neigel, J.E. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). *American Journal of Botany* 83: 430–439.
- Kai, G., Zheng, J., Zhang, L., Pang, Y., Liao, Z., Li, Z., Zhao, L., Sun, X. & Tang, K. 2003. Molecular cloning of a new lectin gene from *Z. grandiflora*. *Mitochondrial DNA* 14: 335–338.
- Kaila, L. & Stahls, G. 2006. DNA barcodes: Evaluating the potential of COI to differentiate closely related species of *Elachista* (Lepidoptera: Gelechioidea: Elachistidae) from Australia. *Zootaxa* 1170: 1–26.
- Kato, Y. 1960. Mitotic Abnormalities in the root meristem and ovular tissue of *Clivia miniata* Regel and the seasonal variation of their occurrences. *Cytologia* 25: 313–320.
- Kearney, M. 2002. Fragmentary taxa, missing data, and ambiguity: mistaken assumptions and conclusions. *Systematic Biology* 51: 369–381.
- Kellogg, E. & Juliano, N.D. 1997. The structure and function of RuBisCO and their implications for systematic studies. *American Journal of Botany* 84: 413–428.
- Kellogg, E. & Linder, H.P. 1995. Phylogeny of Poales. *Monocotyledons: systematics and evolution* (eds P.J. Rudall, P.J. Cribb, D.F. Cutler & C.J. Humphries), pp. 511–542. Royal Botanic Gardens, Kew.
- Kesanakurti, P.R., Fazekas, A.J., Burgess, K.S., Percy, D.M., Newmaster, S.G., Graham, S.W., Barrett, S.C.H., Hajibabaei, M. & Husband, B.C. 2011. Spatial patterns of plant diversity below-ground as revealed by DNA barcoding. *Molecular Ecology* 20: 1289–1302.
- Kim, S.T., Sultan, S.E. & Donoghue, M.J. 2008. Allopolyploid speciation in *Persicaria* (Polygonaceae): Insights from a low-copy nuclear region. *Proceedings of the National Academy of Sciences* 105: 12370.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

- Kleynhans, R. 2006. *Lachenalia* spp. *Flower Breeding and Genetics: Issues, challenges and opportunities for the 21st century* (ed N.O. Anderson), pp. 491–516. Springer, Berlin.
- Kleynhans, R. 2009. Back to basics for new crop development. *Acta Horticulturae* 836: 185–191.
- Kleynhans, R. 2011. Potential new lines in the Hyacinthaceae. *Acta Horticulturae* 886: 139–145.
- Kleynhans, R. & Hancke, F.L. 2002. Problems and breeding strategies for the development of new *Lachenalia* cultivars. *Acta Horticulturae* 570: 233–240.
- Kleynhans, R. & Spies, J.J. 1999. Chromosome number and morphological variation in *Lachenalia bulbifera* (Hyacinthaceae). *South African Journal of Botany* 65: 357–360.
- Kleynhans, R. & Spies, J.J. 2000. Evaluation of genetic variation in *Lachenalia bulbifera* (Hyacinthaceae) using RAPDs. *Euphytica* 115: 141–147.
- Kleynhans, R., Spies, J.J. & Spies, P. 2009. Cross-ability in the genus *Lachenalia*. *Acta Horticulturae* 813: 385–392.
- Kleynhans, C.J., Thirion, C. & Moolman, J. 2005. *A Level I River Ecoregion Classification System for South Africa*. Resource Quality Services, Department of Water Affairs and Forestry, Lesotho and Swaziland, Pretoria, South Africa.
- Knight, C.A. & Ackerly, D.D. 2002. Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. *Ecology Letters* 5: 66–76.
- Knight, C.A., Molinari, N.A. & Petrov, D.A. 2005. The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany* 95: 177–190.
- Knox, R.B. & Singh, M.B. 1987. New perspectives in pollen biology and fertilization. *Annals of Botany* 60: 15–37.
- Köhler, F. 2007. From DNA taxonomy to barcoding - how a vague idea evolved into a biosystematic tool. *Zoosystematics and Evolution* 83: 44–51.
- Koopowitz, H. 2002. *Clivias*. Timber Press, Incorporated. Portland, USA.
- Kress, W.J. & Erickson, D.L. 2007. A two-locus global DNA barcode for land plants: the coding *rbcl* gene complements the non-coding *trnH-psbA* spacer region. *PLoS One* 2: e508.
- Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O. & Bermingham, E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences* 106: 18621–18626.
- Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. & Janzen, D.H. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* 102: 8369.

- Kumar, S., Carlsen, T., Mevik, B.-H., Enger, P., Blaallid, R., Shalchian-Tabrizi, K. & Kauserud, H. 2011. CLOTU: An online pipeline for processing and clustering of 454 amplicon reads into OTUs followed by taxonomic annotation. *BMC Bioinformatics* 12: 182.
- Kumari, V., Bansal, A., Aminedi, R., Taneja, D. & Das, N. 2012. Simplified extraction of good quality genomic DNA from a variety of plant materials. *African Journal of Biotechnology* 11: 6420–6427.
- Kwong, S., Srivathsan, A. & Meier, R. 2012. An update on DNA barcoding: low species coverage and numerous unidentified sequences. *Cladistics* 28: 639–644.
- Lahaye, R., Van Der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G. & Savolainen, V. 2008. DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences* 105: 2923–2928.
- Langlois, A., Mulholland, D.A., Duncan, G.D., Crouch, N.R. & Edwards, T.J. 2005. A novel 3-benzylchromone from the South African *Lachenalia rubida* (Hyacinthaceae). *Biochemical Systematics and Ecology* 33: 961–966.
- Leven, G. 1981. *Antimicrobiële en Antivirale Screening van Hogere Planten en Studie van het Antiviraal Werkingsmechanisme van Lycorine, een Alkaloïde uit Clivia miniata R.* Ph. D., Universitaire Instelling Antwerpen.
- Levin, R.A., Wagner, W.L., Hoch, P.C., Nepokroeff, M., Pires, J.C., Zimmer, E.A. & Sytsma, K.J. 2003. Family-level relationships of Onagraceae based on chloroplast *rbcl* and *ndhF* data. *American Journal of Botany* 90: 107–115.
- Li, H.-Q., Chen, J.-Y., Wang, S. & Xiong, S.-Z. 2012. Evaluation of six candidate DNA barcoding loci in *Ficus* (Moraceae) of China. *Molecular Ecology Resources* 12: 783–790.
- Li, D.-Z., Gao, L.-M., Li, H.-T., Wang, H., Ge, X.-J., Liu, J.-Q., Chen, Z.-D., Zhou, S.-L., Chen, S.-L., Yang, J.-B., Fu, C.-X., Zeng, C.-X., Yan, H.-F., Zhu, Y.-J., Sun, Y.-S., Chen, S.-Y., Zhao, L., Wang, K., Yang, T. & Duan, G.-W. 2011a. Comparative analysis of a large dataset indicates that internal transcribed spacer (*ITS*) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences* 108: 19641–19646.
- Li, Y., Gao, L.M., Poudel, R.C., Li, D.Z. & Forrest, A. 2011b. High universality of *matK* primers for barcoding gymnosperms. *Journal of Systematics and Evolution* 49: 169–175.
- Li, F.W., Kuo, L.Y., Rothfels, C.J., Ebihara, A., Chiou, W.L., Windham, M.D. & Pryer, K.M. 2011c. *rbcl* and *matK* earn two thumbs up as the core DNA barcode for ferns. *PLoS One* 6: e26597.
- Li, M., Wong, K.-L., Chan, W.-H., Li, J.-X., But, P.P.-H., Cao, H. & Shaw, P.-C. 2012b. Establishment of DNA barcodes for the identification of the botanical sources of the Chinese “cooling” beverage. *Food Control* 25: 758–766.



- Linck, A.J. & Blaydes, G.W. 1960. Demonstration of the chemotropism of pollen tubes *in vitro* in four plant species. *Ohio Journal of Science* 60: 274–278.
- Linder, H.P., Meadows, M.E. & Cowling, R.M. 1992. History of the Cape Flora, *In Cowling, R.M. (ed.) The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town, South Africa.
- Lindley, J. 1828. *Clivia nobilis*. *Edwards's Botanical Register* 14: t.1182.
- Little, D.P. 2010. A unified index of sequence quality and contig overlap for DNA barcoding. *Bioinformatics* 26: 2780–2781.
- Little, D.P. 2011. DNA barcode sequence identification incorporating taxonomic hierarchy and within taxon variability. *PLoS one* 6: e20552.
- Liu, C., Liang, D., Gao, T., Pang, X., Song, J., Yao, H., Han, J., Liu, Z., Guan, X., Jiang, K., Li, H. & Chen, S. 2011a. PTIGS-IdIt, a system for species identification by DNA sequences of the *psbA-trnH* intergenic spacer region. *BMC Bioinformatics* 12: S4.
- Liu, J., Möller, M., Gao, L.-M., Zhang, D.-Q. & Li, D.-Z. 2011b. DNA barcoding for the discrimination of Eurasian yews (*Taxus* L., Taxaceae) and the discovery of cryptic species. *Molecular Ecology Resources* 11: 89–100.
- Liu, Y., YAN, H.F., Cao, T. & GE, X.J. 2010. Evaluation of 10 plant barcodes in Bryophyta (Mosses). *Journal of Systematics and Evolution* 48: 36–46.
- Lou, M. & Brian Golding, G. 2010. Assigning sequences to species in the absence of large interspecific differences. *Molecular Phylogenetics and Evolution* 56: 187–194.
- Lowenstein, J.H., Amato, G. & Kolokotronis, S.-O. 2009. The Real maccoyii: Identifying Tuna Sushi with DNA Barcodes – contrasting characteristic attributes and genetic distances. *PLoS ONE* 4: e7866.
- Lubbinge, J. 1980. *Lachenalia* Breeding I. Introduction. *Acta Horticulturae* 109: 289–295.
- Luo, K., Chen, S.L., Chen, K.L., Song, J.Y., Yao, H., Ma, X., Zhu, Y.J., Pang, X.H., Yu, H., Li, X.W. & Liu, Z. 2010. Assessment of candidate plant DNA barcodes using the Rutaceae family. *Science China Life Sciences* 53: 701–708.
- Maddison, D.R., Swofford, D.L. & Maddison, W.P. 1997. NEXUS: an extensible file format for systematic information. *Systematic Biology* 46: 590–621.
- Mallet, J. 2007. Hybrid speciation. *Nature* 446: 279–283.
- Mander, M. 1997. *Medicinal Plant Marketing and Strategies for Sustaining the Plant Supply in the Bushbuckridge Area and Mpumalanga Province*. DANCED/DWAF, Nelspruit, South Africa.

- Manning, J.C., Goldblatt, P. & Fay, M.F. 2004a. A revised generic synopsis of Hyacinthaceae in sub-Saharan Africa, based on molecular evidence, including new combinations and the new tribe Pseudoprosperaeae. *Edinburgh Journal of Botany* 60: 533–568.
- Manning, J.C., Goldblatt, P. & Fay, M.F. 2004b. A revised generic synopsis of Hyacinthaceae in sub-saharan Africa, based on molecular evidence, including new combinations and the new tribe Pseudoprosperaeae. *Edinburgh Journal of Botany* 60: 533–568.
- Manning, J.C., Goldblatt, P. & Snijman, D. 2002. *The Color Encyclopedia of Cape Bulbs*. Timber Press. Cambridge, UK.
- Masters, B., Fan, V. & Ross, H. 2010. Species delimitation plugin manual. URL [www.cebl.auckland.ac.nz/~hros001/Software/SpDelim/](http://www.cebl.auckland.ac.nz/~hros001/Software/SpDelim/) [accessed 13 June 2013]
- Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of Angiosperms. *Science* 264: 421–424.
- Matsumoto, S., Kouchi, M., Yabuki, J., Kusunoki, M., Ueda, Y. & Fukui, H. 1998. Phylogenetic analyses of the genus *Rosa* using the *matK* sequence: molecular evidence for the narrow genetic background of modern roses. *Scientia Horticulturae* 77: 73–82.
- Meerow, A.W. 1995. Towards a phylogeny of the Amaryllidaceae. *Monocotyledons: systematics and evolution* (eds P.J. Rudall, P.J. Cribb, D.F. Cutler & C.J. Humphries), Royal Botanic Gardens, Kew.
- Meerow, A.W. & Clayton, J.R. 2004. Generic relationships among the baccate-fruited Amaryllidaceae (tribe Haemantheae) inferred from plastid and nuclear non-coding DNA sequences. *Plant Systematics and Evolution* 244: 141–155.
- Meerow, A.W., Fay, M.F., Guy, C.L., Li, Q.B., Zaman, F.Q. & Chase, M.W. 1999a. Systematics of Amaryllidaceae based on cladistic analysis of plastid sequence data. *American Journal of Botany* 86: 1325–1345.
- Meerow, A.W., Fay, M.F., Guy, C.L., Li, Q.B., Zaman, F.Q. & Chase, M.W. 1999b. Systematics of Amaryllidaceae based on cladistic analysis of plastid sequence data. *American Journal of Botany* 86: 1325–1345.
- Meerow, A.W. & Snijman, D.A. 1998. Amaryllidaceae. *Flowering Plants. Monocotyledons: Lilianae (except Orchidaceae)* The family and genera of vascular plants., 1st ed (ed K. Kubitzki), pp. 83–110. Springer.
- Meerow, A.W. & Snijman, D.A. 2001. Phylogeny of Amaryllidaceae tribe Amaryllideae based on nrDNA ITS sequences and morphology. *American Journal of Botany* 88: 2321–2330.
- Meerow, A.W. & Snijman, D.A. 2006. The never-ending story: multigene approaches to the phylogeny of amaryllidaceae. *Aliso* 22: 355366.
- Mehlis, B. 1965. *Die Alkaloide von Clivia miniata*. Humboldt-University, Faculty of Mathematics and Natural Sciences, Berlin.

- Meier, R., Shiyang, K., Vaidya, G. & Ng, P.K.L. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728.
- Meier, R., Zhang, G. & Ali, F. 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology* 57: 809–813.
- Melchior, H. 1964. Liliiflorae. *Engler’s Syllabus der Pflanzenfamilien 2. Angiospermen, übersicht über die florenggebiete der erde* p. 666. Berlin-Nikolassee Bornträger, Berlin.
- Meyer, C.P. & Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS biology* 3: e422.
- Moffett, A.A. 1936. The cytology of *Lachenalia*. *Cytologia* 7: 490–498.
- Mogford, D.J. 1978. Centromeric heterochromatin in *Lachenalia tricolor* (L.) Thunb. *Journal of South African Botany* 44: 111–117.
- Moore, F.W. 1905. *Lachenalia* hybrids. *The Gardeners’ Chronicle* 37: 210–211.
- Moritz, C. & Cicero, C. 2004. DNA Barcoding: Promise and pitfalls. *PLoS Biol* 2: e354.
- Muellner, A.N., Schaefer, H. & Lahaye, R. 2011. Evaluation of candidate DNA barcoding loci for economically important timber species of the mahogany family (Meliaceae). *Molecular Ecology Resources* 11: 450–460.
- Müller, T., Philippi, N., Dandekar, T., Schultz, J. & Wolf, M. 2007. Distinguishing species. *RNA* 13: 1469–1472.
- Müller-Doblies, D. & Müller-Doblies, U. 1996. Tribes and subtribes and some species combinations in Amaryllidaceae J. St.-Hil. Emend. R Dahlgren et al 1985. *Feddes Repertorium* 107: S.c.1 – S.c.9.
- Müller-Doblies, U. & Müller-Doblies, D. 1997. A partial revision of the tribe Massonieae (Hyacinthaceae) 1. Survey, including three novelties from Namibia: A new genus, a second species in the monotypic *Whiteheadia*, and a new combination in *Massonia*. *Feddes Repertorium* 108: 49–96.
- Müller-Doblies, U., Nordenstam, B. & Müller-Doblies, D. 1987. A second species in *Lachenalia* subgen. *Brachyscypha* (Hyacinthaceae): *Lachenalia barkeriana* sp. nov. from southern Little Namaqualand. *South African Journal of Botany* 53: 481–488.
- Munch, K., Boomsma, W., Huelsenbeck, J.P., Willerslev, E. & Nielsen, R. 2008a. Statistical Assignment of DNA Sequences using Bayesian Phylogenetics. *Systematic Biology* 57: 750–757.
- Munch, K., Boomsma, W., Willerslev, E. & Nielsen, R. 2008b. Fast phylogenetic DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 3997–4002.

- Murdock, L.L. & Shade, R.E. 2002. Lectins and Protease Inhibitors as Plant Defenses against insects. *Journal of Agricultural and Food Chemistry* 50: 6605–6611.
- Murray, B.G., Ran, Y., De Lange, P.J., Hammett, K.R.W., Truter, J.T. & Swanevelder, Z.H. 2004. A new species of *Clivia* (Amaryllidaceae) endemic to the Pondoland Centre of Endemism, South Africa. *Botanical Journal of the Linnean Society* 146: 369–374.
- Murray, B.G., Wong, C. & Hammett, K.R.. 2011. The karyotype of *Clivia mirabilis* analyzed by differential banding and fluorescence *in situ* hybridization. *Plant Systematics and Evolution* 293: 193–196.
- Muturi, C.N., Ouma, J.O., Malele, I.I., Ngure, R.M., Rutto, J.J., Mithöfer, K.M., Enyaru, J. & Masiga, D.K. 2011. Tracking the feeding patterns of Tsetse flies (*Glossina* genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS ONE* 6: e17284.
- Nandi, S. 1973. Chromosome studies in several genera of Amaryllidaceae with special reference to the status of the tribe Zephyrantheae. *Journal of Cytology and Genetics* 7-8: 24–35.
- Navarro, S.P., Jurado-Rivera, J.A., Gómez-Zurita, J., Lyal, C.H.C. & Vogler, A.P. 2010. DNA profiling of host–herbivore interactions in tropical forests. *Ecological Entomology* 35: 18–32.
- Nei, M. & Kumar, S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press.
- Neuhaus, H. & Link, G. 1987. The chloroplast tRNA<sup>Lys</sup>(UUU) gene from mustard (*Sinapis alba*) contains a class II intron potentially coding for a maturase-related polypeptide. *Current Genetics* 11: 251–257.
- Newmaster, S.G., Fazekas, A.J. & Ragupathy, S. 2006. DNA barcoding in land plants: evaluation of *rbcL* in a multigene tiered approach. *Canadian Journal of Botany* 84: 335–341.
- Neyland, R. & Urbatsch, L.E. 1996. Phylogeny of subfamily Epidendroideae (Orchidaceae) inferred from *ndhF* chloroplast gene sequences. *American Journal of Botany* 83: 1195–1206
- Nichols, R. 2001. Gene trees and species trees are not the same. *Trends in Ecology & Evolution* 16: 358–364.
- Nielsen, R. & Matz, M. 2006. Statistical approaches for DNA barcoding. *Systematic Biology* 55: 162–169.
- Nishimuran, M., Takabe, T., Sugiyama, T. & Akazawa, T. 1973. Structure and function of chloroplast proteins XIX. Dissociation of Spinach leaf Ribulose-1, 5-diphosphate Carboxylase by Mercuribenzoate. *Journal of Biochemistry* 74: 945–954.
- Niu, W.-H., Xu, Y.-B. & Liu, J.-H. 1986. A preliminary study on the induction of polyploidy *Clivia miniata* Regel. *Acta Horticulturae Sinica* 13: 61–63.

- Nock, C.J., Waters, D.L.E., Edwards, M.A., Bowen, S.G., Rice, N., Cordeiro, G.M. & Henry, R.J. 2011. Chloroplast genome sequences from total DNA for plant identification. *Plant Biotechnology Journal* 9: 328–333.
- Nordenstam, B. 1982. Chromosome numbers of southern African plants 2. *Journal of South African Botany* 48: 273–275.
- Nylander, J.A.A., Olsson, U., Alström, P. & Sanmartín, I. 2008. Accounting for phylogenetic uncertainty in biogeography: A Bayesian approach to dispersal-vicariance analysis of the Thrushes (Aves: *Turdus*). *Systematic Biology* 57: 257–268.
- Ödeen, A. & Håstad, O. 2010. Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A* 196: 91–96.
- Olszewska, M.J. & Osiecka, R. 1982. The relationship between 2C DNA content, life-cycle type, systematic position, and the level of DNA endoreplication in nuclei of parenchyma cells during growth and differentiation of roots in some monocotyledonous species. *Biochemie und Physiologie der Pflanzen* 177: 319–336.
- Ornduff, R. & Watters, P.. 1978. Chromosome numbers in *Lachenalia* (Liliaceae). *Journal of South African Botany* 44: 387–390.
- Otto, F. 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. *Methods in cell biology* Flow cytometry. (eds Z. Darzynkiewicz & H.A. Crissman), pp. 105–110. Academic Press, Inc., New York, USA.
- Otto, S.P. & Whitton, J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401–437.
- Palmer, J.D., Adams, K.L., Cho, Y., Parkinson, C.L., Qiu, Y.-L. & Song, K. 2000. Dynamic evolution of plant mitochondrial genomes: Mobile genes and introns and highly variable mutation rates. *Proceedings of the National Academy of Sciences of the United States of America* 97: 6960–6966.
- Paradis, E., Claude, J. & Strimmer, K. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.
- Pawluczyk, M., Weiss, J., Vicente-Colomer, M.J. & Egea-Cortines, M. 2011. Two alleles of *rpoB* and *rpoC1* distinguish an endemic European population from *Cistus heterophyllus* and its putative hybrid (*C. × clausonis*) with *C. albidus*. *Plant Systematics and Evolution* 298: 409–419.
- Pelletier, S.W. 1999. *Alkaloids: Chemical and Biological Perspectives, Volume 15: Chemical and Biological Perspectives*. Elsevier.
- Perry, P. 1985. The restructuring of the family Liliaceae. *Veld and Flora*: 66–68.
- Petit, R. & Excoffier, L. 2009. Gene flow and species delimitation. *Trends in Ecology & Evolution* 24: 386–393.

- Pfossner, M. & Speta, F. 1999a. Phylogenetics of Hyacinthaceae based on plastid DNA sequences. *Annals of the Missouri Botanical Garden*: 852–875.
- Pfossner, M. & Speta, F. 1999b. Phylogenetics of hyacinthaceae based on plastid DNA sequences. *Annals of the Missouri Botanical Garden* 86: 852–875.
- Pfossner, M., Wetschnig, W., Ungar, S. & Prenner, G. 2003. Phylogenetic relationships among genera of Massonieae (Hyacinthaceae) inferred from plastid DNA and seed morphology. *Journal of Plant Research* 116: 115–132.
- Pietsch, J., Koch, I., Hermanns-Clausen, M., Hüller, G., Wagner, R. & Dressler, J. 2008. Pediatric plant exposures in Germany, 1998–2004. *Clinical Toxicology* 46: 686–691.
- Pohl, T.L.S. 1999. *The chemical investigation of Ledebouria ovatifolia, Clivia caulescens and Haemanthus pauculifolius*. M.Sc., University of Natal, Durban, South Africa.
- Porco, D., Decaëns, T., Deharveng, L., James, S.W., Skarżyński, D., Erséus, C., Butt, K.R., Richard, B. & Hebert, P.D.N. 2012. Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America. *Biological Invasions* 1–12.
- Powell, K.S., Gatehouse, J.A., Gatehouse, A.M.R. & Hilder, V.A. 1993. Antimetabolic effects of plant lectins and plant fungal enzymes on the nymphal stages of two important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*. *Entomologia Experimentalis et Applicata*. 66: 119–126.
- Pryer, K.M., Schuettpelz, E., Huiet, L., Grusz, A.L., Rothfels, C.J., Avent, T., Schwartz, D. & Windham, M.D. 2010. DNA barcoding exposes a case of mistaken identity in the fern horticultural trade. *Molecular Ecology Resources* 10: 979–985.
- Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2011a. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*. doi: 10.1111/j.1365-294X.2011.05239.x.
- Puillandre, N., Macpherson, E., Lambourdière, J., Cruaud, C., Boisselier-Dubayle, M.-C. & Samadi, S. 2011b. Barcoding type specimens helps to identify synonyms and an unnamed new species in *Eumunida* Smith, 1883 (Decapoda : Eumunidae). *Invertebrate Systematics* 25: 322–333.
- Pujol, J. 1990. *NaturAfrica: The Herbalist Handbook : African Flora, Medicinal Plants*. J. Pujol Natural Healers Foundation.
- Pusztai, A., Ewen, S.W.B., Grant, G., Peumans, W.J., vanDamme, E.J.M., Rubio, L. & Bardocz, S. 1990. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion* 46: 308–316.
- Pyle, M.M. & Adams, R.P. 1989. *In situ* preservation of DNA in plant specimens. *Taxon* 38: 576–581.

- Quandt, D., Muller, K., Stech, M., Frahm, J.-P., Frey, W., Hilu, K.W. & Borsch, T. 2004. Molecular evolution of the chloroplast *trnL-F* region in land plants. *Monographs in Systematic Botany* 98: 13–40.
- Rach, J., DeSalle, R., Sarkar, I.N., Schierwater, B. & Hadrys, H. 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in *Odonata*. *Proceedings of the Royal Society B: Biological Sciences* 275: 237–247.
- Ramón, M.F., Ballesteros, S., Martínez-Arrieta, R. & Cabrera, J. 2003. Plant and herb exposures in Spain. *Journal of Toxicology* 41: 383–564.
- Ramsey, J. & Schemske, D.W. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589–639.
- Ran, Y. 2000. *Genetic analysis of the genus Clivia*. Ph. D., University of Auckland.
- Ran, Y., Murray, B.G. & Hammett, K.R.W. 1999. Karyotype analysis of the genus *Clivia* by giemsa and fluorochrome banding and *in situ* hybridization. *Euphytica* 106: 139–147.
- Ran, Y., Hammett, K.R.W & Murray, B.G. 2001a. Hybrid identification in *Clivia* (Amaryllidaceae) using chromosome banding and genomic *in situ* hybridization. *Annals of Botany* 87: 457–462.
- Ran, Y., Hammett, K.R.W & Murray, B.G. 2001b. Phylogenetic analysis and karyotype evolution in the genus *Clivia* (Amaryllidaceae). *Annals of Botany* 87: 823–830.
- Ran, Y., Murray, B.G. & Hammett, K.R.W. 2001c. Evaluating genetic relationships between and within *Clivia* species using RAPDs. *Scientia Horticulturae* 90: 167–179.
- Ratnasingham, S. & Hebert, P.D. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364.
- Razafimandimbison, S.G., Kainulainen, K., Wong, K.M., Beaver, K. & Bremer, B. 2011. Molecular support for a basal grade of morphologically distinct, monotypic genera in the species-rich vanguerieae alliance (Rubiaceae, Ixoroideae): Its systematic and conservation implications. *Taxon* 60: 941–952.
- Regel, E. 1864. *Clivia miniata* Lindl. Amaryllideae. *Gartenflora* 14: 131, t.434.
- Reinten, E.Y., Coetzee, J.H. & van Wyk, B.-E. 2011. The potential of South African indigenous plants for the international cut flower trade. *South African Journal of Botany* 77: 934–946.
- Renner, S.S. 1999. Circumscription and phylogeny of the Laurales: evidence from molecular and morphological data. *American Journal of Botany* 86: 1301–1315.
- Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P. & Coissac, E. 2011. EcoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research* 39: e145.

- Riley, H.P. 1962. Chromosome studies in some South African monocotyledons. *Canadian Journal of Genetic Cytology* 4: 40–55.
- Rogstad, S.H. 1992. Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. *Taxon*: 701–708.
- Rosenberg, N.A. 2007. Statistical tests for taxonomic distinctiveness from observations of monophyly. *Evolution* 61: 317–323.
- Ross, H.A., Murugan, S. & Li, W.L.S. 2008. Testing the reliability of genetic methods of species identification via simulation. *Systematic Biology* 57: 216–230.
- Rourke, J.P. 2002. *Clivia mirabilis* (Amaryllidaceae: Haemantheae) a new species from Northern Cape, South Africa. *Bothalia* 32: 1–7.
- Roy, S., Tyagi, A., Shukla, V., Kumar, A., Singh, U.M., Chaudhary, L.B., Datt, B., Bag, S.K., Singh, P.K., Nair, N.K., Husain, T. & Tuli, R. 2010. Universal plant DNA barcode loci may not work in complex groups: A case study with Indian *Berberis* species. *PLoS ONE* 5: e13674.
- Rubinoff, D., Cameron, S. & Will, K. 2006. Are plant DNA barcodes a search for the Holy Grail? *Trends in Ecology & Evolution* 21: 1–2.
- Ruhl, M.W., Wolf, M. & Jenkins, T.M. 2010. Compensatory base changes illuminate morphologically difficult taxonomy. *Molecular Phylogenetics and Evolution* 54: 664–669.
- Rutner, A.C.A. & Lane, M.D.M. 1967. Nonidentical subunits of ribulose diphosphate carboxylase. *Biochemical and Biophysical Research Communications* 28: 531.
- Saghai-Maroo, M.A., Soliman, K.M., Jorgensen, R.A. & Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences of the United States of America* 81: 8014–8018.
- Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- Salisbury, R.A. 1866. *The genera of plants. A fragment containing part of Liriogamae*. J. van Voorst, London.
- SANBI. 2012. Red list of South African Plants. URL <http://redlist.sanbi.org/> [accessed 11 October 2012]
- Sandionigi, A., Galimberti, A., Labra, M., Ferri, E., Panunzi, E., De Mattia, F. & Casiraghi, M. 2012. Analytical approaches for DNA barcoding data—how to find a way for plants? *Plant Biosystems* 146: 805–813.



- Sang, T., Crawford, D. & Stuessy, T. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1120.
- SAPA. 2010. Barcodes to fight cycad smuggling. *IOL news*.
- Sarkar, I.N., Planet, P.J., Bael, T.E., Stanley, S.E., Siddall, M., DeSalle, R. & Figurski, D.H. 2002a. Characteristic attributes in cancer microarrays. *Journal of Biomedical Informatics* 35: 111–122.
- Sarkar, I.N., Planet, P.J. & Desalle, R. 2008. CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources* 8: 1256–1259.
- Sarkar, I.N., Thornton, J.W., Planet, P.J., Figurski, D.H., Schierwater, B. & DeSalle, R. 2002b. An automated phylogenetic key for classifying homeoboxes. *Molecular Phylogenetics and Evolution* 24: 388–399.
- Sarkar, I.N. & Trizna, M. 2011. The Barcode of Life Data Portal: bridging the biodiversity informatics divide for DNA barcoding. *PLoS one* 6: e14689.
- Satô, D. 1938. Karyotype alteration and phylogeny. IV. Karyotypes in Amaryllidaceae with special reference to the SAT-chromosome. *Cytologia* 9: 203–242.
- Satô, D. 1942. Karyotype alteration and phylogeny in Liliaceae and allied families. *Japanese Journal of Botany* 12: 57–161.
- Saunders, G.W. & McDonald, B. 2010. DNA barcoding reveals multiple overlooked Australian species of the red algal order Rhodymeniales (Florideophyceae), with resurrection of *Halopeltis* J. Agardh and description of *Pseudohalopeltis* gen. nov. *Botany* 88: 639–667.
- Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K. & Lane, R. 2005. Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1805–1811.
- Seberg, O. & Petersen, G. 2009. How many loci does it take to DNA Barcode a *Crocus*? *PLoS ONE* 4: e4598.
- Serino, G. & Maliga, P. 1998. RNA Polymerase subunits encoded by the plastid *rpo* genes are not shared with the Nucleus-Encoded Plastid Enzyme. *Plant Physiology* 117: 1165–1170.
- Sewram, V., Raynor, M.W., Mulholland, D.A. & Raidoo, D.M. 2001. Supercritical fluid extraction and analysis of compounds from *Clivia miniata* for uterotonic activity. *Planta Medica* 67: 451–455.
- Sewram, V., Raynor, M.W., Raidoo, D.M. & Mulholland, D.A. 1998. Coupling SFE to uterotonic bioassay: an on-line approach to analysing medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis* 18: 305–318.

- Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.
- Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94: 275–288.
- Shen, J., Shen, Y., Li, W., Shang, Y., Ding, J. & Li, R. 2007. Studies on pollen viability and fertilization of *Clivia miniata* Regel. *Acta Horticulturae Sinica* 34: 1249.
- Shi, L.-C., Zhang, J., Han, J.-P., Song, J.-Y., Yao, H., Zhu, Y.-J., Li, J.-C., Wang, Z.-Z., Xiao, W., Lin, Y.-L., Xie, C.-X., Qian, Z.-Z. & Chen, S.-L. 2011. Testing the potential of proposed DNA barcodes for species identification of Zingiberaceae. *Journal of Systematics and Evolution* 49: 261–266.
- Shrestha, S., Graham, G.C., Loch, D.S. & Adkins, S.W. 2010. Molecular identification of weedy *Sporobolus* species by PCR-RFLP. *Weed Research* 50: 383–387.
- Simpson, M.G. 2010. *Plant Systematics*. Academic Press. Oxford, UK.
- Singer, G.A.C. & Hajibabaei, M. 2009. iBarcode. org: web-based molecular biodiversity analysis. *BMC bioinformatics* 10: S14.
- Slabbinck, B., Dawyndt, P., Martens, M., Vos, P.D. & Baets, B.D. 2008. TaxonGap: a visualization tool for intra- and inter-species variation among individual biomarkers. *Bioinformatics* 24: 866–867.
- Smith, A.A. 1895. *A Contribution to South African Materia Medica; Chiefly from Plants in Use Among the Natives*. Juta, Cape Town, South Africa.
- Smith, M.A., Bertrand, C., Crosby, K., Eveleigh, E.S., Fernandez-Triana, J., Fisher, B.L., Gibbs, J., Hajibabaei, M., Hallwachs, W., Hind, K., Hrcek, J., Huang, D.-W., Janda, M., Janzen, D.H., Li, Y., Miller, S.E., Packer, L., Quicke, D., Ratnasingham, S., Rodriguez, J., Rougerie, R., Shaw, M.R., Sheffield, C., Stahlhut, J.K., Steinke, D., Whitfield, J., Wood, M. & Zhou, X. 2012. *Wolbachia* and DNA Barcoding insects: patterns, potential, and problems. *PLoS ONE* 7: e36514.
- Smith, G.F., Buys, M., Walters, M., Herbert, D. & Hamer, M. 2008. Taxonomic research in South Africa: the state of the discipline. *South African Journal of Science* 104: 254–256.
- Snijman, D.A. & Archer, R.H. 2003. Amaryllidaceae. *Plants of southern Africa: an annotated checklist*. p. 1231. Strelitzia 14, National Botanical Institute, Pretoria.
- Snijman, D.A. & Meerow, A.W. 2010. Floral and macroecological evolution within *Cyrtanthus* (Amaryllidaceae): Inferences from combined analyses of plastid *ndhF* and nrDNA *ITS* sequences. *South African Journal of Botany* 76: 217–238.

- Snijman, D.A. & Victor, J. 2003. Threatened Amaryllidaceae of South Africa. *Herbertia* 58: 91–109.
- Soltis, P.S. & Soltis, D.E. 2009. The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60: 561–588.
- Sparrow, A.H. & Miksche, J.P. 1961. Correlation of nuclear volume and DNA content with higher plant tolerance to chronic radiation. *Science* 134: 282–283.
- Spies, J.J., Du Preez, J.L., Minnaar, A. & Kleynhans, R. 2000. Hyacinthaceae. Chromosome studies on African plants. 13. *Lachenalia mutabilis*, *L. pustulata* and *L. unicolor*. *Bothalia* 30: 106.
- Spies, J.J., Van Rooyen, P. & Kleynhans, R. 2002. The subgeneric delimitation of *Lachenalia* (Hyacinthaceae). *Acta Horticulturae* 570: 225–232.
- Spies, J.J., Spies, P., Reinecke, S.M.C., Kleynhans, R., Duncan, G.D. & Edwards, T.J. 2008. *Lachenalia* (ed K Marhold). *Taxon* 57: 554–555.
- Spies, J.J., Spies, P., Reinecke, S.M.C., Minnaar, A., Du Preez, J.L. & Kleynhans, R. 2009. *Lachenalia* (eds K Marhold and I Breitwieser). *Taxon* 58: 1288–1289.
- Spies, P. 2004. *Phylogenetic relationships of the genus Lachenalia with other related Liliaceous taxa*. M.Sc. thesis, Department of Genetics, University of the Free State.
- Stamatakis, A., Aberer, A.J., Goll, C., Smith, S.A., Berger, S.A. & Izquierdo-Carrasco, F. 2012. RAxML-Light: a tool for computing terabyte phylogenies. *Bioinformatics* 28: 2064–2066.
- Starr, J.R., Naczi, R.F. & Chouinard, B.N. 2009. Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). *Molecular Ecology Resources* 9: 151–163.
- Stebbins, G.L. 1950. *Variations and Evolution in Plants*. Columbia University Press. New York, USA.
- Stebbins, G.L. 1971. *Processes of Organic Evolution*. Prentice-Hall, Englewood Cliffs, New Jersey.
- Steenkamp, V. 2003. Traditional herbal remedies used by South African women for gynaecological complaints. *Journal of Ethnopharmacology* 86: 97–108.
- Stegmann, S. 2011. *Genetic variation of Clivia caulescens*. M.Sc. thesis, Department of Genetics, University of the Free State, Bloemfontein.
- Steinke, D., Vences, M., Salzburger, W. & Meyer, A. 2005. Taxl: a software tool for DNA barcoding using distance methods. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1975–1980.
- Stern, R.F., Horak, A., Andrew, R.L., Coffroth, M.-A., Andersen, R.A., Küpper, F.C., Jameson, I., Hoppenrath, M., Véron, B., Kasai, F., Brand, J., James, E.R. & Keeling, P.J. 2010.

- Environmental barcoding reveals massive Dinoflagellate diversity in marine environments. *PLoS ONE* 5: e13991.
- Stoeckle, M. 2003. Taxonomy, DNA, and the bar code of life. *BioScience* 53: 796–797.
- Stoeckle, M., Janzen, D., Hallwachs, W., Hanken, J. & Baker, J. 2003. *Taxonomy, DNA, and the Barcode of Life*. Draft Conference Report, Banbury Center, Cold Spring Harbor Laboratory, New York, NY.
- Stoeckle, M., Waggoner, P. & Ausubel, J. 2004. *Consortium for the Barcode of Life, Inaugural Meeting*. Draft, Smithsonian Institution, Washington DC.
- Stuessy, T.F. & König, C. 2008. Patrocladistic classification. *Taxon*: 594–601.
- Stuut, J.B.W., Crosta, X., Van der Borg, K. & Schneider, R. 2004. Relationship between Antarctic sea ice and southwest African climate during the late Quaternary. *Geology* 32: 909–912.
- Sun, Y.L. & Hong, S.K. 2012. Phylogenetic relationship and evolution analysis of the peony *Paeonia* species using multi-locus deoxyribonucleic acid (DNA) barcodes. *Journal of Medicinal Plants Research* 6: 5048–5058.
- Sun, X.-Q., Zhu, Y.-J., Guo, J.-L., Peng, B., Bai, M.-M. & Hang, Y.-Y. 2012. DNA Barcoding the Dioscorea in China, a vital group in the evolution of Monocotyledon: use of *matK* gene for species discrimination. *PLoS ONE* 7: e32057.
- Sveshnikova, L.I. & Zemskova, E.A. 1988. Chromosome numbers in some members of the Amaryllidaceae. *Botanicheskii Zhurnal* 73: 1207–1208.
- Swanevelder, D. 2003. *Diversity and population structure of Clivia miniata Lindl. (Amaryllidaceae): Evidence from molecular genetics and ecology*. M.Sc., Department of Botany, University of Pretoria, Pretoria, South Africa.
- Swanevelder, Z.H. & Fisher, R.C. 2009. *Clivia: Nature and Nurture*. Pretoria: Briza.
- Swanevelder, Z.H., Forbes-Hardinge, A., Truter, J.T. & van Wyk, A.E. 2006. A new variety of *Clivia robusta*. *Bothalia* 36: 66–67.
- Swofford, D.L. 2003. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermet, T., Corthier, G., Brochmann, C. & Willerslev, E. 2007. Power and limitations of the chloroplast *trnL* (UAA) intron for plant DNA barcoding. *Nucleic Acids Research* 35: e14–e14.
- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

- Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular Biology and Evolution* 9: 678–687.
- Tamura, K. & Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
- Tang, G.P. 1973. Behaviour of the vegetative nuclei and generative cell during the development of the microgametophyte of *Clivia nobilis* *in vitro*. *Acta Botanica Sinica* 15: 12–21.
- Tang, G.P. 1979. Variation of generative cell and vegetative nucleus in developing male gametophyte of *Clivia nobilis* cultured *in vitro*. II Cytochemical observations on adenosine triphosphatase distribution. *Acta Botanica Sinica* 21: 107–116.
- Tate, J.A. & Simpson, B.B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* 28: 723–737.
- Theodoridis, S., Stefanaki, A., Tezcan, M., Aki, C., Kokkini, S. & Vlachonasios, K.E. 2012. DNA barcoding in native plants of the Labiatae (Lamiaceae) family from Chios Island (Greece) and the adjacent Çeşme-Karaburun Peninsula (Turkey). *Molecular Ecology Resources* 12: 620–633.
- Therman, E. 1956. Chromocentres in the mitosis of Liliaceae. *Archivum Societatis Zoologicae Botanicae Fennicae Vanamo* 11: 189–193.
- Tolley, K.A., Chase, B.M. & Forest, F. 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. *Journal of Biogeography* 35: 1402–1414.
- Traub, H.P. 1963. *The genera of Amaryllidaceae*. American Plant Life Society, La Jolla, California.
- Tyagi, A., Bag, S.K., Shukla, V., Roy, S. & Tuli, R. 2010. Oligonucleotide frequencies of barcoding loci can discriminate species across kingdoms. *PLoS ONE* 5: e12330.
- Vaidya, G., Lohman, D.J. & Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- Van der Westhuizen, H.M. 2010. *Genetic variation in the most primitive Clivia species*. MSc thesis, Department of Genetics, University of the Free State, South Africa.

- Vanhaecke, D., Garcia de Leaniz, C., Gajardo, G., Young, K., Sanzana, J., Orellana, G., Fowler, D., Howes, P., Monzon-Arguello, C. & Consuegra, S. 2012. DNA barcoding and microsatellites help species delimitation and hybrid identification in endangered Galaxiid Fishes. *PLoS ONE* 7: e32939.
- Van Rooyen, P., Spies, J.J. & Kleynhans, R. 2002. The species delimitation of *Lachenalia unifolia* and *L. hirta*. *Acta Horticulturae* 570: 395–401.
- Veale, D.J.H., Furman, K.I. & Oliver, D.W. 1992. South African traditional herbal medicines used during pregnancy and childbirth. *Journal of Ethnopharmacology* 36: 185–191.
- Veale, D.J.H., Oliver, D.W., Arangies, N.S. & Furman, K.I. 1989. Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. *Journal of Ethnopharmacology* 27: 341–346.
- Veale, D.J.H., Oliver, D.W. & Havlik, I. 2000. The effects of herbal oxytocics on the isolated “stripped” myometrium model. *Life Sciences* 67: 1381–1388.
- Van Velzen, R., Weitschek, E., Felici, G. & Bakker, F.T. 2012. DNA Barcoding of recently diverged species: relative performance of matching methods. *PloS one* 7: e30490.
- Von Staden, L., Raimondo, D. & Dayaram, A. 2013. Taxonomic research priorities for the conservation of the South African flora. *South African Journal of Science* 109: 1–10.
- Vidic, T., Greilhuber, J. & Vilhar, B. 2003. Abstract 31 of the Second Plant Genome Size Meeting held at The Royal Botanic Gardens, Kew. (<http://www.kew.org/cval/abstracts/cvalAbstract31.html>).
- Viladomat, F., Bastida, J., Codina, C., Nair, J.J. & Campbell, W. 1997. Alkaloids of the South African Amaryllidaceae. *Recent Research Development in Phytochemistry* 1: 131–171.
- Vinogradov, A.E. 2003. Selfish DNA is maladaptive: evidence from the plant Red List. *Trends in Genetics* 19: 609–614.
- Von Cräutlein, M., Korpelainen, H., Pietiläinen, M. & Rikkinen, J. 2011. DNA barcoding: a tool for improved taxon identification and detection of species diversity. *Biodiversity and Conservation* 20: 373–389.
- Vosa, C.G. 1987. Chromosome studies in the genus *Gethyllis* (Amaryllidaceae). *Caryologia* 39: 251–7.
- Vosa, C.G. & Marchi, P.D. 1980. Chromosome analysis of *Haemanthus* and *Scadoxus* (Amaryllidaceae). *Plant Systematics and Evolution* 135: 119–126.
- Voshell, S.M., Baldini, R.M., Kumar, R., Tatalovich, N. & Hilu, K.W. 2011. Canary grasses (*Phalaris*, Poaceae): molecular phylogenetics, polyploidy and floret evolution. *Taxon* 60: 1306–1316.
- Wakasugi, T., Sugita, M., Tsudzuki, T. & Sugiura, M. 1998. Updated gene map of Tobacco chloroplast DNA. *Plant Molecular Biology Reporter* 16: 231–241.

- Wallace, L.J., Boilard, S.M.A.L., Eagle, S.H.C., Spall, J.L., Shokralla, S. & Hajibabaei, M. 2012. DNA barcodes for everyday life: Routine authentication of Natural Health Products. *Food Research International* 49: 446–452.
- Wallman, J.F. & Donnellan, S.C. 2001. The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. *Forensic Science International* 120: 60–67.
- Wang, Y., Wang, X., Tang, H., Tan, X., Ficklin, S.P., Feltus, F.A. & Paterson, A.H. 2011. Modes of gene duplication contribute differently to genetic novelty and redundancy, but show parallels across divergent Angiosperms. *PLoS ONE* 6: e28150.
- Watson, W. 1899. *Clivia miniata* var. *citrina*. *The Garden* 56: 338–339, t. 1246.
- Weitschek, E., Van Velzen, R., Felici, G. & Bertolazzi, P. 2013. BLOG 2.0: a software system for character-based species classification with DNA Barcode sequences. What it does, how to use it. *Molecular Ecology Resources* 13: 1043–1046.
- Wendel, J.F., Schnabel, A. & Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences* 92: 280–284.
- Wheeler, T.J. 2009. Large-Scale Neighbor-Joining with NINJA. *Algorithms in Bioinformatics* Lecture notes in computer science. (eds S.L. Salzberg & T. Warnow), pp. 375–389. Springer Berlin Heidelberg, Berlin, Heidelberg.
- White, T., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Protocols: a guide to methods and applications* pp. 315–322. Academic Press, New York, USA.
- Whitlock, B.A., Hale, A.M. & Groff, P.A. 2010. Intraspecific inversions pose a challenge for the *trnH-psbA* plant DNA barcode. *PLoS one* 5: e11533.
- Wiens, J.J. 2006. Missing data and the design of phylogenetic analyses. *Journal of Biomedical Informatics* 39: 34–42.
- Will, K.W., Mishler, B.D. & Wheeler, Q.D. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* 54: 844–851.
- Will, K.W. & Rubinoff, D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20: 47–55.
- Williams, V.L., Balkwill, K. & Witkowski, E.T.F. 2001. A lexicon of plants traded in the Witwatersrand umuthi shops, South Africa. *Bothalia* 31: 71–98.
- Wink, M. 2008. Ecological roles of alkaloids. *Modern Alkaloids. Structure, isolation, synthesis and biology*. p. 689. Wiley-VCH, Weinheim.
- Wittlake, E.H. 1940. Chromosomes of *Clivia Cyrtanthiflora*. *Herbertia* 7: 166–167.

- Won, H. & Renner, S.S. 2005. The chloroplast *trnT-trnF* region in the seed plant lineage Gnetales. *Journal of Molecular Evolution* 61: 425–436.
- Xiang, X.G.U.O., Hiu, H., Wang, W. & Jin, X.H.U.A. 2011. DNA barcoding of the recently evolved genus *Holcoglossum* (Orchidaceae: Aeridinae): a test of DNA barcode candidates. *Molecular Ecology Resources* 11: 1012–1021.
- Xue, C.Y. & Li, D.Z. 2011. Use of DNA barcode sensu lato to identify traditional Tibetan medicinal plant *Gentianopsis paludosa* (Gentianaceae). *Journal of Systematics and Evolution* 49: 267–270.
- Yan, C., Burleigh, J.G. & Eulenstein, O. 2005. Identifying optimal incomplete phylogenetic data sets from sequence databases. *Molecular Phylogenetics and Evolution* 35: 528–535.
- Yancy, H.F., Zemlak, T.S., Mason, J.A., Washington, J.D., Tenge, B.J., Nguyen, N.-L.T., Barnett, J.D., Savary, W.E., Hill, W.E., Moore, M.M., Fry, F.S., Randolph, S.C., Rogers, P.L. & Hebert, P.D.N. 2008. Potential Use of DNA Barcodes in Regulatory Science: Applications of the Regulatory Fish Encyclopedia. Text. URL <http://auto287311.library.ingentaconnect.com/content/iafp/jfp/2008/00000071/00000001/art00033> [accessed 19 October 2012].
- Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. & Chen, S. 2010. Use of *ITS2* region as the universal DNA barcode for plants and animals. *PLoS one* 5: e13102.
- Yassin, A., Markow, T.A., Narechania, A., O'Grady, P.M. & DeSalle, R. 2010. The genus *Drosophila* as a model for testing tree-and character-based methods of species identification using DNA barcoding. *Molecular Phylogenetics and Evolution* 57: 509–517.
- Yoshinaga, K., Iinuma, H., Masuzawa, T. & Uedal, K. 1996. Extensive RNA editing of U to C in addition to C to U substitution in the *rbcl* Transcripts of Hornwort chloroplasts and the origin of RNA editing in Green Plants. *Nucleic Acids Research* 24: 1008–1014.
- Yousif, F., Hifnawy, M.S., Soliman, G., Boulos, L., Labib, T., Mahmoud, S., Ramzy, F., Yousif, M., Hassan, I., Mahmoud, K., El-Hallouty, S.M., El-Gendy, M., Gohar, L., El-Manawaty, M., Fayyad, W & El-Menshawhi, B.S. 2007. Large-scale *in vitro* screening of Egyptian native and cultivated plants for schistosomicidal activity. *Pharmaceutical Biology* 45: 501–510.
- Yu, J., Xue, J.-H. & Zhou, S.-L. 2011. New universal *matK* primers for DNA barcoding angiosperms. *Journal of Systematics and Evolution* 49: 176–181.
- Zhu, C., Bai, C., Sanahuja, G., Yuan, D., Farré, G., Naqvi, S., Shi, L., Capell, T. & Christou, P. 2010. The regulation of carotenoid pigmentation in flowers. *Archives of Biochemistry and Biophysics* 504: 132–141.
- Zonneveld, B.J.M. 2002. The systematic value of nuclear DNA content in *Clivia*. *Herbertia* 57: 41–48.



- Zonneveld, B.J.M. 2010. New record holders for maximum genome size in Eudicots and Monocots. *Journal of Botany* 2010: 1–4.
- Zonneveld, B.J.M., Leitch, I.J. & Bennett, M.D. 2005. First nuclear DNA amounts in more than 300 angiosperms. *Annals of Botany* 96: 229–244.
- Zůbková, M. & Sladký, Z. 1975. The possibility of obtaining seeds following placental pollination *in vitro*. *Biologia Plantarum* 17: 276–280.



- *The End* -

- *Die einde* ~

# Appendices

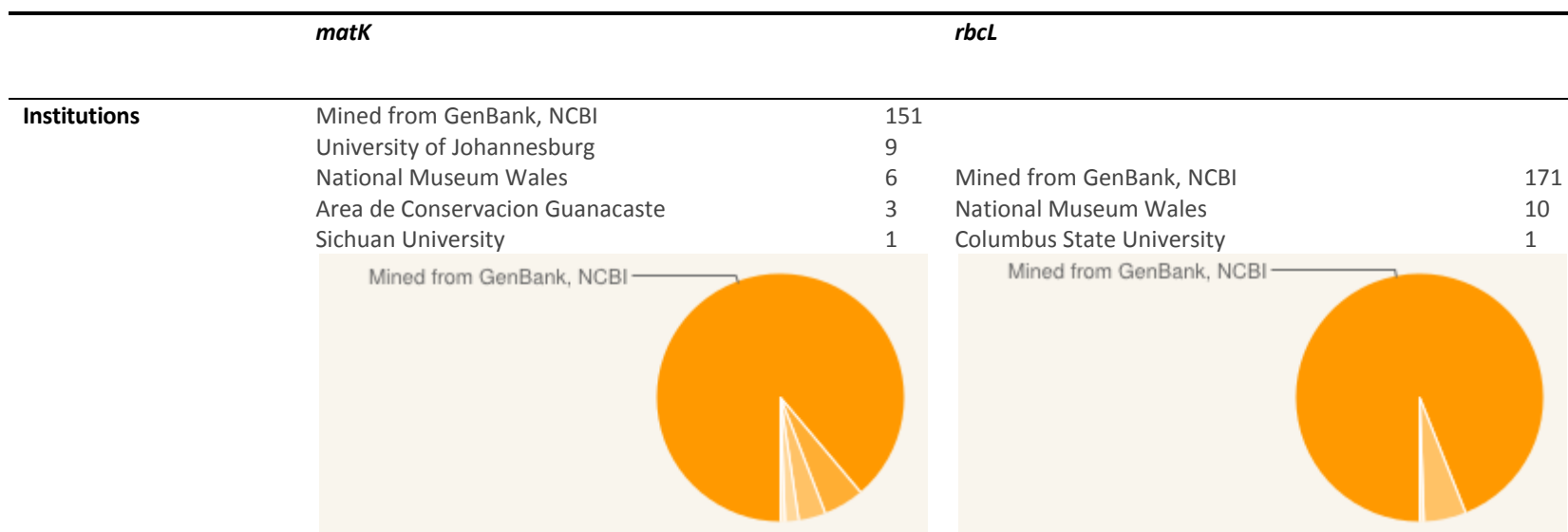
# Appendix A

## ***Distribution of the sequences deposited in public databases***

---

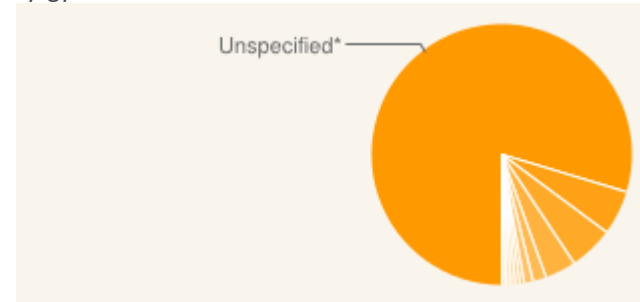
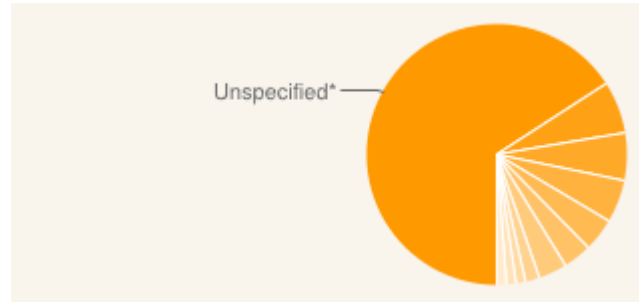
The summary constructed by BOLD ([www.boldsystems.org](http://www.boldsystems.org)). All online sequence depositories were search to determine the number of sequence depositories that contributed to specific gene regions and families. The number of countries contributing to published sequences as well as the number of species/genera represented in publications of *matK* and *rbcl* is presented in pie charts.

---

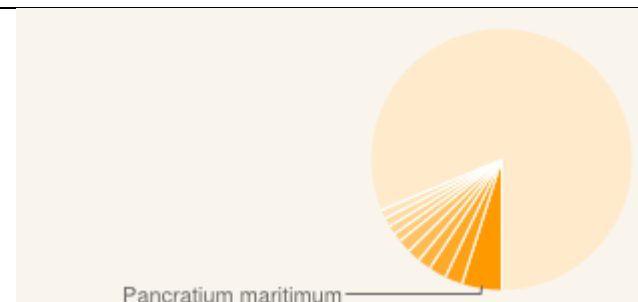
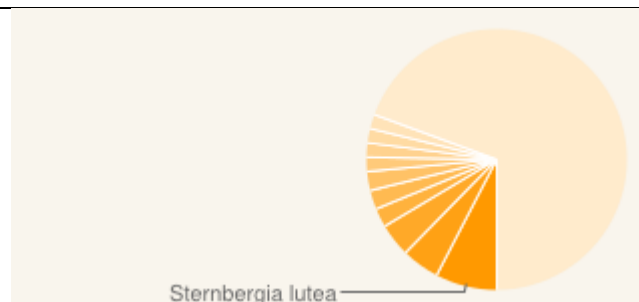
Amaryllidaceae *matK*

**Countries**

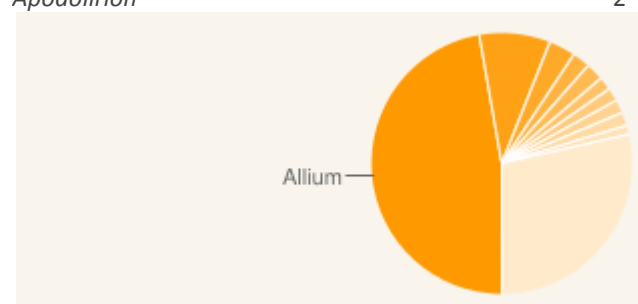
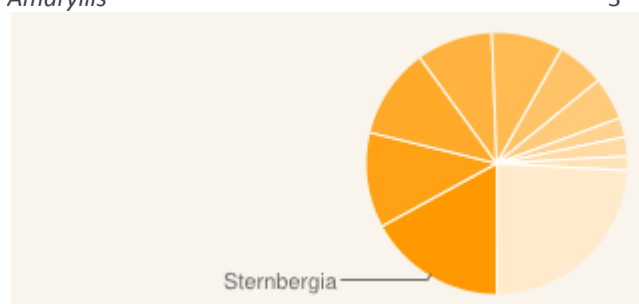
|                |     |                |     |
|----------------|-----|----------------|-----|
| Unspecified*   | 112 | Unspecified*   | 145 |
| China          | 11  | Italy          | 10  |
| South Africa   | 10  | United Kingdom | 10  |
| Italy          | 9   | Portugal       | 7   |
| Portugal       | 7   | Israel         | 3   |
| United Kingdom | 6   | United States  | 2   |
| Japan          | 6   | South Africa   | 1   |
| Costa Rica     | 3   | Spain          | 1   |
| Spain          | 2   | Greece         | 1   |
| Greece         | 2   | Kyrgyzstan     | 1   |

**Species**

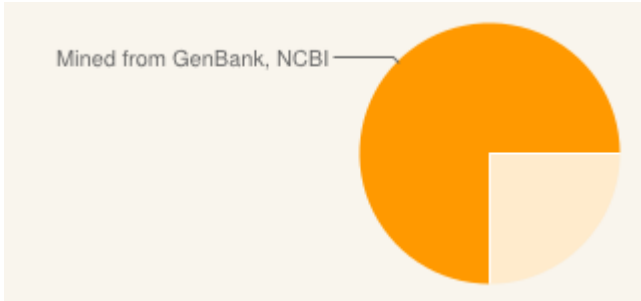
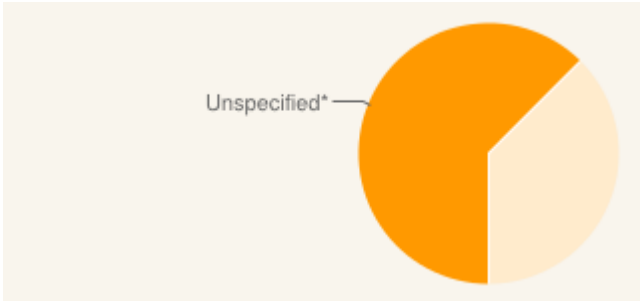
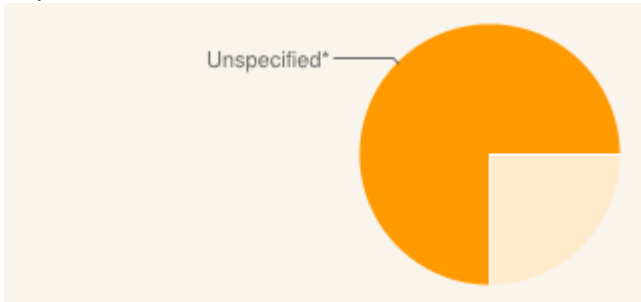
|                                |    |                             |   |
|--------------------------------|----|-----------------------------|---|
| <i>Sternbergia lutea</i>       | 13 | <i>Pancratium maritimum</i> | 9 |
| <i>Pancratium maritimum</i>    | 8  | <i>Allium ursinum</i>       | 4 |
| <i>Sternbergia sicula</i>      | 7  | <i>Allium cepa</i>          | 4 |
| <i>Pancratium illyricum</i>    | 4  | <i>Agapanthus africanus</i> | 3 |
| <i>Lycoris radiata</i>         | 4  | <i>Allium ampeloprasum</i>  | 3 |
| <i>Unspecified*</i>            | 4  | <i>Allium altaicum</i>      | 3 |
| <i>Pancratium canariense</i>   | 3  | <i>Allium triquetrum</i>    | 2 |
| <i>Sternbergia greuteriana</i> | 3  | <i>Allium neriniflorum</i>  | 2 |
| <i>Hymenocallis littoralis</i> | 3  | <i>Pancratium illyricum</i> | 2 |
| <i>Allium ursinum</i>          | 3  | <i>Allium schoenoprasum</i> | 2 |



| Genera |                     |    |                     |    |
|--------|---------------------|----|---------------------|----|
|        | <i>Sternbergia</i>  | 29 | <i>Allium</i>       | 86 |
|        | <i>Pancratium</i>   | 20 | <i>Pancratium</i>   | 16 |
|        | <i>Lycoris</i>      | 19 | <i>Narcissus</i>    | 6  |
|        | <i>Allium</i>       | 16 | <i>Agapanthus</i>   | 4  |
|        | <i>Galanthus</i>    | 15 | <i>Nothoscordum</i> | 4  |
|        | <i>Acis</i>         | 10 | <i>Lycoris</i>      | 3  |
|        | <i>Narcissus</i>    | 9  | <i>Clivia</i>       | 3  |
|        | <i>Hymenocallis</i> | 4  | <i>Ismene</i>       | 3  |
|        | <i>Leucojum</i>     | 4  | <i>Ipheion</i>      | 3  |
|        | <i>Amaryllis</i>    | 3  | <i>Apodolirion</i>  | 2  |



## Hyacinthaceae

|                     | <i>matK</i>                                                                         | <i>rbcl</i>                                                                         |
|---------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| <b>Institutions</b> | Mined from GenBank, NCBI 3<br>Palermo Botanical Garden, Herbarium M... 1            | Unspecified* 5<br>India 3                                                           |
|                     |   |  |
| <b>Countries</b>    | Unspecified* 3<br>Italy 1                                                           | Unknown                                                                             |
|                     |  |                                                                                     |

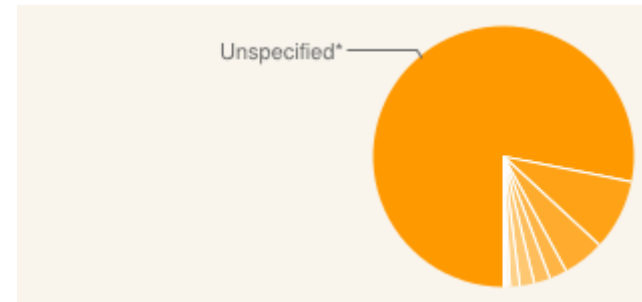
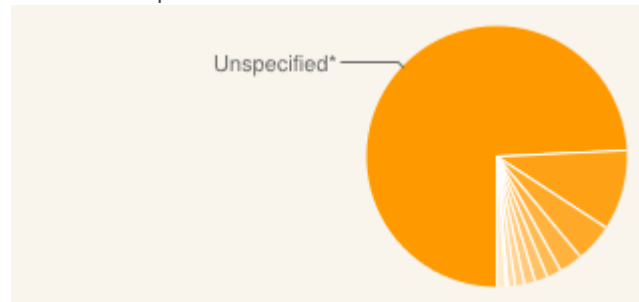




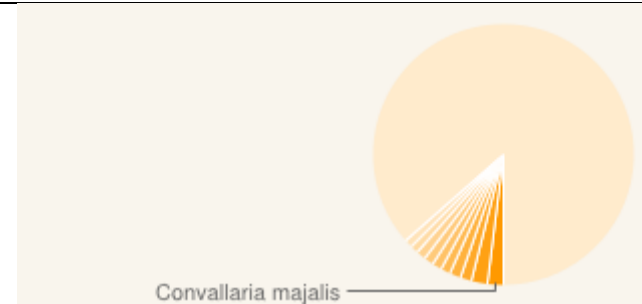
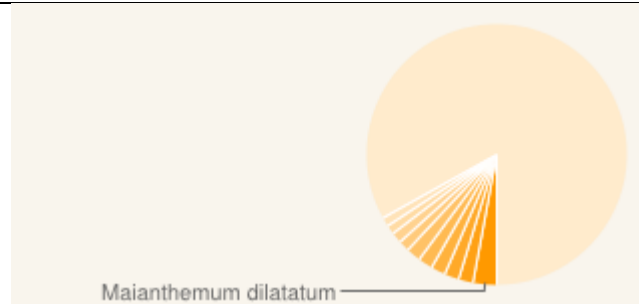
Asparagaceae *matK*:

|                     | <i>matK</i>                              |     | <i>rbcl</i>                              |     |
|---------------------|------------------------------------------|-----|------------------------------------------|-----|
| <b>Institutions</b> | Mined from GenBank, NCBI                 | 365 | Mined from GenBank, NCBI                 | 423 |
|                     | Chinese Academy of Sciences, Kunming ... | 31  | Chinese Academy of Sciences, Kunming ... | 33  |
|                     | National Museum Wales                    | 13  | National Museum Wales                    | 22  |
|                     | Smithsonian Institution, United State... | 10  | Smithsonian Institution, United State... | 8   |
|                     | University of Johannesburg               | 6   | Paul Hebert Centre for DNA Barcoding ... | 6   |
|                     | Area de Conservacion Guanacaste          | 5   | National Botanic Garden of Wales         | 4   |
|                     | University of Guelph, OAC Herbarium      | 5   | Chinese Academy of Sciences, Institut... | 4   |
|                     | Chinese Academy of Sciences, Institut... | 4   | Gujarat Biodiversity Gene Bank           | 2   |
|                     | Biodiversity Institute of Ontario        | 1   | Columbus State University                | 1   |
|                     | Australian Tropical Herbarium            | 1   |                                          |     |
|                     |                                          |     |                                          |     |

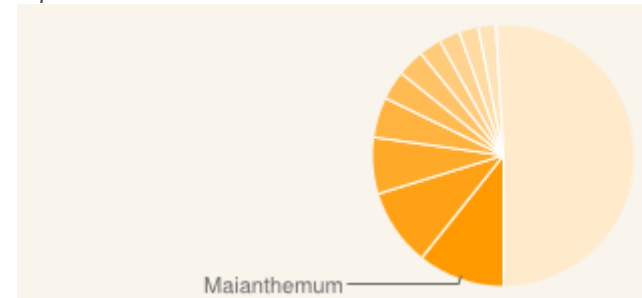
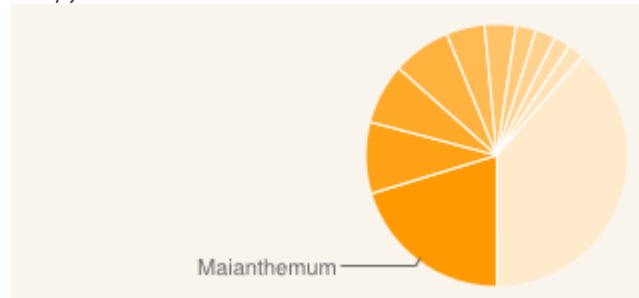
|                  |                    |     |                  |     |
|------------------|--------------------|-----|------------------|-----|
| <b>Countries</b> | Unspecified*       | 329 | Unspecified*     | 393 |
|                  | China              | 44  | China            | 44  |
|                  | Mexico             | 21  | United Kingdom   | 26  |
|                  | United Kingdom     | 13  | United States    | 11  |
|                  | Portugal           | 8   | India            | 10  |
|                  | Canada             | 7   | Portugal         | 9   |
|                  | South Africa       | 6   | Papua New Guinea | 6   |
|                  | Costa Rica         | 5   | Mexico           | 2   |
|                  | Italy              | 4   | Italy            | 1   |
|                  | Dominican Republic | 1   | Canada           | 1   |



|                |                                  |    |                                  |    |
|----------------|----------------------------------|----|----------------------------------|----|
| <b>Species</b> | <i>Maianthemum dilatatum</i>     | 13 | <i>Convallaria majalis</i>       | 10 |
|                | <i>Polygonatum cirrhifolium</i>  | 8  | <i>Polygonatum cirrhifolium</i>  | 10 |
|                | <i>Maianthemum bifolium</i>      | 8  | <i>Maianthemum japonicum</i>     | 7  |
|                | <i>Maianthemum canadense</i>     | 8  | <i>Hyacinthoides non-scripta</i> | 7  |
|                | <i>Convallaria majalis</i>       | 8  | <i>Maianthemum henryi</i>        | 6  |
|                | <i>Maianthemum japonicum</i>     | 8  | <i>Ruscus aculeatus</i>          | 6  |
|                | <i>Maianthemum atropurpureum</i> | 6  | <i>Polygonatum humile</i>        | 6  |
|                | <i>Maianthemum henryi</i>        | 6  | <i>Asparagus officinalis</i>     | 6  |
|                | <i>Asparagus cochinchinensis</i> | 5  | <i>Anemarrhena asphodeloides</i> | 5  |
|                | Unspecified*                     | 5  | <i>Liriope spicata</i>           | 5  |



| Genera |                     |    |                     |    |
|--------|---------------------|----|---------------------|----|
|        | <i>Maianthemum</i>  | 90 | <i>Maianthemum</i>  | 54 |
|        | <i>Polygonatum</i>  | 39 | <i>Ornithogalum</i> | 48 |
|        | <i>Ornithogalum</i> | 33 | <i>Polygonatum</i>  | 35 |
|        | <i>Agave</i>        | 32 | <i>Asparagus</i>    | 25 |
|        | <i>Asparagus</i>    | 21 | <i>Albuca</i>       | 18 |
|        | <i>Dipcadi</i>      | 17 | <i>Dipcadi</i>      | 17 |
|        | <i>Tupistra</i>     | 11 | <i>Ophiopogon</i>   | 14 |
|        | <i>Ophiopogon</i>   | 11 | <i>Chlorophytum</i> | 13 |
|        | <i>Chlorophytum</i> | 9  | <i>Dracaena</i>     | 12 |
|        | <i>Campylandra</i>  | 9  | <i>Tupistra</i>     | 11 |



# Appendix B

## *Aligned Clivia sequences*

---

| Accession          | Gene | Length      | Sequence   | Accession  | Gene       | Length     | Sequence | Accession | Gene | Length | Sequence |
|--------------------|------|-------------|------------|------------|------------|------------|----------|-----------|------|--------|----------|
| C._aff._caulescens | 8575 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG | 50       |           |      |        |          |
| C._aff._gardeniil  | 8325 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._gardeniil  | 8576 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._gardeniil  | 8577 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._gardeniil  | 9000 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._gardeniil  | 9001 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._robusta    | 8579 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._robusta    | 8582 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._robusta    | 8585 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._aff._robusta    | 8593 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._caulescens      | 8479 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._caulescens      | 8490 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._caulescens      | 8495 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8369 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8374 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8403 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8405 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8418 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8444 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._gardeniil       | 8523 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8391 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8394 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8396 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8406 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8408 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8410 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8419 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8526 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8558 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8573 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8574 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._miniata         | 8992 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8943 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8944 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8947 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8950 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8951 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8954 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8955 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8958 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._mirabilis       | 8963 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._x_nimbicola     | 8553 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._x_nimbicola     | 8578 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._x_nimbicola     | 8594 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._x_nimbicola     | 8595 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._nobilis         | 8932 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._nobilis         | 8933 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGAATTAT | TACTTATTGG |          |           |      |        |          |
| C._nobilis         | 8934 | AAAAAAAAAAT | GGTTAATGAT | ACAATCAACC | AATGA      |            |          |           |      |        |          |

| Accession          | Gene | Protein    | Accession  | Gene       | Protein    | Accession  | Gene | Protein | Accession | Gene | Protein |
|--------------------|------|------------|------------|------------|------------|------------|------|---------|-----------|------|---------|
| C. aff. caulescens | 8375 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA | 100  |         |           |      |         |
| C. aff. gardeniil  | 8325 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. gardeniil  | 8576 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. gardeniil  | 8577 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. gardeniil  | 9000 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. gardeniil  | 9001 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. robusta    | 8579 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. robusta    | 8582 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. robusta    | 8585 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. aff. robusta    | 8593 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. caulescens      | 8479 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. caulescens      | 8490 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. caulescens      | 8495 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8369 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8374 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8403 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8405 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8418 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8444 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. gardenii        | 8523 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8391 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8394 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8396 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8406 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8408 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8410 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8419 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8526 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8558 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8573 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8574 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. miniata         | 8992 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8943 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8944 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8947 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8950 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8951 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8954 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8955 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8958 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. mirabilis       | 8963 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. x nimbicola     | 8553 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. x nimbicola     | 8578 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. x nimbicola     | 8594 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. x nimbicola     | 8595 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. nobilis         | 8932 | ATCACTAAAA | TATATCGAGT | CGAAGTAACT | AAAACCTCGA | ATAAAAATAA |      |         |           |      |         |
| C. nobilis         | 893  |            |            |            |            |            |      |         |           |      |         |

*C. aff. gardeniil*\_9001 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. aff. robusta*\_8579 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. aff. robusta*\_8582 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. aff. robusta*\_8585 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. aff. robusta*\_8593 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. caulescens*\_8479 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. caulescens*\_8490 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. caulescens*\_8495 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8369 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8374 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8403 TAAATAATAT AAATATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8405 TAAATAATAT AAATATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8418 TAAATAATAT AAATATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8444 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. gardenii*\_8523 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8391 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8394 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8396 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8406 TAAATAATAT AAATATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8408 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8410 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8419 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8526 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8558 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8573 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8574 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. miniata*\_8992 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8943 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8944 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8947 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8950 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8951 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8954 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8955 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8958 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. mirabilis*\_8963 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. x nimbicola*\_8553 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. x nimbicola*\_8578 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. x nimbicola*\_8594 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. x nimbicola*\_8595 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8932 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8933 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8934 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8936 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8937 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8938 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8939 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8940 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8941 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. nobilis*\_8945 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. robusta*\_8415 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. robusta*\_8440 TAAATAATAT AAATATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. robusta*\_8442 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*C. robusta*\_8462 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*Cr. vansonii*\_8964 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*G. lanuginosa*\_9051 TAAATMA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT  
*G. lanuginosa*\_9055 TAAATAA--- ---TATAGAT AGGGGTAACC CCTATATAAC TAGTATATAT

*C. aff. caulescens*\_8575 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT 200  
*C. aff. gardeniil*\_8325 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. gardeniil*\_8576 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. gardeniil*\_8577 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. gardeniil*\_9000 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. gardeniil*\_9001 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. robusta*\_8579 CTAATATCAC ATATACATTT GTTTTTTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. robusta*\_8582 CTAATATCAC ATATACATTT GTTTTTTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. robusta*\_8585 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT  
*C. aff. robusta*\_8593 CTAATATCAC ATATACATTT GTTTCCTTTCA TAACGTAAAC CGCGCGCATT

|                    |      |            |            |            |            |            |
|--------------------|------|------------|------------|------------|------------|------------|
| C._caulescens      | 8479 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._caulescens      | 8490 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._caulescens      | 8495 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8369 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8374 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8403 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8405 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8418 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8444 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._gardenii        | 8523 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8391 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8394 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8396 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8406 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8408 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8410 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8419 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8526 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8558 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8573 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8574 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._miniata         | 8992 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8943 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8944 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8947 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8950 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8951 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8954 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8955 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8958 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._mirabilis       | 8963 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._x_nimbicola     | 8553 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._x_nimbicola     | 8578 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._x_nimbicola     | 8594 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._x_nimbicola     | 8595 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8932 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8933 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8934 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8936 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8937 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8938 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8939 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8940 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8941 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._nobilis         | 8945 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._robusta         | 8415 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._robusta         | 8440 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._robusta         | 8442 | CTAATATCAC | ATATACATT  | GTTTTTTTCA | TAACGTAAAC | CGCGCGCATT |
| C._robusta         | 8462 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| Cr._vansonii       | 8964 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| G._lanuginosa      | 9051 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| G._lanuginosa      | 9055 | CTAATATCAC | ATATACATT  | GTTTCCTTCA | TAACGTAAAC | CGCGCGCATT |
| C._aff._caulescens | 8575 | TTCTATTGAA | TAGGATTCTA | GAATAATTCT | TCGAAAGATA | TACATACAGG |
| C._aff._gardeniil  | 8325 | TTCTATTGAA | TCGGATTCTA | GAATAATTCT | TCGAAAGATA | TACATACAGG |
| C._aff._gardeniil  | 8576 | TTCTATTGAA | TCGGATTCTA | GAATAATTCT | TCGAAAGATA | TACATACAGG |
| C._aff._gardeniil  | 8577 | TTCTATTGAA | TCGGATTCTA | GAATAATTCT | TCGAAAGATA | TACATACAGG |
| C._aff._gardeniil  | 9000 | TTCTATTGAA | TCGGATTCTA | GAATAATTCT | TC         |            |



[illegible]

|                           |      |            |             |            |            |            |
|---------------------------|------|------------|-------------|------------|------------|------------|
| <i>C. miniata</i>         | 8391 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8394 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8396 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8406 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8408 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8410 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8419 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8526 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8558 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8573 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8574 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. miniata</i>         | 8992 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8943 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8944 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8947 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8950 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8951 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8954 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8955 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8958 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. mirabilis</i>       | 8963 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. x nimbicola</i>     | 8553 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. x nimbicola</i>     | 8578 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. x nimbicola</i>     | 8594 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. x nimbicola</i>     | 8595 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8932 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8933 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8934 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8936 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8937 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8938 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8939 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8940 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8941 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. nobilis</i>         | 8945 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. robusta</i>         | 8415 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. robusta</i>         | 8440 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. robusta</i>         | 8442 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>C. robusta</i>         | 8462 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>Cr. vansonii</i>       | 8964 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>G. lanuginosa</i>      | 9051 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
| <i>G. lanuginosa</i>      | 9055 | GGCTGTGGCT | GGACTTATAG  | ACATTACATA | TATCTAGTGT | GACCCCCCTA |
|                           |      |            |             |            |            |            |
| <i>C. aff. caulescens</i> | 8575 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. gardeniil</i>  | 8325 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. gardeniil</i>  | 8576 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. gardeniil</i>  | 8577 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. gardeniil</i>  | 9000 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. gardeniil</i>  | 9001 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. robusta</i>    | 8579 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. robusta</i>    | 8582 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. robusta</i>    | 8585 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. aff. robusta</i>    | 8593 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. caulescens</i>      | 8479 | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCCTCCT | ACAACTCTAG |
| <i>C. caulescens</i>      |      |            |             |            |            |            |

| Accession               | Species    | Gene        | Protein    | Accession  | Species    | Gene | Protein |
|-------------------------|------------|-------------|------------|------------|------------|------|---------|
| C. miniata_8410         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8419         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8526         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8558         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8573         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8574         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. miniata_8992         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8943       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8944       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8947       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8950       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8951       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8954       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8955       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8958       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. mirabilis_8963       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. x nimbiicola_8553    | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. x nimbiicola_8578    | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. x nimbiicola_8594    | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. x nimbiicola_8595    | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8932         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8933         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8934         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8936         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8937         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8938         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8939         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8940         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8941         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. nobilis_8945         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. robusta_8415         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. robusta_8440         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. robusta_8442         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. robusta_8462         | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| Cr. vansonii_8964       | ACCCATCCAC | CATTTTCGTAA | TATCGTCTAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| G. lanuginosa_9051      | ACCCATCCAC | CATTTTCGTAA | TATCGTATAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| G. lanuginosa_9055      | ACCCATCCAC | CATTTTCGTAA | TATCGTATAT | CTTTCTCCT  | ACAACTCTAG |      |         |
| C. aff. caulescens_8575 | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      | 400     |
| C. aff. gardeniil_8325  | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. gardeniil_8576  | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. gardeniil_8577  | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. gardeniil_9000  | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. gardeniil_9001  | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. robusta_8579    | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. robusta_8582    | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. robusta_8585    | TCGTATA--- | -TACATATGT  | ATTTCTATCT | AT-TATGTTT | CCCAACTAAG |      |         |
| C. aff. robusta_8593</  |            |             |            |            |            |      |         |

```

C._miniata_8574 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._miniata_8992 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8943 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8944 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8947 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8950 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8951 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8954 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8955 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8958 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._mirabilis_8963 TCGTATATAC ATACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._x_nimbicola_8553 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._x_nimbicola_8578 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._x_nimbicola_8594 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._x_nimbicola_8595 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8932 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8933 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8934 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8936 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8937 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8938 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8939 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8940 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8941 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._nobilis_8945 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._robusta_8415 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._robusta_8440 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._robusta_8442 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
C._robusta_8462 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
Cr._vansonii_8964 TCGTATA--- -TACATATGT ATTTCTATCT AT-TATGTTT CCCTAACTAAG
G._lanuginosa_9051 TCGTATATAC ATATATATGT ATTTCTATCT ATGTATGTTT CCCTAACTAAG
G._lanuginosa_9055 TCGTATATAC ATATATATGT ATTTCTATCT ATGTATGTTT CCCTAACTAAG

C._aff._caulescens_8575 AACCAAATAG ATTTTCCTGA ACCACGCATT 430
C._aff._gardeniil_8325 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._gardeniil_8576 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._gardeniil_8577 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._gardeniil_9000 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._gardeniil_9001 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._robusta_8579 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._robusta_8582 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._robusta_8585 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._aff._robusta_8593 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._caulescens_8479 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._caulescens_8490 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._caulescens_8495 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8369 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8374 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8403 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8405 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8418 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8444 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._gardeniil_8523 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8391 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8394 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8396 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8406 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8408 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8410 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8419 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8526 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8558 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8573 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8574 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._miniata_8992 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8943 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8944 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8947 AACCAAATAG ATTTTCCTGA ACCACGCATT

```

```

C._mirabilis_8950 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8951 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8954 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8955 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8958 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._mirabilis_8963 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._x_nimbicola_8553 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._x_nimbicola_8578 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._x_nimbicola_8594 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._x_nimbicola_8595 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8932 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8933 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8934 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8936 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8937 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8938 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8939 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8940 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8941 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._nobilis_8945 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._robusta_8415 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._robusta_8440 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._robusta_8442 AACCAAATAG ATTTTCCTGA ACCACGCATT
C._robusta_8462 AACCAAATAG ATTTTCCTGA ACCACGCATT
Cr._vansonii_8964 AACCAAATAG ATTTTCCCGA ACCACGCATT
G._lanuginosa_9051 AACCAAATAG ATTTTCCTGA ACCACGCATT
G._lanuginosa_9055 AACCAAATAG ATTTTCCTGA ACCACGCATT

```

Appendix B2 – *Clivia ITS2*

|                            |            |            |             |            |            |     |
|----------------------------|------------|------------|-------------|------------|------------|-----|
| <i>C. gardenii</i> _8403   | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG | 50  |
| <i>C. gardenii</i> _8418   | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. gardenii</i> _8374   | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. gardenii</i> _8523   | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. robusta</i> _8415    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. miniata</i> _8406    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. miniata</i> _8391    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. miniata</i> _8394    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. miniata</i> _8992    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. miniata</i> _8419    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. caulescens</i> _9049 | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | CGACGCTCCG |     |
| <i>C. nobilis</i> _8932    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | CGACGCTCCG |     |
| <i>C. nobilis</i> _8936    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | CGACGCTCCG |     |
| <i>C. nobilis</i> _8933    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | CGACGCTCCG |     |
| <i>C. nobilis</i> _8937    | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | CGACGCTCCG |     |
| <i>C. mirabilis</i> _8953  | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>C. mirabilis</i> _8963  | TATCTGGCCA | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>Cr. vansonii</i> _8964  | TTTCTGGCTG | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGACGCTCCG |     |
| <i>G. sp. nov</i> _2062    | TATCTGGCCG | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGGCGCTCTG |     |
| <i>Gethyllis</i> _4312     | TATCTGGCCG | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGGCGCTCTG |     |
| <i>Gethyllis</i> _3834     | TACCTGGCCG | AGGGCACGCC | TGCCTGGGCG  | TCACGCCTAC | TGGCGCTCTG |     |
|                            |            |            |             |            |            |     |
| <i>C. gardenii</i> _8403   | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA | 100 |
| <i>C. gardenii</i> _8418   | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. gardenii</i> _8374   | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. gardenii</i> _8523   | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. robusta</i> _8415    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. miniata</i> _8406    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. miniata</i> _8391    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. miniata</i> _8394    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. miniata</i> _8992    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. miniata</i> _8419    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. caulescens</i> _9049 | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. nobilis</i> _8932    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. nobilis</i> _8936    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. nobilis</i> _8933    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. nobilis</i> _8937    | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. mirabilis</i> _8953  | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>C. mirabilis</i> _8963  | TGCCTCTTGC | CCCCTCACCC | TGCGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>Cr. vansonii</i> _8964  | TGCTTCTTGC | CCCCTCACCC | TGTGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>G. sp. nov</i> _2062    | TGCCTCTTGC | ACCATCGCCT | TGTGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>Gethyllis</i> _4312     | TGCCTCTTGC | ACCATCGCCT | TGTGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
| <i>Gethyllis</i> _3834     | TGCCTCTTGC | ACCATCGCCT | TGTGCGGTTCG | GCGGCAGGCA | CGGATGCGGA |     |
|                            |            |            |             |            |            |     |
| <i>C. gardenii</i> _8403   | GATTGGCCCC | -CC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT | 150 |
| <i>C. gardenii</i> _8418   | GATTGGCCCC | -CC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. gardenii</i> _8374   | GATTGGCCCC | -CC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. gardenii</i> _8523   | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. robusta</i> _8415    | GATTGGCCCC | -CC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. miniata</i> _8406    | GATTGGCCCC | -CC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. miniata</i> _8391    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. miniata</i> _8394    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. miniata</i> _8992    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. miniata</i> _8419    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. caulescens</i> _9049 | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | AGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. nobilis</i> _8932    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. nobilis</i> _8936    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. nobilis</i> _8933    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. nobilis</i> _8937    | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. mirabilis</i> _8953  | GAATGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>C. mirabilis</i> _8963  | GAATGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>Cr. vansonii</i> _8964  | GATTGGCCCC | ACC-ACGCAT | CGCTGCGCGG  | CGGGTCGAAG | CGCGTGCCGT |     |
| <i>G. sp. nov</i> _2062    | GATTGGCTC- | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>Gethyllis</i> _4312     | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |
| <i>Gethyllis</i> _3834     | GATTGGCCCC | -CCCACGCAT | CGCTGCGCGG  | CGGGTCGAAG | TGCGGGCCGT |     |

|                            |            |            |            |            |            |     |
|----------------------------|------------|------------|------------|------------|------------|-----|
| <i>C. gardenii</i> _8403   | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG | 200 |
| <i>C. gardenii</i> _8418   | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. gardenii</i> _8374   | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. gardenii</i> _8523   | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. robusta</i> _8415    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. miniata</i> _8406    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. miniata</i> _8391    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. miniata</i> _8394    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. miniata</i> _8992    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. miniata</i> _8419    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. caulescens</i> _9049 | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. nobilis</i> _8932    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. nobilis</i> _8936    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. nobilis</i> _8933    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. nobilis</i> _8937    | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. mirabilis</i> _8953  | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGAT | TGACACGCAC | GACGCCGCTG |     |
| <i>C. mirabilis</i> _8963  | CGGCAGGGTC | AGACGCGGCG | ACTGTTGGTT | TGACACGCAC | GACGCCGCTG |     |
| <i>Cr. vansonii</i> _8964  | CGGCTGGGTC | AGACGCGGCG | AGTGGTGGAC | TGACGCGCAC | GCCGCCGCTG |     |
| <i>G. sp. nov</i> _2062    | TGGCAGGGCC | AGACGCGGCG | AGTGGTGGAT | TGACACGCAC | GACGTCGTCG |     |
| <i>Gethyllis</i> _4312     | TGGCTGGGCC | AGACGCGGCG | AGTGGTGGAT | TGACACGCAC | GACGCCGTTG |     |
| <i>Gethyllis</i> _3834     | TGGCTGGGCC | AGACGCGGCG | AGTGGTGGAT | TGACACGCAC | GACGTCGTTG |     |
| <i>C. gardenii</i> _8403   | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG | 250 |
| <i>C. gardenii</i> _8418   | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. gardenii</i> _8374   | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. gardenii</i> _8523   | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. robusta</i> _8415    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. miniata</i> _8406    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. miniata</i> _8391    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. miniata</i> _8394    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. miniata</i> _8992    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. miniata</i> _8419    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. caulescens</i> _9049 | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. nobilis</i> _8932    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. nobilis</i> _8936    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. nobilis</i> _8933    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. nobilis</i> _8937    | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. mirabilis</i> _8953  | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>C. mirabilis</i> _8963  | AAGTG--ACC | CTAGATCGGG | CGGTGCACCG | GAGGAACCCA | CGCCGACGGG |     |
| <i>Cr. vansonii</i> _8964  | AAGTGAGAGC | CTGGATCGAC | CGGCGCACCG | GAGGAACCCA | CGCTGACTGG |     |
| <i>G. sp. nov</i> _2062    | G-GTG--ACC | CTAGCTCGGA | CGGTGCGCCG | GAGGAACCCA | TGTCGACGGG |     |
| <i>Gethyllis</i> _4312     | A-GTG--AAC | CTAGCTCGGG | CGGTGCACTG | -AGGAACCCA | CGTCGACGGG |     |
| <i>Gethyllis</i> _3834     | A-GTG--AAC | CTAGCTCGGG | CGGTGCACTG | GAGGAACCCA | CGTCGACGGG |     |
| <i>C. gardenii</i> _8403   | CGCCATGTGA | GC         | 262        |            |            |     |
| <i>C. gardenii</i> _8418   | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. gardenii</i> _8374   | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. gardenii</i> _8523   | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. robusta</i> _8415    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. miniata</i> _8406    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. miniata</i> _8391    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. miniata</i> _8394    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. miniata</i> _8992    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. miniata</i> _8419    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. caulescens</i> _9049 | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. nobilis</i> _8932    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. nobilis</i> _8936    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. nobilis</i> _8933    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. nobilis</i> _8937    | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. mirabilis</i> _8953  | CGCCATGTGA | GC         |            |            |            |     |
| <i>C. mirabilis</i> _8963  | CGCCATGTGA | GC         |            |            |            |     |
| <i>Cr. vansonii</i> _8964  | CGCCATGCGA | GC         |            |            |            |     |
| <i>G. sp. nov</i> _2062    | CGCCACGCGA | GG         |            |            |            |     |
| <i>Gethyllis</i> _4312     | CGCCACGCGA | GC         |            |            |            |     |
| <i>Gethyllis</i> _3834     | CGCCATGCGA | GC         |            |            |            |     |

### Appendix B3 – *Clivia matK* (CCDB and UFS sequences)

50



|                                 |            |            |             |            |                |
|---------------------------------|------------|------------|-------------|------------|----------------|
| <i>C._gardenii_8887*</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._gardenii_8888*</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._gardenii_8889*</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._gardenii_8892*</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8391*</i>         | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8394*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8396*</i>         | TTCAAAAGAA | AAAAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8406*</i>         | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8408*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8410*</i>         | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8419*</i>         | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8558*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8573</i>          | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8574</i>          | TTCAAAAGAA | AATAAAAGAC | TCTTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8616*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8617</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8637*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8781*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8943</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8944</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8947*</i>       | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8950</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8951</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8953*</i>       | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8954</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8955*</i>       | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8958*</i>       | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._mirabilis_8963</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8932*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8933*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8936*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8937*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8938</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8939</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8940*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._nobilis_8941</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_5518</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_8365*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_8415*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_8440*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_8442*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._robusta_8462*</i>         | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGCGT     |
| <i>C._robusta_8817</i>          | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8552*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8553*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8554*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8555*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8556*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8566*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8570*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>C._x_nimbicola_8578*</i>     | TTCAAAAGAA | AATAAAAGAC | TATTTTGTTT  | CCTATACAAT | TTTTATGTGT     |
| <i>Cr._vansonii_8964</i>        | TTCAAAAGAA | AATAAAAGAC | TATTTTCGGTT | CCTATACAAT | TTTTATGTGT     |
| <i>C._miniata_8667*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA 100 |
| <i>C._aff._miniata_8327*</i>    | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._gardenii_8615*</i>        | TTGAATGTGA | ATTTTTATTT | TTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8469*</i>    | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8470*</i>    | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8780</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._caulescens_8575*</i> | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8325</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._gardeniil_8576</i>   | TTGAATGTGA | ATTTTTATTT | TTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._gardeniil_8577</i>   | TTGAATGTGA | ATTTTTATTT | TTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_9000</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_9001</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._gardeniil2_8781</i>  | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8319</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |
| <i>C._aff._miniata_8324</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC  | GTAAACAATC | TTCTTATTTA     |



|                                 |            |            |            |            |                |
|---------------------------------|------------|------------|------------|------------|----------------|
| <i>C._mirabilis_8943</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8944</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8947*</i>       | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8950</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8951</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8953*</i>       | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8954</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8955*</i>       | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8958*</i>       | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._mirabilis_8963</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8932*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8933*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8936*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8937*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8938</i>          | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8939</i>          | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8940*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._nobilis_8941</i>          | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_5518</i>          | TTGAATGTGA | ATTTTTATTT | TTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8365*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8415*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8440*</i>         | TTGAATGTGA | ATTTTTATTT | TTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8442*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8462*</i>         | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._robusta_8817</i>          | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8552*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8553*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8554*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8555*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8556*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8566*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8570*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>C._x_nimbicola_8578*</i>     | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | TTCTTATTTA     |
| <i>Cr._vansonii_8964</i>        | TTGAATGTGA | ATTTTTATTT | GTTTTTATTC | GTAAACAATC | CTCTTATTTA     |
|                                 |            |            |            |            |                |
| <i>C._miniata_8667*</i>         | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA 150 |
| <i>C._aff._miniata_8327*</i>    | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._gardenii_8615*</i>        | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8469*</i>    | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8470*</i>    | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8780</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._caulescens_8575*</i> | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8325</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._gardeniil_8576</i>   | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._gardeniil_8577</i>   | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_9000</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_9001</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._gardeniil2_8781</i>  | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8319</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8324</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8660</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8686</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_8689</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._miniata_9002</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8579</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8582</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8585</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8593</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8803</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8804</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8805</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8807</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8808</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8809</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8811</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._aff._robusta_8814</i>     | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._caulescens_8479</i>       | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |
| <i>C._caulescens_8417*</i>      | CGATTAACAT | CTTTTGGAAC | TTTTCTTGAG | CGAACACATT | TCTATGGAAA     |

[illegible]

|                           |       |            |            |            |            |            |     |
|---------------------------|-------|------------|------------|------------|------------|------------|-----|
| <i>C. robusta</i>         | 5518  | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8365* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8415* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8440* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8442* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8462* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. robusta</i>         | 8817  | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8552* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8553* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8554* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8555* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8556* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8566* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8570* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>C. x nimbicola</i>     | 8578* | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
| <i>Cr. vansonii</i>       | 8964  | CGATTAACAT | CTTTTGAAC  | TTTTCTTGAG | CGAACACATT | TCTATGAAA  |     |
|                           |       |            |            |            |            |            |     |
| <i>C. miniata</i>         | 8667* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT | 200 |
| <i>C. aff. miniata</i>    | 8327* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. gardenii</i>        | 8615* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8469* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8470* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8780  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. caulescens</i> | 8575* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8325  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. gardeniil</i>  | 8576  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. gardeniil</i>  | 8577  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 9000  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 9001  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. gardenii2</i>  | 8781  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8319  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8324  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8660  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8686  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 8689  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. miniata</i>    | 9002  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8579  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8582  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8585  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8593  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8803  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8804  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8805  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8807  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8808  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8809  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8811  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. aff. robusta</i>    | 8814  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. caulescens</i>      | 8479  | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. caulescens</i>      | 8417* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. caulescens</i>      | 8487* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |
| <i>C. caulescens</i>      | 8488* | AATAGAACAT | CTTCAAATAG | AAAAATTTAT | AGTAATATGT | CGTAACAATT |     |

[illegible]

[illegible]

[illegible]



[illegible]

|                                  |            |            |            |            |                |
|----------------------------------|------------|------------|------------|------------|----------------|
| <i>C. mirabilis</i> _8951        | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. mirabilis</i> _8953*       | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. mirabilis</i> _8954        | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. mirabilis</i> _8955*       | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. mirabilis</i> _8958*       | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. mirabilis</i> _8963        | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8932*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8933*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8936*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8937*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8938          | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8939          | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8940*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. nobilis</i> _8941          | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _5518          | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8365*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8415*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8440*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8442*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8462*         | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. robusta</i> _8817          | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8552*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8553*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8554*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8555*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8556*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8566*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8570*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>C. x nimbiicola</i> _8578*    | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
| <i>Cr. vansonii</i> _8964        | TATCAAGGAA | AAGCAATTCT | TGCTTCAAAG | GGGACTCATC | TTCTGATGAC     |
|                                  |            |            |            |            |                |
| <i>C. miniata</i> _8667*         | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT 350 |
| <i>C. aff. miniata</i> _8327*    | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. gardenii</i> _8615*        | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8469*    | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8470*    | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8780     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. caulescens</i> _8575* | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8325     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. gardeniil</i> _8576   | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. gardeniil</i> _8577   | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _9000     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _9001     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. gardeniil2</i> _8781  | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8319     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8324     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8660     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8686     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _8689     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. miniata</i> _9002     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8579     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8582     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8585     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8593     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8803     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8804     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8805     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8807     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8808     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8809     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8811     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. aff. robusta</i> _8814     | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8479       | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8417*      | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8487*      | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8488*      | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8490       | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |
| <i>C. caulescens</i> _8494*      | GAAATGGAAA | TATCATTTTG | TCAATTTCTG | GCAATATTAT | TTTCACTTTT     |

[illegible]

```

C._robusta_8442* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._robusta_8462* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._robusta_8817 GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8552* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8553* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8554* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8555* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8556* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8566* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8570* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
C._x_nimbicola_8578* GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT
Cr._vansonii_8964 GAAATGGAAA TATCATTTTG TCAATTTCTG GCAATATTAT TTTCACTTTT

C._miniata_8667* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC 400
C._aff._miniata_8327* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._gardenii_8615* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8469* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8470* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8780 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._caulescens_8575* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8325 ----- AATTATCAAA CTATTCTTTC
C._aff._gardeniil_8576 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._gardeniil_8577 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_9000 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_9001 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._gardeniil_8781 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8319 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8324 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8660 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8686 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_8689 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._miniata_9002 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8579 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8582 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8585 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8593 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8803 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8804 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8805 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8807 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8808 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8809 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8811 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._aff._robusta_8814 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8479 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8417* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8487* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8488* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8490 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8494* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8495 GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8496* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8497* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8498* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8499* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8500* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8501* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8502* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8503* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8504* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8557* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8561* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8562* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8571* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._caulescens_8609* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._gardenii_8367* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._gardenii_8368* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC
C._gardenii_8369* GGTCTCAACC GTACAGGATC CATATAAATA AATTATCAAA CTATTCTTTC

```

|                              |            |            |            |            |                |
|------------------------------|------------|------------|------------|------------|----------------|
| <i>C._gardenii_8374*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTCTTCTTTC     |
| <i>C._gardenii_8376*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8403*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8405*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8418*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8444*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8523</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._gardenii_8780*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8884*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._gardenii_8885*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._gardenii_8887*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._gardenii_8888*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._gardenii_8889*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._gardenii_8892*</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8391*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8394*</i>      | GGTCTCAACC | ATACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8396*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8406*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._miniata_8408*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8410*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8419*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8558*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8573</i>       | GGTCTCAACC | GTACAGGATC | CATATAAGTA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8574</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8616*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._miniata_8617</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8637*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._miniata_8781*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8943</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8944</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8947*</i>    | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8950</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8951</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8953*</i>    | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8954</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8955*</i>    | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8958*</i>    | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._mirabilis_8963</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8932*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8933*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8936*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8937*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8938</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8939</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8940*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._nobilis_8941</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_5518</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_8365*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTCTCAAA | CTATTCTTTC     |
| <i>C._robusta_8415*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_8440*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_8442*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_8462*</i>      | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._robusta_8817</i>       | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8552*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8553*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8554*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8555*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8556*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8566*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8570*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>C._x_nimbicola_8578*</i>  | GGTCTCAACC | GTACAGGATC | CATATAAATA | AATTATCAAA | CTATTCTTTC     |
| <i>Cr._vansonii_8964</i>     | GGTCTCAACC | GTACAGGATC | CATATAAATC | AATTATCAAA | CTATTCTTTC     |
| <i>C._miniata_8667*</i>      | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG 450 |
| <i>C._aff._miniata_8327*</i> | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._gardenii_8615*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._aff._miniata_8469*</i> | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._aff._miniata_8470*</i> | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |

[illegible]

|                                 |            |            |            |            |                |
|---------------------------------|------------|------------|------------|------------|----------------|
| <i>C._miniata_8408*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8410*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8419*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8558*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8573</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8574</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8616*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8617</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8637*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._miniata_8781*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8943</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8944</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8947*</i>       | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8950</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8951</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8953*</i>       | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8954</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8955*</i>       | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8958*</i>       | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._mirabilis_8963</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AGAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8932*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8933*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8936*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8937*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8938</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8939</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8940*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._nobilis_8941</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_5518</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8365*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8415*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8440*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8442*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8462*</i>         | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._robusta_8817</i>          | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8552*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8553*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8554*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8555*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8556*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8566*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8570*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>C._x_nimbicola_8578*</i>     | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | ATAAATTCTT | CGGCAGTAAG     |
| <i>Cr._vansonii_8964</i>        | TATTTTCTGG | GTTATCTTTC | AAGTCTACTA | AAAAATTCTT | CGGCAGTAAG     |
|                                 |            |            |            |            |                |
| <i>C._miniata_8667*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT 500 |
| <i>C._aff._miniata_8327*</i>    | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._gardenii_8615*</i>        | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8469*</i>    | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8470*</i>    | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8780</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._caulescens_8575*</i> | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8325</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._gardeniil_8576</i>   | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._gardeniil_8577</i>   | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_9000</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_9001</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._gardeniil2_8781</i>  | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8319</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8324</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8660</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8686</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_8689</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._miniata_9002</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._robusta_8579</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._robusta_8582</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._robusta_8585</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |
| <i>C._aff._robusta_8593</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT     |

[illegible]



|                                 |            |            |            |            |            |
|---------------------------------|------------|------------|------------|------------|------------|
| <i>C._mirabilis_8958*</i>       | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._mirabilis_8963</i>        | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8932*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8933*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8936*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8937*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8938</i>          | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8939</i>          | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8940*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._nobilis_8941</i>          | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_5518</i>          | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8365*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8415*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8440*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8442*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8462*</i>         | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._robusta_8817</i>          | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._x_nimbicola_8552*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8553*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8554*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8555*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8556*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8566*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8570*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>C._x_nimbicola_8578*</i>     | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCATT | ACTAAGAAAT |
| <i>Cr._vansonii_8964</i>        | GAATCAAATG | TTAGAGAATT | CATTTCTAAT | AGATACCGTT | ACTAAGAAAT |
| <i>C._miniata_8667*</i>         | TTGATACCAT | AGTCCCAG   | 518        |            |            |
| <i>C._aff._miniata_8327*</i>    | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._gardenii_8615*</i>        | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8469*</i>    | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8470*</i>    | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8780</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._caulescens_8575*</i> | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8325</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._gardeniil_8576</i>   | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._gardeniil_8577</i>   | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_9000</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_9001</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._gardenii2_8781</i>   | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8319</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8324</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8660</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8686</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_8689</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._miniata_9002</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8579</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8582</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8585</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8593</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8803</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8804</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8805</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8807</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8808</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8809</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8811</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._aff._robusta_8814</i>     | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8479</i>       | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8417*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8487*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8488*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8490</i>       | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8494*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8495</i>       | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8496*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8497*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |
| <i>C._caulescens_8498*</i>      | TTGATACCAT | AGTCCCAG   |            |            |            |

*C.\_caulescens*\_8499\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8500\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8501\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8502\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8503\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8504\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8557\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8561\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8562\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8571\* TTGATACCAT AGTCCCAG  
*C.\_caulescens*\_8609\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8367\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8368\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8369\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8374\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8376\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8403\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8405\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8418\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8444\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8523 TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8780\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8884\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8885\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8887\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8888\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8889\* TTGATACCAT AGTCCCAG  
*C.\_gardenii*\_8892\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8391\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8394\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8396\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8406\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8408\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8410\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8419\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8558\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8573 TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8574 TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8616\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8617 TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8637\* TTGATACCAT AGTCCCAG  
*C.\_miniata*\_8781\* TTGATACCAT AGTCCCAG  
*C.\_mirabilis*\_8943 TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8944 TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8947\* TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8950 TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8951 TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8953\* TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8954 TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8955\* TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8958\* TTGATACCAT AGCCCCAG  
*C.\_mirabilis*\_8963 TTGATACCAT AGCCCCAG  
*C.\_nobilis*\_8932\* TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8933\* TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8936\* TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8937\* TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8938 TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8939 TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8940\* TTGATACCAT AGTCCCAG  
*C.\_nobilis*\_8941 TTGATACCAT AGTCCCAG  
*C.\_robusta*\_5518 TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8365\* TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8415\* TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8440\* TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8442\* TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8462\* TTGATACCAT AGTCCCAG  
*C.\_robusta*\_8817 TTGATACCAT AGTCCCAG  
*C.\_x\_nimbicola*\_8552\* TTGATACCAT AGTCCCAG

```
C._x_nimbicola_8553* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8554* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8555* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8556* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8566* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8570* TTGATACCAT AGTCCCAG  
C._x_nimbicola_8578* TTGATACCAT AGTCCCAG  
Cr._vansonii_8964 TTGATACCAT AGTCCCAG
```

Appendix B4 – *Clivia psbA-trnH*

|                                   |            |            |            |            |            |     |
|-----------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C. gardenii</i> _8523          | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG | 50  |
| <i>C. gardenii</i> _8403          | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. gardenii</i> _8374          | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. miniata</i> _8394           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. miniata</i> _8406           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. miniata</i> _8419           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. x nimbicola</i> _8595       | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. nobilis</i> _8933           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. nobilis</i> _8936           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. nobilis</i> _8937           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. mirabilis</i> _8953         | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. robusta</i> _8415           | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>Gethyllis lanigunose</i> _3554 | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>Gethyllis setosa</i> _3227     | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>Gethyllis sp. nov.</i> _2062   | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>Gethyllis</i> _4169            | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>Cryptostephanus</i> _8964      | GATATTCTAT | TGGACATAGA | ATGCCAATCT | TTAAAATGTA | AAAAAAGGAG |     |
| <i>C. gardenii</i> _8523          | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA | 100 |
| <i>C. gardenii</i> _8403          | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. gardenii</i> _8374          | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. miniata</i> _8394           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. miniata</i> _8406           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. miniata</i> _8419           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. x nimbicola</i> _8595       | TAATCAGCTG | TGGCACGTTT | GCTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. nobilis</i> _8933           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. nobilis</i> _8936           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. nobilis</i> _8937           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. mirabilis</i> _8953         | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. robusta</i> _8415           | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>Gethyllis lanigunose</i> _3554 | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>Gethyllis setosa</i> _3227     | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>Gethyllis sp. nov.</i> _2062   | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>Gethyllis</i> _4169            | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>Cryptostephanus</i> _8964      | TAATCAGCTG | TGGCACGTTT | ACTAAAAAAA | AATCCTTTTG | TAGCTAATCA |     |
| <i>C. gardenii</i> _8523          | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT | 150 |
| <i>C. gardenii</i> _8403          | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. gardenii</i> _8374          | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. miniata</i> _8394           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. miniata</i> _8406           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. miniata</i> _8419           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. x nimbicola</i> _8595       | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. nobilis</i> _8933           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. nobilis</i> _8936           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. nobilis</i> _8937           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. mirabilis</i> _8953         | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. robusta</i> _8415           | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>Gethyllis lanigunose</i> _3554 | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>Gethyllis setosa</i> _3227     | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>Gethyllis sp. nov.</i> _2062   | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>Gethyllis</i> _4169            | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>Cryptostephanus</i> _8964      | TTTATTGAGA | AAAATTGAAA | AACTCAACAT | GAGGGAG-GA | GAAAGAA-AT |     |
| <i>C. gardenii</i> _8523          | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC | 200 |
| <i>C. gardenii</i> _8403          | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. gardenii</i> _8374          | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. miniata</i> _8394           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. miniata</i> _8406           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. miniata</i> _8419           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. x nimbicola</i> _8595       | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. nobilis</i> _8933           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. nobilis</i> _8936           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. nobilis</i> _8937           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. mirabilis</i> _8953         | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C. robusta</i> _8415           | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>Gethyllis lanigunose</i> _3554 | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>Gethyllis setosa</i> _3227     | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>Gethyllis sp. nov.</i> _2062   | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |

|                                   |            |            |            |            |            |     |
|-----------------------------------|------------|------------|------------|------------|------------|-----|
| <i>Gethyllis</i> _4169            | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>Cryptostephanus</i> _8964      | AATAGTAACT | TGGTCTCGGG | CATCTACCAT | TATACCCAAA | ATGATTGGCC |     |
| <i>C._gardenii</i> _8523          | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA | 250 |
| <i>C._gardenii</i> _8403          | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._gardenii</i> _8374          | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._miniata</i> _8394           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._miniata</i> _8406           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._miniata</i> _8419           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._x_nimbicola</i> _8595       | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._nobilis</i> _8933           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._nobilis</i> _8936           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._nobilis</i> _8937           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._mirabilis</i> _8953         | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._robusta</i> _8415           | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>Gethyllis_lanigunose</i> _3554 | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>Gethyllis_setosa</i> _3227     | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>Gethyllis_sp._nov.</i> _2062   | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>Gethyllis</i> _4169            | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>Cryptostephanus</i> _8964      | ATACAATCGC | TATTCATAAT | GGAAAGGAAC | ATTTACCTAT | TTATATAACA |     |
| <i>C._gardenii</i> _8523          | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGGCTTT | 300 |
| <i>C._gardenii</i> _8403          | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._gardenii</i> _8374          | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._miniata</i> _8394           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._miniata</i> _8406           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._miniata</i> _8419           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._x_nimbicola</i> _8595       | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._nobilis</i> _8933           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._nobilis</i> _8936           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._nobilis</i> _8937           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._mirabilis</i> _8953         | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._robusta</i> _8415           | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>Gethyllis_lanigunose</i> _3554 | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>Gethyllis_setosa</i> _3227     | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>Gethyllis_sp._nov.</i> _2062   | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>Gethyllis</i> _4169            | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>Cryptostephanus</i> _8964      | GATCGTATGG | TAGGTCACAA | ATTGGGAGAA | TTCGCGCCTA | CTCTGACTTT |     |
| <i>C._gardenii</i> _8523          | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA | 350 |
| <i>C._gardenii</i> _8403          | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._gardenii</i> _8374          | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._miniata</i> _8394           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._miniata</i> _8406           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._miniata</i> _8419           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._x_nimbicola</i> _8595       | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._nobilis</i> _8933           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._nobilis</i> _8936           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._nobilis</i> _8937           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._mirabilis</i> _8953         | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._robusta</i> _8415           | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>Gethyllis_lanigunose</i> _3554 | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>Gethyllis_setosa</i> _3227     | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>Gethyllis_sp._nov.</i> _2062   | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>Gethyllis</i> _4169            | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>Cryptostephanus</i> _8964      | CGCGAGACAT | GCGAGAAACG | ATAATAAATC | TCGTCGTTAA | TTTGAATAAA |     |
| <i>C._gardenii</i> _8523          | AAAAAAAAAA | AAA-GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC | 400 |
| <i>C._gardenii</i> _8403          | AAAAAAAAAA | ----GATACT | TATCATTTCA | TTCATTGGCG | GGGGATAACC |     |
| <i>C._gardenii</i> _8374          | AAAAAAAAAA | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._miniata</i> _8394           | AAAAAA--   | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._miniata</i> _8406           | AAAAAAAAAA | ----GATACT | TATCATTTCA | TTCATTGGCG | GGGGATAACC |     |
| <i>C._miniata</i> _8419           | AAAAAAAAAA | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._x_nimbicola</i> _8595       | AAAAAAAAAA | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._nobilis</i> _8933           | AAAAAA--   | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._nobilis</i> _8936           | AAAAAA--   | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._nobilis</i> _8937           | AAAAAA--   | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._mirabilis</i> _8953         | AAAAAAAAAA | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._robusta</i> _8415           | AAAAAAAAAA | GA----TACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>Gethyllis_lanigunose</i> _3554 | AAAAAA--   | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>Gethyllis_setosa</i> _3227     | AAAAAA--   | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>Gethyllis_sp._nov.</i> _2062   | AAAAAA--   | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |

|                                   |            |            |            |            |            |     |
|-----------------------------------|------------|------------|------------|------------|------------|-----|
| <i>Gethyllis</i> _4169            | AAAAAAA--- | ----GATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>Cryptostephanus</i> _8964      | CGAAAAAAA- | GAAAGATACT | TATCA----- | TTCATTGGCG | GGGGATAACC |     |
| <i>C._gardenii</i> _8523          | T-----     | -----      | -----      | -----      | -----      | 450 |
| <i>C._gardenii</i> _8403          | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._gardenii</i> _8374          | TTATGAT--- | -----      | -----      | -----      | -----      |     |
| <i>C._miniata</i> _8394           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._miniata</i> _8406           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._miniata</i> _8419           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._x_nimbicola</i> _8595       | TTATGATAAA | GAACTCGAAT | -----      | -----      | -----      |     |
| <i>C._nobilis</i> _8933           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._nobilis</i> _8936           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._nobilis</i> _8937           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._mirabilis</i> _8953         | TTATGATAAA | GAACTCGAAT | TCGGGTAGAG | AAGTCAAAGT | TTTAGCTCAA |     |
| <i>C._robusta</i> _8415           | T-----     | -----      | -----      | -----      | -----      |     |
| <i>Gethyllis_lanigunose</i> _3554 | TTATGATAAA | GAACTCGAAT | TCGGGTAGAG | AAGTAAA--- | -----      |     |
| <i>Gethyllis_setosa</i> _3227     | TTATGATAAA | GAACTCGAAT | TCGGGTAGAG | AAGTAAA--- | -----      |     |
| <i>Gethyllis_sp._nov.</i> _2062   | TTATGATAAA | GAACTCGAAT | TCGGGTAGAG | AAGTAAA--- | -----      |     |
| <i>Gethyllis</i> _4169            | TTATGATAAA | GAACTCGAAT | TCGGGTAGAG | AAGTAAA--- | -----      |     |
| <i>Cryptostephanus</i> _8964      | T-----     | -----      | -----      | -----      | -----      |     |
| <i>C._gardenii</i> _8523          | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- | 500 |
| <i>C._gardenii</i> _8403          | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._gardenii</i> _8374          | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._miniata</i> _8394           | -----      | -----      | -----AGGG  | A-----CCC  | AATATC---- |     |
| <i>C._miniata</i> _8406           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._miniata</i> _8419           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._x_nimbicola</i> _8595       | -----      | -----      | -----      | AGCGATACCC | AATATC---- |     |
| <i>C._nobilis</i> _8933           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._nobilis</i> _8936           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._nobilis</i> _8937           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>C._mirabilis</i> _8953         | CATATATGTA | TGGAAGATGA | AGGGGAA-GG | AGCGATACCC | AATATCTATC |     |
| <i>C._robusta</i> _8415           | -----      | -----      | -----AGGG  | AGCGATACCC | AATATC---- |     |
| <i>Gethyllis_lanigunose</i> _3554 | -----      | -----      | -----AGCG  | AGCTATACCC | AATATC---- |     |
| <i>Gethyllis_setosa</i> _3227     | -----      | -----      | -----AGCG  | AGCTATACCC | AATATC---- |     |
| <i>Gethyllis_sp._nov.</i> _2062   | -----      | -----      | -----AGCG  | AGCTATACCC | AATATC---- |     |
| <i>Gethyllis</i> _4169            | -----      | -----      | -----AGCG  | AGCTATACCC | AATATC---- |     |
| <i>Cryptostephanus</i> _8964      | -----      | -----      | -----AGGG  | AGCTATACCC | AATATCTATC |     |
| <i>C._gardenii</i> _8523          | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA | 550 |
| <i>C._gardenii</i> _8403          | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._gardenii</i> _8374          | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._miniata</i> _8394           | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._miniata</i> _8406           | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._miniata</i> _8419           | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._x_nimbicola</i> _8595       | TTAAGAAAAC | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._nobilis</i> _8933           | TTAAGAAAAC | AAGAT-AGAT | ATTGGGTATC | ACTCCTTCAA | CGATTCTTAA |     |
| <i>C._nobilis</i> _8936           | TTAAGAAAAC | AAGAT-AGAT | ATTGGGTATC | ACTCCTTCAA | CGATTCTTAA |     |
| <i>C._nobilis</i> _8937           | TTAAGAAAAC | AAGAT-AGAT | ATTGGGTATC | ACTCCTTCAA | CGATTCTTAA |     |
| <i>C._mirabilis</i> _8953         | TTGTTTTCTT | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._robusta</i> _8415           | TTGTTTTCTT | AAGAT----- | ATTGGGTATC | GCTCCTTCAA | CGATTCTTAA |     |
| <i>Gethyllis_lanigunose</i> _3554 | TTGTTTTTTT | AAGAT----- | ATTGGGTATG | GCTCCTTCAA | CGATTCTTAA |     |
| <i>Gethyllis_setosa</i> _3227     | TTGTTTTTTT | AAGAT----- | ATTGGGTATG | GCTCCTTCAA | CGATTCTTAA |     |
| <i>Gethyllis_sp._nov.</i> _2062   | TTGTTTTTTT | AAGAT----- | ATTGGGTATG | GCTCCTTCAA | CGATTCTTAA |     |
| <i>Gethyllis</i> _4169            | TTGTTTTTTT | AAGAT----- | ATTGGGTATG | GCTCCTTCAA | CGATTCTTAA |     |
| <i>Cryptostephanus</i> _8964      | TTGTTTTATT | AAGTTAAGAT | ATTGGGTATG | GCTCCTTCAA | CGATTCTTAA |     |
| <i>C._gardenii</i> _8523          | TACA       | 554        |            |            |            |     |
| <i>C._gardenii</i> _8403          | TACA       |            |            |            |            |     |
| <i>C._gardenii</i> _8374          | TACA       |            |            |            |            |     |
| <i>C._miniata</i> _8394           | TACA       |            |            |            |            |     |
| <i>C._miniata</i> _8406           | TACA       |            |            |            |            |     |
| <i>C._miniata</i> _8419           | TACA       |            |            |            |            |     |
| <i>C._x_nimbicola</i> _8595       | TACA       |            |            |            |            |     |
| <i>C._nobilis</i> _8933           | TACA       |            |            |            |            |     |
| <i>C._nobilis</i> _8936           | TACA       |            |            |            |            |     |
| <i>C._nobilis</i> _8937           | TACA       |            |            |            |            |     |
| <i>C._mirabilis</i> _8953         | TACA       |            |            |            |            |     |
| <i>C._robusta</i> _8415           | TACA       |            |            |            |            |     |
| <i>Gethyllis_lanigunose</i> _3554 | TACA       |            |            |            |            |     |
| <i>Gethyllis_setosa</i> _3227     | TACA       |            |            |            |            |     |
| <i>Gethyllis_sp._nov.</i> _2062   | TACA       |            |            |            |            |     |

*Gethyllis*\_4169 TACA  
*Cryptostephanus*\_8964 TACA

## Appendix B5 – *Clivia rbcLa*

50



[illegible]

[illegible]

| Accession | Gene | Length | GC Content | GC Skew | GC Bias | GC Bias2 | GC Bias4 | GC Bias8 | GC Bias16 | GC Bias32 | GC Bias64 | GC Bias128 | GC Bias256 | GC Bias512 | GC Bias1024 | GC Bias2048 | GC Bias4096 | GC Bias8192 | GC Bias16384 | GC Bias32768 | GC Bias65536 | GC Bias131072 | GC Bias262144 | GC Bias524288 | GC Bias1048576 | GC Bias2097152 | GC Bias4194304 | GC Bias8388608 | GC Bias16777216 | GC Bias33554432 | GC Bias67108864 | GC Bias134217728 | GC Bias268435456 | GC Bias536870912 | GC Bias1073741824 | GC Bias2147483648 | GC Bias4294967296 | GC Bias8589934592 | GC Bias17179869184 | GC Bias34359738368 | GC Bias68719476736 | GC Bias137438953472 | GC Bias274877906944 | GC Bias549755813888 | GC Bias1099511627776 | GC Bias2199023255552 | GC Bias4398046511104 | GC Bias8796093022208 | GC Bias17592186044416 | GC Bias35184372088832 | GC Bias70368744177664 | GC Bias140737488355328 | GC Bias281474976710656 | GC Bias562949953421312 | GC Bias1125899906842624 | GC Bias2251799813685248 | GC Bias4503599627370496 | GC Bias9007199254740992 | GC Bias18014398509481984 | GC Bias36028797018963968 | GC Bias72057594037927936 | GC Bias144115188075855872 | GC Bias288230376151711744 | GC Bias576460752303423488 | GC Bias1152921504606846976 | GC Bias2305843009213693952 | GC Bias4611686018427387904 | GC Bias9223372036854775808 | GC Bias18446744073709551616 | GC Bias36893488147419103232 | GC Bias73786976294838206464 | GC Bias147573952589676412928 | GC Bias295147905179352825856 | GC Bias590295810358705651712 | GC Bias1180591620717411303424 | GC Bias2361183241434822606848 | GC Bias4722366482869645213696 | GC Bias9444732965739290427392 | GC Bias18889465931478580854784 | GC Bias37778931862957161709568 | GC Bias75557863725914323419136 | GC Bias151115727451828646838272 | GC Bias302231454903657293676544 | GC Bias604462909807314587353088 | GC Bias1208925819614629174706176 | GC Bias2417851639229258349412352 | GC Bias4835703278458516698824704 | GC Bias9671406556917033397649408 | GC Bias19342813113834066795298816 | GC Bias38685626227668133590597632 | GC Bias77371252455336267181195264 | GC Bias154742504910672534362390528 | GC Bias309485009821345068724781056 | GC Bias618970019642690137449562112 | GC Bias1237940039285380274899124224 | GC Bias2475880078570760549798248448 | GC Bias4951760157141521099596496896 | GC Bias9903520314283042199192993792 | GC Bias19807040628566084398385987584 | GC Bias39614081257132168796771975168 | GC Bias79228162514264337593543950336 | GC Bias158456325028528675187087900672 | GC Bias316912650057057350374175801344 | GC Bias633825300114114700748351602688 | GC Bias1267650600228229401496703205376 | GC Bias2535301200456458802993406410752 | GC Bias5070602400912917605986812821504 | GC Bias10141204801825835211973625643008 | GC Bias20282409603651670423947251286016 | GC Bias40564819207303340847894502572032 | GC Bias81129638414606681695789005144064 | GC Bias162259276829213363391578010288128 | GC Bias324518553658426726783156020576256 | GC Bias649037107316853453566312041152512 | GC Bias1298074214633707307132624082305024 | GC Bias2596148429267414614265248164610048 | GC Bias5192296858534829228530496329220096 | GC Bias10384593717069658457060992658440192 | GC Bias20769187434139316914121985316880384 | GC Bias41538374868278633828243970633760768 | GC Bias83076749736557267656487941267521536 | GC Bias166153499473114535312975882535043072 | GC Bias332306998946229070625951765070086144 | GC Bias664613997892458141251903530140172288 | GC Bias1329227995784916282503807060280344576 | GC Bias2658455991569832565007614120560689152 | GC Bias5316911983139665130015228241121378304 | GC Bias10633823966279330260030456482242756608 | GC Bias21267647932558660520060912964485513216 | GC Bias42535295865117321040121825928971026432 | GC Bias85070591730234642080243651857942052864 | GC Bias170141183460469284160487303715884105728 | GC Bias340282366920938568320974607431768211456 | GC Bias680564733841877136641949214863536422912 | GC Bias1361129467683754273283898429727072845824 | GC Bias272225893536750854656779685945414569152 | GC Bias544451787073501709313559371890829138304 | GC Bias1088903574147003418627118743781658276608 | GC Bias2177807148294006837254237487563316552192 | GC Bias4355614296588013674508474975126633104384 | GC Bias8711228593176027349016949950253266208768 | GC Bias1742245718635205469803389990050652441536 | GC Bias348449143727041093960677998010 |
|-----------|------|--------|------------|---------|---------|----------|----------|----------|-----------|-----------|-----------|------------|------------|------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|---------------------------------------|
|-----------|------|--------|------------|---------|---------|----------|----------|----------|-----------|-----------|-----------|------------|------------|------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|---------------------------------------|

[illegible]

|                       |      |            |            |            |            |            |     |
|-----------------------|------|------------|------------|------------|------------|------------|-----|
| <i>Clivia</i> sp.     | 8890 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA | 200 |
| <i>C. robusta</i>     | 8817 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. robusta</i>     | 8462 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. robusta</i>     | 8442 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. robusta</i>     | 8440 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. robusta</i>     | 8415 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. nobilis</i>     | 8940 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GGTGCTACCA |     |
| <i>C. nobilis</i>     | 8937 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GGTGCTACCA |     |
| <i>C. nobilis</i>     | 8936 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GGTGCTACCA |     |
| <i>C. nobilis</i>     | 8933 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GGTGCTACCA |     |
| <i>C. nobilis</i>     | 8932 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GGTGCTACCA |     |
| <i>C. x nimbicola</i> | 8578 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8570 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8566 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8556 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8555 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8554 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8553 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. x nimbicola</i> | 8552 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. mirabilis</i>   | 8958 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. mirabilis</i>   | 8955 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. mirabilis</i>   | 8953 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. mirabilis</i>   | 8947 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. miniata</i>     | 8788 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. miniata</i>     | 8787 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |

[illegible]

|                           |      |            |            |            |            |            |     |
|---------------------------|------|------------|------------|------------|------------|------------|-----|
| <i>C. aff. robusta</i>    | 8803 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 9002 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8689 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8686 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8660 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8470 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8469 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8327 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. miniata</i>    | 8324 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>C. aff. caulescens</i> | 8575 | TGGACTGATG | GACTTACCAG | CCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>Clivia sp.</i>         | 8890 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT | 250 |
| <i>C. robusta</i>         | 8817 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. robusta</i>         | 8462 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. robusta</i>         | 8442 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. robusta</i>         | 8440 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. robusta</i>         | 8415 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. nobilis</i>         | 8940 | CATTGAGGCC | GTTATTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. nobilis</i>         | 8937 | CATTGAGGCC | GTTATTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. nobilis</i>         | 8936 | CATTGAGGCC | GTTATTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. nobilis</i>         | 8933 | CATTGAGGCC | GTTATTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. nobilis</i>         | 8932 | CATTGAGGCC | GTTATTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8578 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8570 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8566 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8556 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8555 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8554 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8553 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. x nimbicola</i>     | 8552 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. mirabilis</i>       | 8958 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. mirabilis</i>       | 8955 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. mirabilis</i>       | 8953 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. mirabilis</i>       | 8947 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8788 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8787 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8786 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8699 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8689 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8688 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8686 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8672 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8667 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8637 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8617 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8616 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8558 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8470 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8469 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8419 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8410 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         | 8408 | CATTGAGGCC | GTTGTTGGGG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT |     |
| <i>C. miniata</i>         |      |            |            |            |            |            |     |

[illegible]

|                       |      |            |            |            |            |            |     |
|-----------------------|------|------------|------------|------------|------------|------------|-----|
| <i>Clivia</i> sp.     | 8890 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC | 300 |
| <i>C. robusta</i>     | 8817 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. robusta</i>     | 8462 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. robusta</i>     | 8442 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. robusta</i>     | 8440 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. robusta</i>     | 8415 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. nobilis</i>     | 8940 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. nobilis</i>     | 8937 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. nobilis</i>     | 8936 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. nobilis</i>     | 8933 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. nobilis</i>     | 8932 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8578 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8570 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8566 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8556 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8555 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8554 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8553 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. x nimbicola</i> | 8552 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. mirabilis</i>   | 8958 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |
| <i>C. mirabilis</i>   | 8955 | ATCCTTTTGA | CCTTTTTTGA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |     |

[illegible]



|                           |      |             |            |            |            |            |
|---------------------------|------|-------------|------------|------------|------------|------------|
| <i>C. aff. robusta</i>    | 8811 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. robusta</i>    | 8808 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. robusta</i>    | 8807 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. robusta</i>    | 8804 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. robusta</i>    | 8803 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 9002 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8689 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8686 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8660 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8470 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8469 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8327 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. miniata</i>    | 8324 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
| <i>C. aff. caulescens</i> | 8575 | ATCCTTTTAGA | CCTTTTTGAA | GAAGGTTCTG | TTACTAACAT | GTTTACTTCC |
|                           |      |             |            |            |            |            |
| <i>Clivia sp.</i>         | 8890 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. robusta</i>         | 8817 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. robusta</i>         | 8462 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. robusta</i>         | 8442 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. robusta</i>         | 8440 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. robusta</i>         | 8415 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. nobilis</i>         | 8940 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. nobilis</i>         | 8937 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. nobilis</i>         | 8936 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. nobilis</i>         | 8933 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. nobilis</i>         | 8932 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. x nimbicola</i>     | 8578 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8570 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8566 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8556 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8555 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8554 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8553 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. x nimbicola</i>     | 8552 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGCTGGA  |
| <i>C. mirabilis</i>       | 8958 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. mirabilis</i>       | 8955 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. mirabilis</i>       | 8953 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. mirabilis</i>       | 8947 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8788 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8787 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8786 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8699 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8689 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8688 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8686 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8672 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8667 | ATTGTGGGTA  | ATGTATTTGG | TTTCAAAGCC | CTACGAGCTC | TACGTCTGGA |
| <i>C. miniata</i>         | 8637 |             |            |            |            |            |

*C. aff. caulescens* 8575 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGCCTGGA

400

[illegible]



```

C._gardenii_8887 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8885 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8884 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8883 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8882 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8789 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8780 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8777 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8747 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8744 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8660 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8615 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8444 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8418 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8405 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8403 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8376 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8374 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8369 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8368 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._gardenii_8367 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8609 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8571 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8562 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8561 ATGGNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
C._caulescens_8557 ATGGCATCCA ANNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
C._caulescens_8504 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8503 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8502 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8501 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8500 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8499 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8498 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8497 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8496 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8494 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8488 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8487 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._caulescens_8417 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8814 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8811 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8808 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8807 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8804 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._robusta_8803 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_9002 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8689 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8686 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8660 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8470 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8469 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8327 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._miniata_8324 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA
C._aff._caulescens_8575 ATGGCATCCA AGTTGAAAGA GATAAATTGA ACAAGTATGG TCGTCCCCTA

Clivia_sp._8890 TTGGGATGTA CTATTAA 467
C._robusta_8817 TTGGGATGTA CTATTAA
C._robusta_8462 TTGGGATGTA CTATTAA
C._robusta_8442 TTGGGATGTA CTATTAA
C._robusta_8440 TTGGGATGTA CTATTAA
C._robusta_8415 TTGGGATGTA CTATTAA
C._nobilis_8940 TTGGGATGTA CTATTAA
C._nobilis_8937 TTGGGATGTA CTATTAA
C._nobilis_8936 TTGGGATGTA CTATTAA
C._nobilis_8933 TTGGGATGTA CTATTAA
C._nobilis_8932 TTGGGATGTA CTATTAA
C._x_nimbicola_8578 TTGGGATGTA CTATTAA
C._x_nimbicola_8570 TTGGGATGTA CTATTAA

```

*C.\_x\_nimbicola*\_8566 TTGGGATGTA CTATTAA  
*C.\_x\_nimbicola*\_8556 TTGGGATGTA CTATTAA  
*C.\_x\_nimbicola*\_8555 TTGGGATGTA CTATTAA  
*C.\_x\_nimbicola*\_8554 TTGGGATGTA CTATTAA  
*C.\_x\_nimbicola*\_8553 TTGGGATGTA CTATTAA  
*C.\_x\_nimbicola*\_8552 TTGGGATGTA CTATTAA  
*C.\_mirabilis*\_8958 TTGGGATGTA CTATTAA  
*C.\_mirabilis*\_8955 TTGGGATGTA CTATTAA  
*C.\_mirabilis*\_8953 TTGGGATGTA CTATTAA  
*C.\_mirabilis*\_8947 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8788 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8787 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8786 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8699 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8689 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8688 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8686 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8672 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8667 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8637 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8617 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8616 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8558 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8470 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8469 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8419 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8410 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8408 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8406 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8396 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8394 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8391 TTGGGATGTA CTATTAA  
*C.\_miniata*\_8327 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8892 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8889 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8888 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8887 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8885 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8884 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8883 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8882 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8789 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8780 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8777 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8747 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8744 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8660 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8615 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8444 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8418 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8405 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8403 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8376 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8374 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8369 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8368 TTGGGATGTA CTATTAA  
*C.\_gardenii*\_8367 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8609 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8571 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8562 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8561 NNNNNNNNNN NNNNNNN  
*C.\_caulescens*\_8557 NNNNNNNNNN NNNNNNN  
*C.\_caulescens*\_8504 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8503 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8502 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8501 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8500 TTGGGATGTA CTATTAA  
*C.\_caulescens*\_8499 TTGGGATGTA CTATTAA

```

C._caulescens_8498 TTGGGATGTA CTATTAA
C._caulescens_8497 TTGGGATGTA CTATTAA
C._caulescens_8496 TTGGGATGTA CTATTAA
C._caulescens_8494 TTGGGATGTA CTATTAA
C._caulescens_8488 TTGGGATGTA CTATTAA
C._caulescens_8487 TTGGGATGTA CTATTAA
C._caulescens_8417 TTGGGATGTA CTATTAA
C._aff._robusta_8814 TTGGGATGTA CTATTAA
C._aff._robusta_8811 TTGGGATGTA CTATTAA
C._aff._robusta_8808 TTGGGATGTA CTATTAA
C._aff._robusta_8807 TTGGGATGTA CTATTAA
C._aff._robusta_8804 TTGGGATGTA CTATTAA
C._aff._robusta_8803 TTGGGATGTA CTATTAA
C._aff._miniata_9002 TTGGGATGTA CTATTAA
C._aff._miniata_8689 TTGGGATGTA CTATTAA
C._aff._miniata_8686 TTGGGATGTA CTATTAA
C._aff._miniata_8660 TTGGGATGTA CTATTAA
C._aff._miniata_8470 TTGGGATGTA CTATTAA
C._aff._miniata_8469 TTGGGATGTA CTATTAA
C._aff._miniata_8327 TTGGGATGTA CTATTAA
C._aff._miniata_8324 TTGGGATGTA CTATTAA
C._aff._caulescens_8575 TTGGGATGTA CTATTAA

```

## Appendix B6 – *Clivia rpl16*

[illegible]



[illegible]

[illegible]

[illegible]

|                           |      |                    |            |             |            |             |     |
|---------------------------|------|--------------------|------------|-------------|------------|-------------|-----|
| <i>C. nobilis</i>         | 8940 | TGCAGAG- <b>TT</b> | CGGGCCTAAC | CATTTCATGGA | GAAGCTATAG | GAACGACGAA  |     |
| <i>C. nobilis</i>         | 8941 | TGCAGAG- <b>TT</b> | CGGGCCTAAC | CATTTCATGGA | GAAGCTATAG | GAACGACGAA  |     |
| <i>C. nobilis</i>         | 8962 | TGCAGAG- <b>TT</b> | CGGGCCTAAC | CATTTCATGGA | GAAGCTATAG | GAACGACGAA  |     |
| <i>C. aff. caulescens</i> | 8575 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG | 400 |
| <i>C. gardenii</i>        | 8366 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. gardenii</i>        | 8369 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. miniata</i>         | 8391 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. miniata</i>         | 8408 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. miniata</i>         | 8573 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. mirabilis</i>       | 8942 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. mirabilis</i>       | 8943 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8944 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8945 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. mirabilis</i>       | 8946 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8947 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8948 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8949 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8950 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8951 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8952 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8953 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8954 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8955 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8956 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8957 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8958 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8959 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8960 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8961 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. mirabilis</i>       | 8963 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TTATTTCATCG |     |
| <i>C. nobilis</i>         | 8932 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8933 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8934 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8935 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8936 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8937 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8938 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8939 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8940 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8941 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. nobilis</i>         | 8962 | ACCTGTGACT         | ATATAAGATT | CTATTAAAAA  | CGAATCCTAA | TGATTTCATCG |     |
| <i>C. aff. caulescens</i> | 8575 | GGTGGGATGG         | CGGAACAAAC | CAAGAACAAA  | TTGAGATTTA | CTCTGAGAGA  | 450 |
| <i>C. gardenii</i>        | 8366 | GGTGGGATGG         | CGGAACAAAC | CAAGAACAAA  | TTGAGATTTA | CTCTGAGAGA  |     |
| <i>C. gardenii</i>        | 8369 | GGTGGGATGG         | CGGAACAAAC | CAAGAACAAA  | TTGAGATTTA | CTCTGAGAGA  |     |
| <i>C. miniata</i>         | 8391 | GGTGGGATGG         | CGGAACAAAC | CAAGAACAAA  | TTGAGATTTA | CTCTGAGAGA  |     |
| <i>C. miniata</i>         | 8    |                    |            |             |            |             |     |

[illegible]

[illegible]

[illegible]

[illegible]



[illegible]

```

C._mirabilis_8950 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8951 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8952 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8953 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8954 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8955 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8956 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8957 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8958 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8959 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8960 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8961 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._mirabilis_8963 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8932 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8933 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8934 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8935 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8936 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8937 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8938 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8939 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8940 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8941 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT
C._nobilis_8962 AGAATGAAGG GAATATCTTG TCGAGGTAAT CATATT

```

## Appendix B7 – *Clivia rpoB*

50

|                           |      |            |            |            |            |            |
|---------------------------|------|------------|------------|------------|------------|------------|
| <i>C. gardenii</i>        | 8405 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. gardenii</i>        | 8523 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 8495 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 8490 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 8479 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 6    | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 43   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 41   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 40   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 38   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 32   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 31   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 25   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 24   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 22   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 20   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 17   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 15   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 14   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 13   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. caulescens</i>      | 10   | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8806 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8805 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8804 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8593 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8585 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8582 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. robusta</i>    | 8579 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>C. aff. caulescens</i> | 8575 | TCGGGGGTTT | CCGTTATAGC | TGAACGCGAA | GGAAAGATCA | TTTATACTGA |
| <i>Cr. vansonii</i>       | 8964 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. gard. x miniata</i> | 8658 | TACTTACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. miniata</i>    | 8470 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. miniata</i>    | 8469 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. miniata</i>    | 8318 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. gardeniil</i>  | 9001 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. gardeniil</i>  | 9000 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. gardeniil</i>  | 8577 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. gardeniil</i>  | 8576 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. aff. gardeniil</i>  | 8325 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. robusta</i>         | 8462 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. robusta</i>         | 8442 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. robusta</i>         | 8440 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. robusta</i>         | 8415 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. nobilis</i>         | 8941 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. nobilis</i>         | 8940 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. nobilis</i>         | 8939 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. nobilis</i>         | 8938 | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C. nobilis</i>         | 893  |            |            |            |            |            |

|                                |            |            |            |            |            |
|--------------------------------|------------|------------|------------|------------|------------|
| <i>C._miniata_8616</i>         | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8574</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8573</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8558</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8526</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8443</i>         | TACTTACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8419</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8410</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8408</i>         | TACTTACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8406</i>         | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8396</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8394</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_8391</i>         | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_42</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_37</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_35</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_34</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_33</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_22</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_21</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._miniata_18</i>           | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8777</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8444</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8418</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8403</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8374</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8369</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8405</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._gardenii_8523</i>        | TACTTACAAG | ATCATTTTAT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_8495</i>      | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_8490</i>      | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_8479</i>      | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_6</i>         | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_43</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_41</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_40</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_38</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_32</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_31</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_25</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_24</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_22</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_20</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_17</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_15</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_14</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_13</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._caulescens_10</i>        | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8806</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8805</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8804</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8593</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8585</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8582</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._robusta_8579</i>    | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>C._aff._caulescens_8575</i> | TACTCACAAG | ATCATTTTCT | CAAGTAATGG | GGACACTATA | AGCATTCCAT |
| <i>Cr._vansonii_8964</i>       | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._gard._x_miniata_8658</i> | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._miniata_8470</i>    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._miniata_8469</i>    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._miniata_8318</i>    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._gardeniil_9001</i>  | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._gardeniil_9000</i>  | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._gardeniil_8577</i>  | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._gardeniil_8576</i>  | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._aff._gardeniil_8325</i>  | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |
| <i>C._robusta_8462</i>         | TAGTTATGTA | TCAACGTTCC | AACAAAAATA | CTTGTATGCA | TCAAAAACCT |

[illegible]

|                                 |            |            |             |            |            |
|---------------------------------|------------|------------|-------------|------------|------------|
| <i>C. caulescens</i> _22        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _20        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _17        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _15        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _14        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _13        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. caulescens</i> _10        | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8806    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8805    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8804    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8593    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8585    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8582    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. robusta</i> _8579    | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>C. aff. caulescens</i> _8575 | TAGTTATGTA | TCAACGTTCC | AACAAAAATA  | CTTGTATGCA | TCAAAAACCT |
| <i>Cr. vansonii</i> _8964       | CGGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. gard. x miniata</i> _8658 | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. miniata</i> _8470    | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. miniata</i> _8469    | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. miniata</i> _8318    | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. gardeniil</i> _9001  | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. gardeniil</i> _9000  | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. gardeniil</i> _8577  | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. gardeniil</i> _8576  | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. aff. gardeniil</i> _8325  | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. robusta</i> _8462         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. robusta</i> _8442         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. robusta</i> _8440         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. robusta</i> _8415         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8941         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8940         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8939         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8938         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8937         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8936         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8933         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. nobilis</i> _8932         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _8595     | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _8594     | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _8578     | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _8553     | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _29       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. x nimbicola</i> _23       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8963       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8958       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8955       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8954       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8951       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8950       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8947       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8944       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. mirabilis</i> _8943       | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. miniata</i> _8992         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. miniata</i> _8616         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. miniata</i> _8574         | CAGGTTCGGC | AGGGTAAATG | CATTA AAAAG | GGACAAATTT | TAGCGGATGG |
| <i>C. miniata</i> _8573         | CAGGTTCGGC | AGGGTAAATG | C           |            |            |

|                                |            |            |            |            |            |     |
|--------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C._miniata_37</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_35</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_34</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_33</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_22</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_21</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._miniata_18</i>           | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8777</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8444</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8418</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8403</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8374</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8369</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8405</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._gardenii_8523</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_8495</i>      | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_8490</i>      | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_8479</i>      | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_6</i>         | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_43</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_41</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_40</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_38</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_32</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_31</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_25</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_24</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_22</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_20</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_17</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_15</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_14</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_13</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._caulescens_10</i>        | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8806</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8805</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8804</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8593</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8585</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8582</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._robusta_8579</i>    | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>C._aff._caulescens_8575</i> | CAGGTTCGGC | AGGGTAAATG | CATTAAAAAG | GGACAAATTT | TAGCGGATGG |     |
| <i>Cr._vansonii_8964</i>       | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG | 250 |
| <i>C._gard._x_miniata_8658</i> | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._miniata_8470</i>    | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._miniata_8469</i>    | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._miniata_8318</i>    | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._gardeniil_9001</i>  | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._gardeniil_9000</i>  | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._gardeniil_8577</i>  | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._gardeniil_8576</i>  | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._aff._gardeniil_8325</i>  | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._robusta_8462</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._robusta_8442</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._robusta_8440</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._robusta_8415</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._nobilis_8941</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8940</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8939</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8938</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8937</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8936</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8933</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._nobilis_8932</i>         | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGTAG |     |
| <i>C._x_nimbicola_8595</i>     | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._x_nimbicola_8594</i>     | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |
| <i>C._x_nimbicola_8578</i>     | TGCGGCTACT | GTTGGTGGGG | AACTCGCTTT | AGGAAAAAAC | GTATTAGCAG |     |



[illegible]

300

|    |                        |      |             |            |            |            |            |     |
|----|------------------------|------|-------------|------------|------------|------------|------------|-----|
|    | <i>Cr. vansonii</i>    | 8964 | GAACGCTCTGG | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA | 350 |
| C. | <i>gard. x miniata</i> | 8658 | GAACGCTCTGG | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. aff. miniata</i> | 8470 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. aff. miniata</i> | 8469 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. aff. miniata</i> | 8318 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>aff. gardeniil</i>  | 9001 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>aff. gardeniil</i>  | 9000 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>aff. gardeniil</i>  | 8577 | GAACGCTCTGG | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>aff. gardeniil</i>  | 8576 | GAACGCTCTGG | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>aff. gardeniil</i>  | 8325 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. robusta</i>      | 8462 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. robusta</i>      | 8442 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. robusta</i>      | 8440 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. robusta</i>      | 8415 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8941 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8940 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8939 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8938 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8937 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8936 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8933 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. nobilis</i>      | 8932 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>x nimbicola</i>     | 8595 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>x nimbicola</i>     | 8594 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>x nimbicola</i>     | 8578 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
| C. | <i>x nimbicola</i>     | 8553 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. x nimbicola</i>  | 29   | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. x nimbicola</i>  | 23   | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8963 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8958 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8955 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8954 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8951 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8950 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8947 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8944 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. mirabilis</i>    | 8943 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. miniata</i>      | 8992 | GAACGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |
|    | <i>C. miniata</i>      | 8616 | GAGCGTCTGG  | TGTATGAAGA | TATTTATACT | TCTTTTCACA | TACGGAAATA |     |

[illegible]

[illegible]

|                                |            |            |            |         |
|--------------------------------|------------|------------|------------|---------|
| <i>C._caulescens_20</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._caulescens_17</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._caulescens_15</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._caulescens_14</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._caulescens_13</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._caulescens_10</i>        | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8806</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8805</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8804</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8593</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8585</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8582</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._robusta_8579</i>    | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |
| <i>C._aff._caulescens_8575</i> | TGAAATTCAG | ACTCATGTGA | CAAGCCAAGG | TCCCGAA |

Appendix B8 – *Clivia rpoC1*

|                                 |             |            |            |            |            |     |
|---------------------------------|-------------|------------|------------|------------|------------|-----|
| <i>C. caulescens</i> _8479      | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT | 50  |
| <i>C. caulescens</i> _8490      | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. caulescens</i> _8495      | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8374        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8405        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8418        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8444        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8369        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8403        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. gardenii</i> _8523        | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. robusta</i> _8579    | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. robusta</i> _8582    | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. robusta</i> _8585    | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. robusta</i> _8593    | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8558         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8391         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8394         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8396         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8992         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8406         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8573         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8574         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8408         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8410         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. miniata</i> _8419         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. x nimbicola</i> _8553     | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. x nimbicola</i> _8554     | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. x nimbicola</i> _8578     | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. x nimbicola</i> _8594     | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. x nimbicola</i> _8595     | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. robusta</i> _8462         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. robusta</i> _8415         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. robusta</i> _8440         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. robusta</i> _8442         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. caulescens</i> _8575 | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. gardeniil</i> _8576  | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. gardeniil</i> _8577  | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. gardeniil</i> _9000  | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. gardeniil</i> _9001  | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. aff. gardeniil</i> _8325  | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. mirabilis</i> _8958       | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. mirabilis</i> _8947       | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. mirabilis</i> _8943       | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. mirabilis</i> _8955       | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. nobilis</i> _8940         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. nobilis</i> _8936         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. nobilis</i> _8933         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. nobilis</i> _8932         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. nobilis</i> _8941         | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>Cr. vansonii</i> _8964       | ACATCAATGT  | GGATTACCTC | GAGAAATAGC | AATAGAGCTT | TTCCAAACAT |     |
| <i>C. caulescens</i> _8479      | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT | 100 |
| <i>C. caulescens</i> _8490      | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. caulescens</i> _8495      | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8374        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8405        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8418        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8444        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8369        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8403        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. gardenii</i> _8523        | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. aff. robusta</i> _8579    | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. aff. robusta</i> _8582    | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. aff. robusta</i> _8585    | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |
| <i>C. aff. robusta</i> _8593    | TTGTAATTTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |     |

|                         |            |            |            |            |            |
|-------------------------|------------|------------|------------|------------|------------|
| C. miniata_8558         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8391         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8394         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8396         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8992         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8406         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8573         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8574         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8408         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8410         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. miniata_8419         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. x nimbicola_8553     | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. x nimbicola_8554     | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. x nimbicola_8578     | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. x nimbicola_8594     | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. x nimbicola_8595     | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. robusta_8462         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. robusta_8415         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. robusta_8440         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. robusta_8442         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. caulescens_8575 | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. gardeniil_8576  | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. gardeniil_8577  | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. gardeniil_9000  | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. gardeniil_9001  | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. aff. gardeniil_8325  | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. mirabilis_8958       | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. mirabilis_8947       | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. mirabilis_8943       | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. mirabilis_8955       | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. nobilis_8940         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. nobilis_8936         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. nobilis_8933         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. nobilis_8932         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| C. nobilis_8941         | TTGTAATTCG | TGGTCTAATC | AGACAACATA | TTGCTTCTAA | CATAGGGATT |
| Cr. vansonii_8964       | TTGTAATTCG | TGGTCTAATC | GGACAACATA | TTGCTTCTAA | CATAGGGATT |
| <br>                    |            |            |            |            |            |
| C. caulescens_8479      | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. caulescens_8490      | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. caulescens_8495      | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8374        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8405        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardeni_8418         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8444        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8369        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8403        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. gardenii_8523        | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. aff. robusta_8579    | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. aff. robusta_8582    | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. aff. robusta_8585    | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. aff. robusta_8593    | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8558         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8391         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8394         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8396         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8992         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8406         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8573         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8574         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8408         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |
| C. miniata_8410         | GCGAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTAT   |            |



|                            |      |             |            |            |            |            |     |
|----------------------------|------|-------------|------------|------------|------------|------------|-----|
| C. <i>_robusta</i>         | 8415 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_robusta</i>         | 8440 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_robusta</i>         | 8442 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._caulescens</i> | 8575 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._gardenii</i>   | 8576 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._gardenii</i>   | 8577 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._gardenii</i>   | 9000 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._gardenii</i>   | 9001 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_aff._gardenii</i>   | 8325 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_mirabilis</i>       | 8958 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_mirabilis</i>       | 8947 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_mirabilis</i>       | 8943 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_mirabilis</i>       | 8955 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_nobilis</i>         | 8940 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_nobilis</i>         | 8936 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_nobilis</i>         | 8933 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_nobilis</i>         | 8932 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| C. <i>_nobilis</i>         | 8941 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
| Cr. <i>_vansonii</i>       | 8964 | GCGAAAAA-GT | AAAATTCGGG | AAAAAGAACC | GATTGTATGG | GAAATACTTC |     |
|                            |      |             |            |            |            |            |     |
| C. <i>_caulescens</i>      | 8479 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG | 200 |
| C. <i>_caulescens</i>      | 8490 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_caulescens</i>      | 8495 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8374 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8405 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8418 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8444 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8369 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8403 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_gardenii</i>        | 8523 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_aff._robusta</i>    | 8579 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_aff._robusta</i>    | 8582 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_aff._robusta</i>    | 8585 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_aff._robusta</i>    | 8593 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8558 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8391 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8394 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8396 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8992 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8406 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8573 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8574 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8408 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8410 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_miniata</i>         | 8419 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_x_nimbicola</i>     | 8553 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_x_nimbicola</i>     | 8554 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_x_nimbicola</i>     | 8578 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_x_nimbicola</i>     | 8594 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_x_nimbicola</i>     | 8595 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_robusta</i>         | 8462 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_robusta</i>         | 8415 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_robusta</i>         | 8440 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATTGT | TGAATAGAGC | ACCCACCCTG |     |
| C. <i>_robusta</i>         | 8442 | AAGAAGTTAT  | GCAGGGGCAT | CCTGTATT   |            |            |     |

```

C._nobilis_8941 AAGAAGTTAT GCAGGGGCAT CCTGTATTGT TGAATAGAGC ACCCACCTG
Cr._vansonii_8964 AAGAAGTTAT GCAGGGGCAT CCTGTATTGT TGAATAGAGC ACCCACCTG

C._caulescens_8479 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG 250
C._caulescens_8490 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._caulescens_8495 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8374 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8405 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8418 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8444 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8369 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8403 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._gardenii_8523 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._robusta_8579 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._robusta_8582 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._robusta_8585 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._robusta_8593 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8558 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8391 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8394 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8396 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8992 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8406 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8573 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8574 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8408 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8410 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._miniata_8419 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._x_nimbicola_8553 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._x_nimbicola_8554 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._x_nimbicola_8578 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._x_nimbicola_8594 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._x_nimbicola_8595 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._robusta_8462 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._robusta_8415 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._robusta_8440 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._robusta_8442 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._caulescens_8575 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._gardeniil_8576 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._gardeniil_8577 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._gardeniil_9000 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._gardeniil_9001 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._aff._gardeniil_8325 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._mirabilis_8958 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._mirabilis_8947 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._mirabilis_8943 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._mirabilis_8955 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._nobilis_8940 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._nobilis_8936 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._nobilis_8933 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._nobilis_8932 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
C._nobilis_8941 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG
Cr._vansonii_8964 CATAGATTGG GCATACAGGC GTTCCAACCC ATTTTAGT-G GAGGGACGCG

C._caulescens_8479 CTATTTGTTT ACAC 264
C._caulescens_8490 CTATTTGTTT ACAC
C._caulescens_8495 CTATTTGTTT ACAC
C._gardenii_8374 CTATTTGTTT ACAC
C._gardenii_8405 CTATTTGTTT ACAC
C._gardenii_8418 CTATTTGTTT ACAC
C._gardenii_8444 CTATTTGTTT ACAC
C._gardenii_8369 CTATTTGTTT ACAC
C._gardenii_8403 CTATTTGTTT ACAC
C._gardenii_8523 CTATTTGTTT ACAC
C._aff._robusta_8579 CTATTTGTTT ACAC
C._aff._robusta_8582 CTATTTGTTT ACAC
C._aff._robusta_8585 CTATTTGTTT ACAC
C._aff._robusta_8593 CTATTTGTTT ACAC

```

*C.\_miniata*\_8558 CTATTTGTTT ACAC  
*C.\_miniata*\_8391 CTATTTGTTT ACAC  
*C.\_miniata*\_8394 CTATTTGTTT ACAC  
*C.\_miniata*\_8396 CTATTTGTTT ACAC  
*C.\_miniata*\_8992 CTATTTGTTT ACAC  
*C.\_miniata*\_8406 CTATTTGTTT ACAC  
*C.\_miniata*\_8573 CTATTTGTTT ACAC  
*C.\_miniata*\_8574 CTATTTGTTT ACAC  
*C.\_miniata*\_8408 CTATTTGTTT ACAC  
*C.\_miniata*\_8410 CTATTTGTTT ACAC  
*C.\_miniata*\_8419 CTATTTGTTT ACAC  
*C.\_x\_nimbicola*\_8553 CTATTTGTTT ACAC  
*C.\_x\_nimbicola*\_8554 CTATTTGTTT ACAC  
*C.\_x\_nimbicola*\_8578 CTATTTGTTT ACAC  
*C.\_x\_nimbicola*\_8594 CTATTTGTTT ACAC  
*C.\_x\_nimbicola*\_8595 CTATTTGTTT ACAC  
*C.\_robusta*\_8462 CTATTTGTTT ACAC  
*C.\_robusta*\_8415 CTATTTGTTT ACAC  
*C.\_robusta*\_8440 CTATTTGTTT ACAC  
*C.\_robusta*\_8442 CTATTTGTTT ACAC  
*C.\_aff.\_caulescens*\_8575 CTATTTGTTT ACAC  
*C.\_aff.\_gardeniil*\_8576 CTATTTGTTT ACAC  
*C.\_aff.\_gardeniil*\_8577 CTATTTGTTT ACAC  
*C.\_aff.\_gardeniil*\_9000 CTATTTGTTT ACAC  
*C.\_aff.\_gardeniil*\_9001 CTATTTGTTT ACAC  
*C.\_aff.\_gardeniil*\_8325 CTATTTGTTT ACAC  
*C.\_mirabilis*\_8958 CTATTTGTTT ACAC  
*C.\_mirabilis*\_8947 CTATTTGTTT ACAC  
*C.\_mirabilis*\_8943 CTATTTGTTT ACAC  
*C.\_mirabilis*\_8955 CTATTTGTTT ACAC  
*C.\_nobilis*\_8940 CTATTTGTTT ACAC  
*C.\_nobilis*\_8936 CTATTTGTTT ACAC  
*C.\_nobilis*\_8933 CTATTTGTTT ACAC  
*C.\_nobilis*\_8932 CTATTTGTTT ACAC  
*C.\_nobilis*\_8941 CTATTTGTTT ACAC  
*Cr.\_vansonii*\_8964 CTATTTGTTT ACAC

Appendix B9 – *Clivia trnL-F*

|                                 |            |             |            |            |            |     |
|---------------------------------|------------|-------------|------------|------------|------------|-----|
| <i>C. caulescens</i> _8479      | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG | 50  |
| <i>C. caulescens</i> _8490      | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. caulescens</i> _8369 | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8374        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8403        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8405        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8418        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8444        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. gardenii</i> _8523        | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. robusta</i> _8593    | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8391         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8394         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8396         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8408         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8410         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8558         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8573         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8574         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. miniata</i> _8992         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. mirabilis</i> _8947       | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. mirabilis</i> _8955       | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. x nimbicola</i> _8578     | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. x nimbicola</i> _8594     | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. x nimbicola</i> _8595     | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. nobilis</i> _8932         | AATGGAAGCT | GTTCTAACGG  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. nobilis</i> _8933         | AATGGAAGCT | GTTCTAACGG  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. nobilis</i> _8940         | AATGGAAGCT | GTTCTAACGG  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. nobilis</i> _8941         | AATGGAAGCT | GTTCTAACGG  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. robusta</i> _8415         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. robusta</i> _8440         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. robusta</i> _8442         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. robusta</i> _8462         | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. caulescens</i> _8575 | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. gardenii</i> _8576   | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. gardenii</i> _9000   | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. aff. gardenii</i> _8325   | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>Cr. vansonii</i> _8964       | AATGGAAGCT | GTTCTAACGA  | ATGGAGTTGA | CTACGTTGCG | TTGGTAACTG |     |
| <i>C. caulescens</i> _8479      | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA | 100 |
| <i>C. caulescens</i> _8490      | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. aff. caulescens</i> _8369 | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8374        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8403        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8405        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8418        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8444        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. gardenii</i> _8523        | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. aff. robusta</i> _8593    | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8391         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8394         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8396         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8408         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8410         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8558         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8573         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8574         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. miniata</i> _8992         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. mirabilis</i> _8947       | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. mirabilis</i> _8955       | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. x nimbicola</i> _8578     | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. x nimbicola</i> _8594     | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. x nimbicola</i> _8595     | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. nobilis</i> _8932         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. nobilis</i> _8933         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C. nobilis</i> _8940         | GCTATCGAAA | TTAAAGAAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |

|                                 |            |            |            |            |            |     |
|---------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C._nobilis</i> _8941         | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._robusta</i> _8415         | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._robusta</i> _8440         | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._robusta</i> _8442         | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._robusta</i> _8462         | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._aff._caulescens</i> _8575 | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._aff._gardenii</i> _8576   | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._aff._gardenii</i> _9000   | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>C._aff._gardenii</i> _8325   | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACGTATA |     |
| <i>Cr._vansonii</i> _8964       | GCTATCGAAA | TTAAAGAAAG | AATGACCTAT | ATATCTAATA | CGTACATATA |     |
|                                 |            |            |            |            |            | 150 |
| <i>C._caulescens</i> _8479      | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._caulescens</i> _8490      | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._aff._caulescens</i> _8369 | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._gardenii</i> _8374        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._gardenii</i> _8403        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._gardenii</i> _8405        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._gardenii</i> _8418        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._gardenii</i> _8444        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._gardenii</i> _8523        | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._aff._robusta</i> _8593    | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._miniata</i> _8391         | CATACTGGCA | TATCAAACAA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._miniata</i> _8394         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._miniata</i> _8396         | CATACTGGCA | TATCAAACAA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._miniata</i> _8408         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._miniata</i> _8410         | CATACTGGCA | TATCAAACAA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._miniata</i> _8558         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._miniata</i> _8573         | CATACTGGCA | TATCAAACAA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._miniata</i> _8574         | CATACTGGCA | TATCAAACAA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._miniata</i> _8992         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._mirabilis</i> _8947       | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._mirabilis</i> _8955       | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._x_nimbicola</i> _8578     | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._x_nimbicola</i> _8594     | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._x_nimbicola</i> _8595     | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._nobilis</i> _8932         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._nobilis</i> _8933         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._nobilis</i> _8940         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._nobilis</i> _8941         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._robusta</i> _8415         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._robusta</i> _8440         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._robusta</i> _8442         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._robusta</i> _8462         | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._aff._caulescens</i> _8575 | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
| <i>C._aff._gardenii</i> _8576   | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._aff._gardenii</i> _9000   | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>C._aff._gardenii</i> _8325   | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATCC |     |
| <i>Cr._vansonii</i> _8964       | CATACTGGCA | TATCAAACGA | TTAATCGCGA | AACGAATCCA | TACCGAATC- |     |
|                                 |            |            |            |            |            | 200 |
| <i>C._caulescens</i> _8479      | -----C     | ATATATATGC | A-ATATATTA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._caulescens</i> _8490      | -----C     | ATATATATGC | A-ATATATTA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._aff._caulescens</i> _8369 | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8374        | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8403        | -----C     | ATATATATGC | ATATATATGC | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8405        | ATATATATGC | ATATATATGC | ATATATATGC | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8418        | ATATATATGC | ATATATATGC | ATATATATGC | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8444        | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._gardenii</i> _8523        | ATATATATGC | ATATATATGC | A-ATATATGC | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._aff._robusta</i> _8593    | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8391         | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8394         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8396         | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8408         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8410         | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8558         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8573         | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8574         | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |
| <i>C._miniata</i> _8992         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG |     |

|                                |            |            |            |            |             |     |
|--------------------------------|------------|------------|------------|------------|-------------|-----|
| <i>C._mirabilis_8947</i>       | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._mirabilis_8955</i>       | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._x_nimbicola_8578</i>     | -----C     | ATATATATGC | A-ATATATTA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._x_nimbicola_8594</i>     | -----C     | ATATATATGC | A-ATATATTA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._x_nimbicola_8595</i>     | -----C     | ATATATATGC | A-ATATATTA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._nobilis_8932</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._nobilis_8933</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._nobilis_8940</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._nobilis_8941</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._robusta_8415</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._robusta_8440</i>         | ATATATATGC | ATATATATGC | ATATATATGC | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._robusta_8442</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._robusta_8462</i>         | ATATATATGC | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._aff._caulescens_8575</i> | -----C     | ATATATATGC | A-ATATATGA | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._aff._gardenii_8576</i>   | ATATATATGC | ATATATATGC | ATATATATGC | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._aff._gardenii_9000</i>   | ATATATATGC | ATATATATGC | A-ATATATGC | AAAATTCAGA | GTTATTGTGG  |     |
| <i>C._aff._gardenii_8325</i>   | ATATATATGC | ATATATATGC | A-ATATATGC | AAAATTCAGA | GTTATTGTGG  |     |
| <i>Cr._vansonii_8964</i>       | -----C     | ATATATAT-- | -----      | -----      | -----       |     |
|                                |            |            |            |            |             |     |
| <i>C._caulescens_8479</i>      | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT | 250 |
| <i>C._caulescens_8490</i>      | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._caulescens_8369</i> | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8374</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8403</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8405</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8418</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8444</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._gardenii_8523</i>        | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._robusta_8593</i>    | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8391</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8394</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8396</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8408</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8410</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8558</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8573</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8574</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._miniata_8992</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._mirabilis_8947</i>       | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._mirabilis_8955</i>       | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._x_nimbicola_8578</i>     | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._x_nimbicola_8594</i>     | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._x_nimbicola_8595</i>     | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._nobilis_8932</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._nobilis_8933</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._nobilis_8940</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._nobilis_8941</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._robusta_8415</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._robusta_8440</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._robusta_8442</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._robusta_8462</i>         | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._caulescens_8575</i> | ATCTATTCCA | ATCGAAGTTA | AGGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._gardenii_8576</i>   | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._gardenii_9000</i>   | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>C._aff._gardenii_8325</i>   | ATCTATTCCA | ATCGAAGTTA | AAGGAAGAAT | CGAATATTCA | GTGATCAAAAT |     |
| <i>Cr._vansonii_8964</i>       | -----      | -----      | -----      | -----      | -----       |     |
|                                |            |            |            |            |             |     |
| <i>C._caulescens_8479</i>      | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  | 300 |
| <i>C._caulescens_8490</i>      | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._aff._caulescens_8369</i> | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8374</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTGAAAAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8403</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8405</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8418</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8444</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._gardenii_8523</i>        | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |
| <i>C._aff._robusta_8593</i>    | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTTAAAAAAA | AAAATGATTA  |     |
| <i>C._miniata_8391</i>         | CATTCATTCC | AGAGTTTGAT | AGACCTTTTT | TTG--AAAAA | AAAATGATTA  |     |

```

C._miniata_8394 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG-AAAAAA AAAATGATTA
C._miniata_8396 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._miniata_8408 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._miniata_8410 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG-AAAAAA AAAATGATTA
C._miniata_8558 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._miniata_8573 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG-AAAAAA AAAATGATTA
C._miniata_8574 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG-AAAAAA AAAATGATTA
C._miniata_8992 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG-AAAAAA AAAATGATTA
C._mirabilis_8947 CATTCAATTC AGAGTTTGAT AGACCTTTTT T-GAAAAAAA AAAATGATTA
C._mirabilis_8955 CATTCAATTC AGAGTTTGAT AGACCTTTTT T-GAAAAAAA AAAATGATTA
C._x_nimbicola_8578 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._x_nimbicola_8594 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._x_nimbicola_8595 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._nobilis_8932 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._nobilis_8933 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._nobilis_8940 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._nobilis_8941 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._robusta_8415 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTTG-AAAAA AAAATGATTA
C._robusta_8440 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._robusta_8442 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTTGAAAAAAA AAAATGATTA
C._robusta_8462 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTGAAAAAAA AAAATGATTA
C._aff._caulescens_8575 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._aff._gardenii_8576 CATTCAATTC AGAGTTTGAT AGACCTTTTT TTG--AAAAA AAAATGATTA
C._aff._gardenii_9000 CATTCAATTC AGAGTTTGAT AGACCTTTTT TT-AAAAAAA AAAATGATTA
C._aff._gardenii_8325 CATTCAATTC AGAGTTTGAT AGACCTTTTT TT-AAAAAAA AAAATGATTA
Cr._vansonii_8964 -----

```

```

C._caulescens_8479 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC 350
C._caulescens_8490 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._caulescens_8369 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8374 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8403 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8405 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8418 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8444 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._gardenii_8523 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._robusta_8593 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8391 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8394 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8396 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8408 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8410 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8558 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8573 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8574 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._miniata_8992 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._mirabilis_8947 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._mirabilis_8955 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._x_nimbicola_8578 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._x_nimbicola_8594 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._x_nimbicola_8595 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._nobilis_8932 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._nobilis_8933 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._nobilis_8940 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._nobilis_8941 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._robusta_8415 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._robusta_8440 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._robusta_8442 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._robusta_8462 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._caulescens_8575 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._gardenii_8576 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._gardenii_9000 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
C._aff._gardenii_8325 ATCGGACGAG AATAAAGAGA GAGTCCCATT CTACATG--- --TCAATACC
Cr._vansonii_8964 -----GAG AATAAAGAGA GAGTCCCATT CTACATGTCA ATTCAATACC

```

```

C._caulescens_8479 GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC TTTAGAAATC 400
C._caulescens_8490 GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC TTTAGAAATC
C._aff._caulescens_8369 GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC TTTAGAAATC

```

| Accession               | Gene       | Protein     | Accession  | Gene       | Protein    | Accession | Gene | Protein |
|-------------------------|------------|-------------|------------|------------|------------|-----------|------|---------|
| C. gardenii_8374        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. gardenii_8403        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. gardenii_8405        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. gardenii_8418        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. gardenii_8444        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. gardenii_8523        | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. aff. robusta_8593    | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8391         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8394         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8396         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8408         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8410         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8558         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8573         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8574         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. miniata_8992         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. mirabilis_8947       | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. mirabilis_8955       | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. x nimbiicola_8578    | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. x nimbiicola_8594    | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. x nimbiicola_8595    | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. nobilis_8932         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. nobilis_8933         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. nobilis_8940         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. nobilis_8941         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. robusta_8415         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. robusta_8440         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. robusta_8442         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. robusta_8462         | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. aff. caulescens_8575 | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. aff. gardenii_8576   | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. aff. gardenii_9000   | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. aff. gardenii_8325   | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| Cr. vansonii_8964       | GACAACAATG | AAATTTTATAG | TAAGAGGAAA | ATCCGTCGAC | TTTAGAAATC |           |      |         |
| C. caulescens_8479      | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT | 450       |      |         |
| C. caulescens_8490      | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. aff. caulescens_8369 | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8374        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8403        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8405        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8418        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8444        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. gardenii_8523        | GT-GAGGGTT | CAAGTCCCTC  | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |           |      |         |
| C. aff. robusta_8593    | GT-G       |             |            |            |            |           |      |         |



|                                 |            |            |            |            |            |     |
|---------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C. aff. gardenii</i> _8576   | GT-GAGGGTT | CAAGTCCCTC | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |     |
| <i>C. aff. gardenii</i> _9000   | GT-GAGGGTT | CAAGTCCCTC | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |     |
| <i>C. aff. gardenii</i> _8325   | GT-GAGGGTT | CAAGTCCCTC | TATCCCCAGT | AAAAAGCCCA | TTTCACTTCT |     |
| <i>Cr. vansonii</i> _8964       | GT-GAGGGTT | CAAGTCCCTC | TATCCCCAGT | AAAAAGCCCA | TTTAACTTCT |     |
| <i>C. caulescens</i> _8479      | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGCGGT | TCAAAGAAAA | 500 |
| <i>C. caulescens</i> _8490      | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGCGGT | TCAAAGAAAA |     |
| <i>C. aff. caulescens</i> _8369 | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGCGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8374        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8403        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8405        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8418        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8444        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. gardenii</i> _8523        | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. aff. robusta</i> _8593    | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8391         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8394         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8396         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8408         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8410         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8558         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8573         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8574         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. miniata</i> _8992         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. mirabilis</i> _8947       | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. mirabilis</i> _8955       | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. x nimbiicola</i> _8578    | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. x nimbiicola</i> _8594    | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. x nimbiicola</i> _8595    | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. nobilis</i> _8932         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. nobilis</i> _8933         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. nobilis</i> _8940         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. nobilis</i> _8941         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. robusta</i> _8415         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. robusta</i> _8440         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. robusta</i> _8442         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. robusta</i> _8462         | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. aff. caulescens</i> _8575 | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. aff. gardenii</i> _8576   | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. aff. gardenii</i> _9000   | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. aff. gardenii</i> _8325   | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>Cr. vansonii</i> _8964       | TAAC----TA | TTTATCCTC- | -TTTTTTTTT | CATAAGTGGT | TCAAAGAAAA |     |
| <i>C. caulescens</i> _8479      | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT | 550 |
| <i>C. caulescens</i> _8490      | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. aff. caulescens</i> _8369 | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8374        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8403        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8405        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8418        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8444        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. gardenii</i> _8523        | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. aff. robusta</i> _8593    | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8391         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8394         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8396         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8408         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8410         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8558         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8573         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8574         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. miniata</i> _8992         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. mirabilis</i> _8947       | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. mirabilis</i> _8955       | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. x nimbiicola</i> _8578    | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. x nimbiicola</i> _8594    | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. x nimbiicola</i> _8595    | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C. nobilis</i> _8932         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |

|                                |            |            |            |            |            |     |
|--------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C._nobilis_8933</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATCG | ATCCGAACAT |     |
| <i>C._nobilis_8940</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATCG | ATCCGAACAT |     |
| <i>C._nobilis_8941</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATCG | ATCCGAACAT |     |
| <i>C._robusta_8415</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._robusta_8440</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._robusta_8442</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._robusta_8462</i>         | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._aff._caulescens_8575</i> | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._aff._gardenii_8576</i>   | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._aff._gardenii_9000</i>   | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>C._aff._gardenii_8325</i>   | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
| <i>Cr._vansonii_8964</i>       | TTCAATATCT | TTCTCATTCA | TTCTACTCTT | TCACAAATGG | ATCCGAACAT |     |
|                                |            |            |            |            |            |     |
| <i>C._caulescens_8479</i>      | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA | 600 |
| <i>C._caulescens_8490</i>      | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._caulescens_8369</i> | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8374</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8403</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8405</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8418</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8444</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._gardenii_8523</i>        | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._robusta_8593</i>    | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8391</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8394</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8396</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8408</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8410</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8558</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8573</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8574</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._miniata_8992</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._mirabilis_8947</i>       | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._mirabilis_8955</i>       | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._x_nimbicola_8578</i>     | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._x_nimbicola_8594</i>     | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._x_nimbicola_8595</i>     | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._nobilis_8932</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._nobilis_8933</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._nobilis_8940</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._nobilis_8941</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._robusta_8415</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._robusta_8440</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._robusta_8442</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._robusta_8462</i>         | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._caulescens_8575</i> | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._gardenii_8576</i>   | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._gardenii_9000</i>   | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>C._aff._gardenii_8325</i>   | AAATCTTTTG | ATCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
| <i>Cr._vansonii_8964</i>       | AAATCTTTTG | CTCTTATACC | AATTTGGTTT | GAATAGATAT | GATACCCGTA |     |
|                                |            |            |            |            |            |     |
| <i>C._caulescens_8479</i>      | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  | 650 |
| <i>C._caulescens_8490</i>      | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._aff._caulescens_8369</i> | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8374</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8403</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8405</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8418</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8444</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._gardenii_8523</i>        | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._aff._robusta_8593</i>    | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8391</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8394</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8396</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8408</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8410</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8558</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |
| <i>C._miniata_8573</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTACAGT  |     |

|                                |            |            |            |            |            |     |
|--------------------------------|------------|------------|------------|------------|------------|-----|
| <i>C._miniata_8574</i>         | CAAATGAACA | TATATGGTCA | AGGGATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._miniata_8992</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._mirabilis_8947</i>       | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._mirabilis_8955</i>       | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._x_nimbicola_8578</i>     | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._x_nimbicola_8594</i>     | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._x_nimbicola_8595</i>     | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._nobilis_8932</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._nobilis_8933</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._nobilis_8940</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._nobilis_8941</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._robusta_8415</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._robusta_8440</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._robusta_8442</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._robusta_8462</i>         | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._aff._caulescens_8575</i> | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._aff._gardenii_8576</i>   | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._aff._gardenii_9000</i>   | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>C._aff._gardenii_8325</i>   | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
| <i>Cr._vansonii_8964</i>       | CAAATGAACA | TATATGGTCA | AGGAATTCCC | ATTATTGAAT | CATTCACAGT |     |
|                                |            |            |            |            |            |     |
| <i>C._caulescens_8479</i>      | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT | 700 |
| <i>C._caulescens_8490</i>      | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._caulescens_8369</i> | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8374</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8403</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8405</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8418</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8444</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._gardenii_8523</i>        | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._robusta_8593</i>    | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8391</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8394</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8396</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8408</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8410</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8558</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8573</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8574</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._miniata_8992</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._mirabilis_8947</i>       | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._mirabilis_8955</i>       | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._x_nimbicola_8578</i>     | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._x_nimbicola_8594</i>     | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._x_nimbicola_8595</i>     | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._nobilis_8932</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._nobilis_8933</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._nobilis_8940</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._nobilis_8941</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._robusta_8415</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._robusta_8440</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._robusta_8442</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._robusta_8462</i>         | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._caulescens_8575</i> | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._gardenii_8576</i>   | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._gardenii_9000</i>   | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>C._aff._gardenii_8325</i>   | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
| <i>Cr._vansonii_8964</i>       | CCATATCATT | ATCCTTACAT | TCACAAAGAA | AGTCTTCTTT | TTGAAAATCT |     |
|                                |            |            |            |            |            |     |
| <i>C._caulescens_8479</i>      | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            | 726 |
| <i>C._caulescens_8490</i>      | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._aff._caulescens_8369</i> | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8374</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8403</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8405</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8418</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8444</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |
| <i>C._gardenii_8523</i>        | AAGAAATTCG | GGGACTAGGT | CCAAAT     |            |            |     |

```

C._aff._robusta_8593 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8391 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8394 AAGAAATTAG GGGACTAGGT CCAAAT
C._miniata_8396 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8408 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8410 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8558 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8573 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8574 AAGAAATTCG GGGACTAGGT CCAAAT
C._miniata_8992 AAGAAATTAG GGGACTAGGT CCAAAT
C._mirabilis_8947 AAGAAATTCG GGGACTAGGT CCAAAT
C._mirabilis_8955 AAGAAATTCG GGGACTAGGT CCAAAT
C._x_nimbicola_8578 AAGAAATTCG GGGACTAGGT CCAAAT
C._x_nimbicola_8594 AAGAAATTCG GGGACTAGGT CCAAAT
C._x_nimbicola_8595 AAGAAATTCG GGGACTAGGT CCAAAT
C._nobilis_8932 AAGAAATTCG GGGACTAGGT CCAAAT
C._nobilis_8933 AAGAAATTCG GGGACTAGGT CCAAAT
C._nobilis_8940 AAGAAATTCG GGGACTAGGT CCAAAT
C._nobilis_8941 AAGAAATTCG GGGACTAGGT CCAAAT
C._robusta_8415 AAGAAATTCG GGGACTAGGT CCAAAT
C._robusta_8440 AAGAAATTCG GGGACTAGGT CCAAAT
C._robusta_8442 AAGAAATTCG GGGACTAGGT CCAAAT
C._robusta_8462 AAGAAATTCG GGGACTAGGT CCAAAT
C._aff._caulescens_8575 AAGAAATTCG GGGACTAGGT CCAAAT
C._aff._gardenii_8576 AAGAAATTCG GGGACTAGGT CCAAAT
C._aff._gardenii_9000 AAGAAATTCG GGGACTAGGT CCAAAT
C._aff._gardenii_8325 AAGAAATTCG GGGACTAGGT CCAAAT
Cr._vansonii_8964 AAGAAATTCG GGGACTAGGT CTAAAT

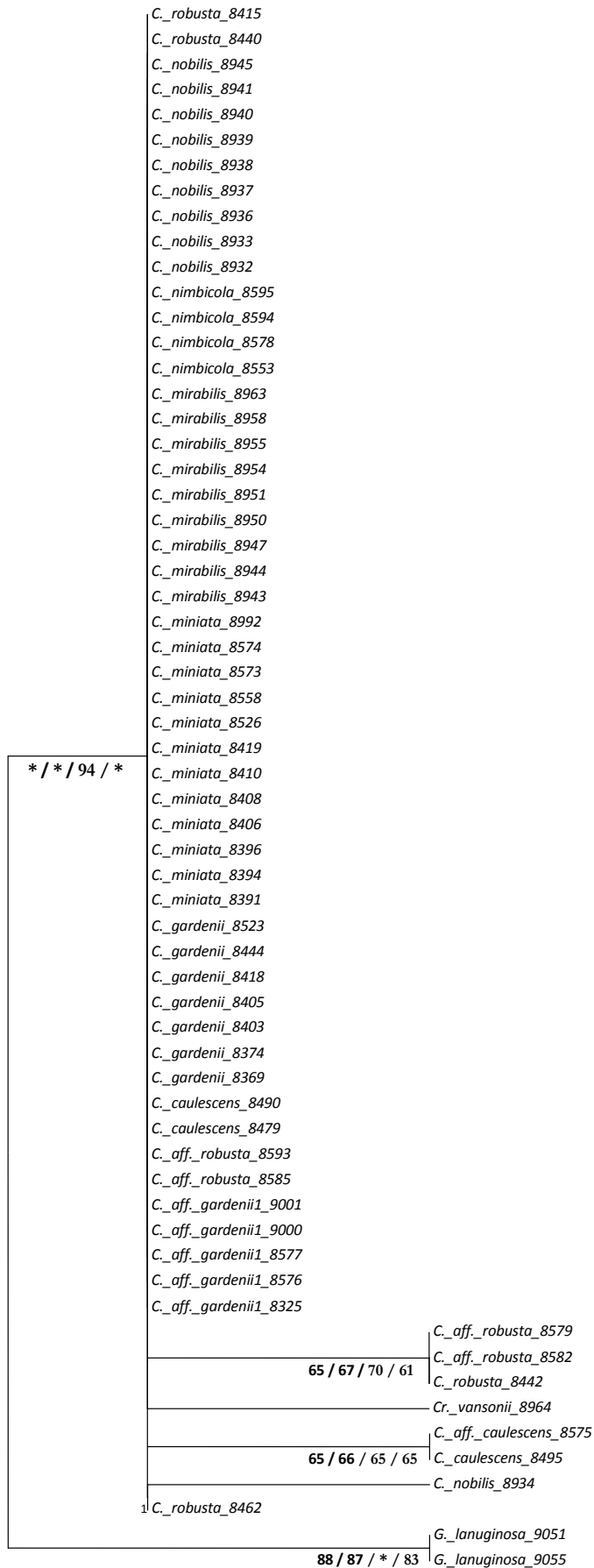
```

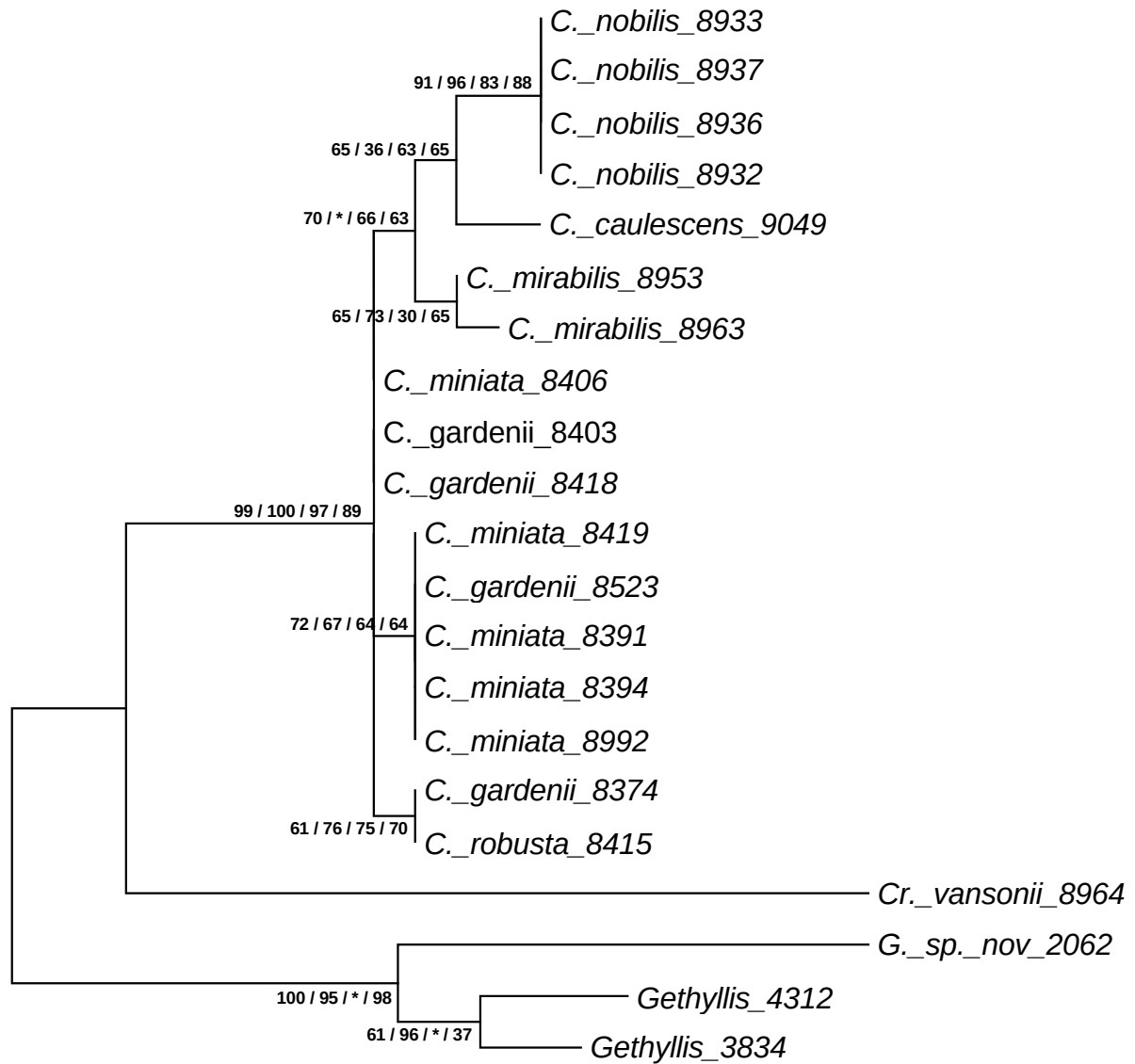
# Appendix C

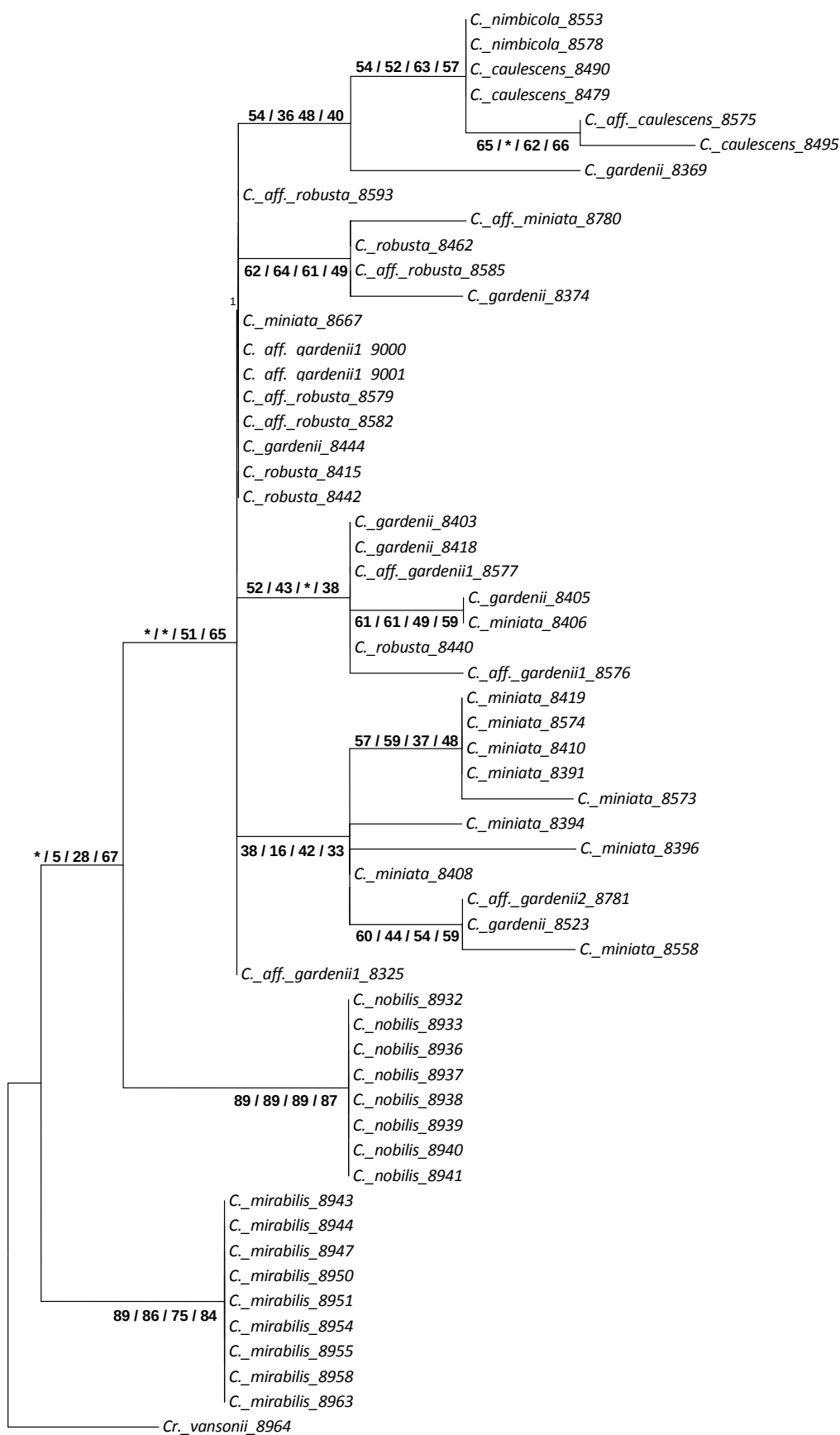
## ***Clivia cladograms***

---

Phylogenetic trees for each gene region were constructed using the Neighbor-Joining (NJ), UPGMA, Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Since the tree topologies of the different methods were highly similar, a cladogram from one of the tree-drawing methods was chosen to represent the phylogenies. The bootstrap and Bayesian Inference values are next to or on the branches in the following order: NJ / UPGMA / MP / ML. An unsupported branch is indicated with an asterisk (\*).

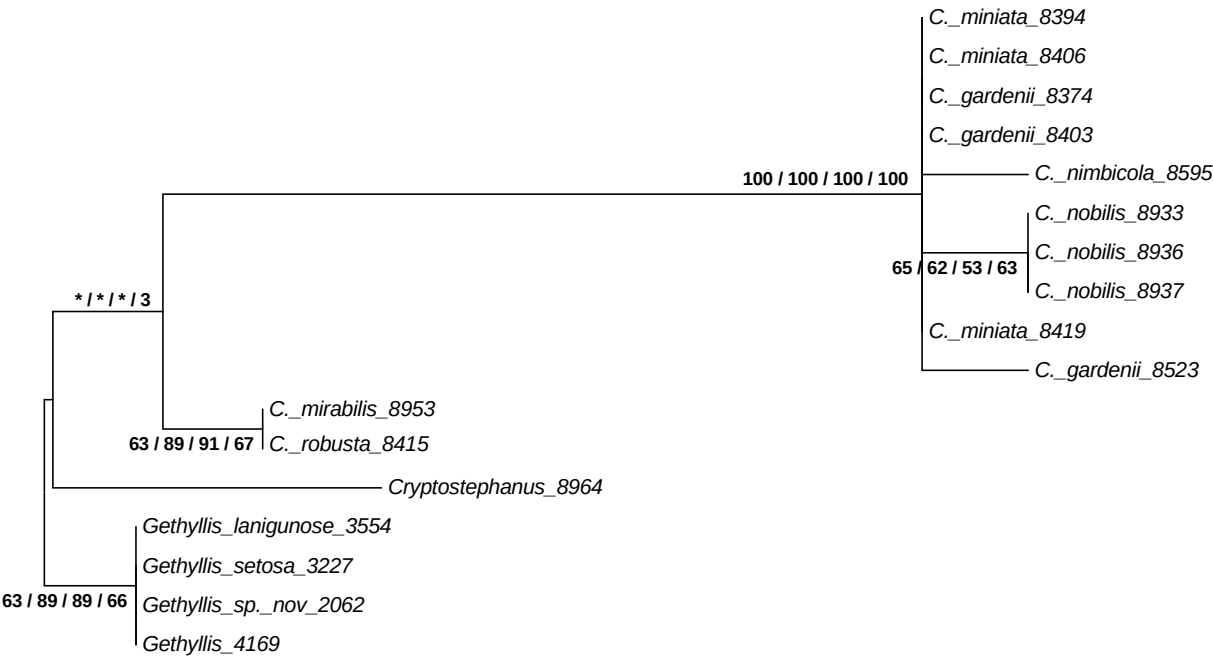
Appendix C1 – *Clivia atpH-atpI*

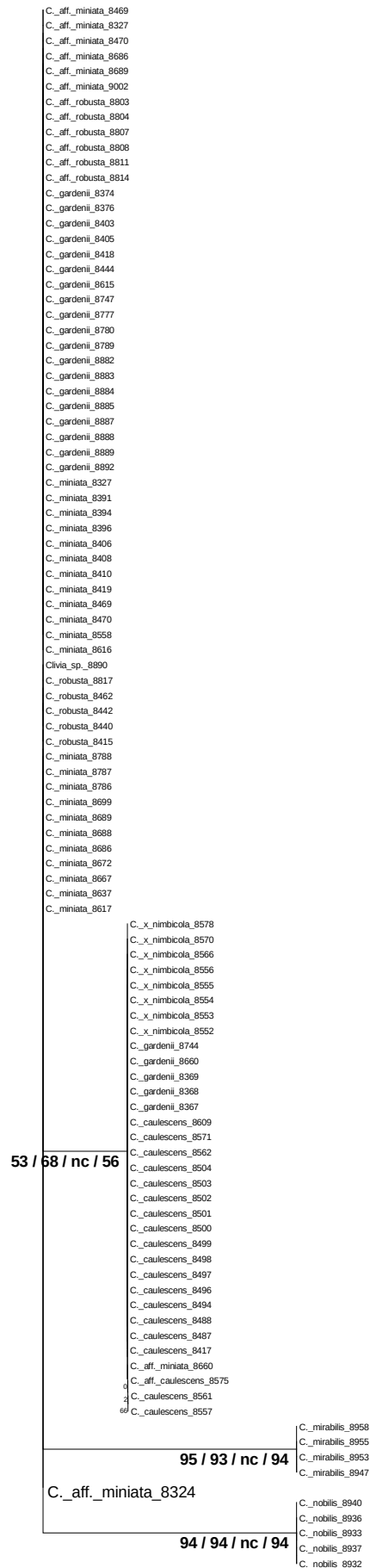
Appendix C2 – *Clivia* ITS2

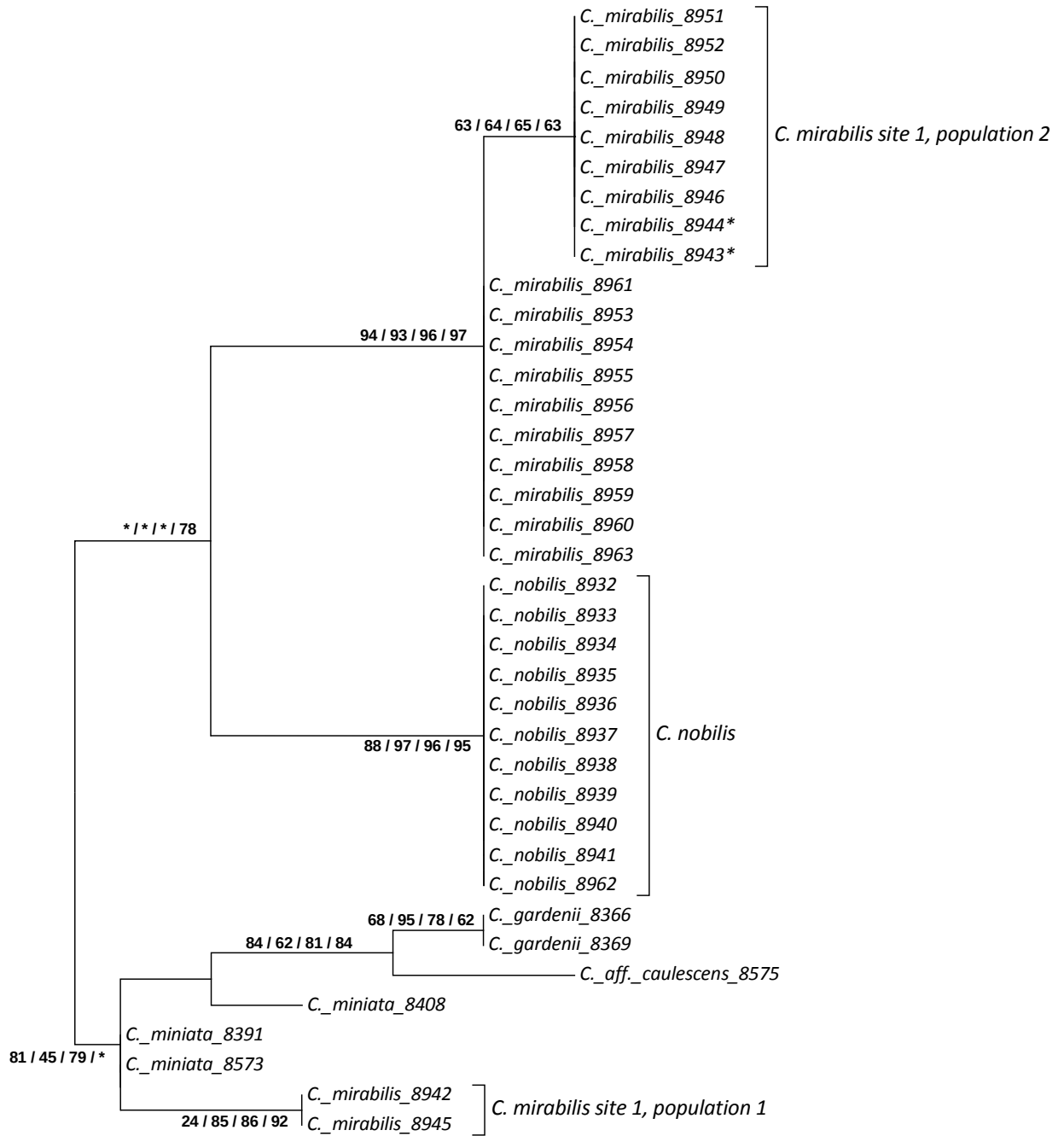
Appendix C3 – *Clivia* *matK*

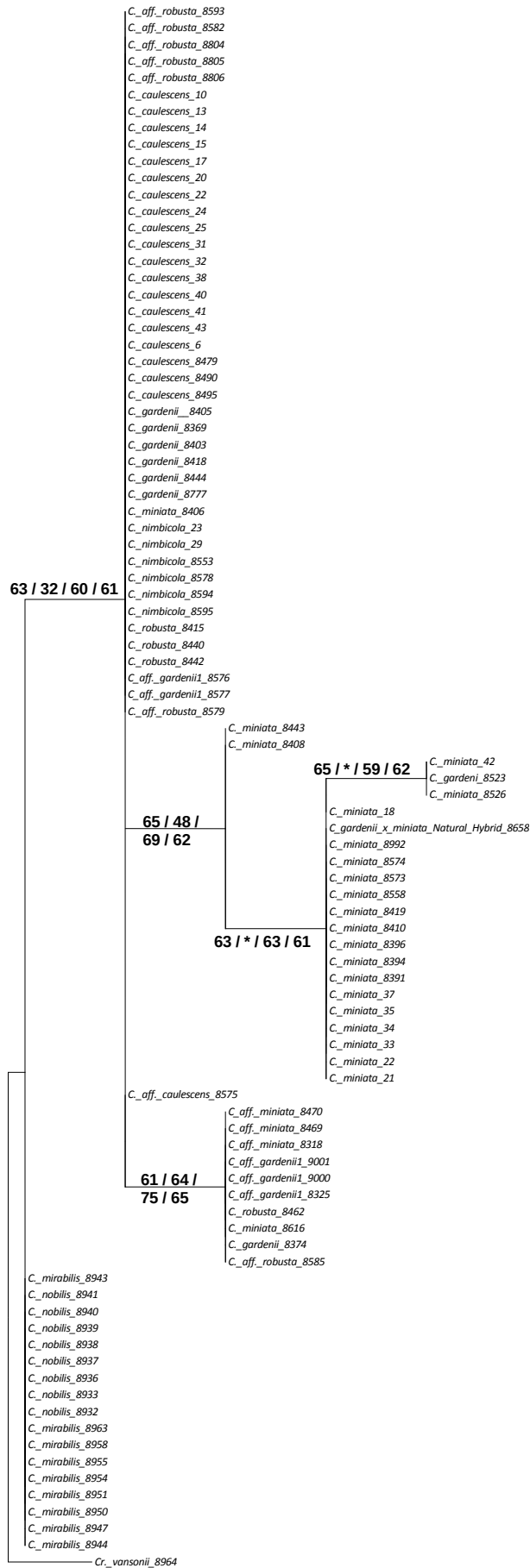


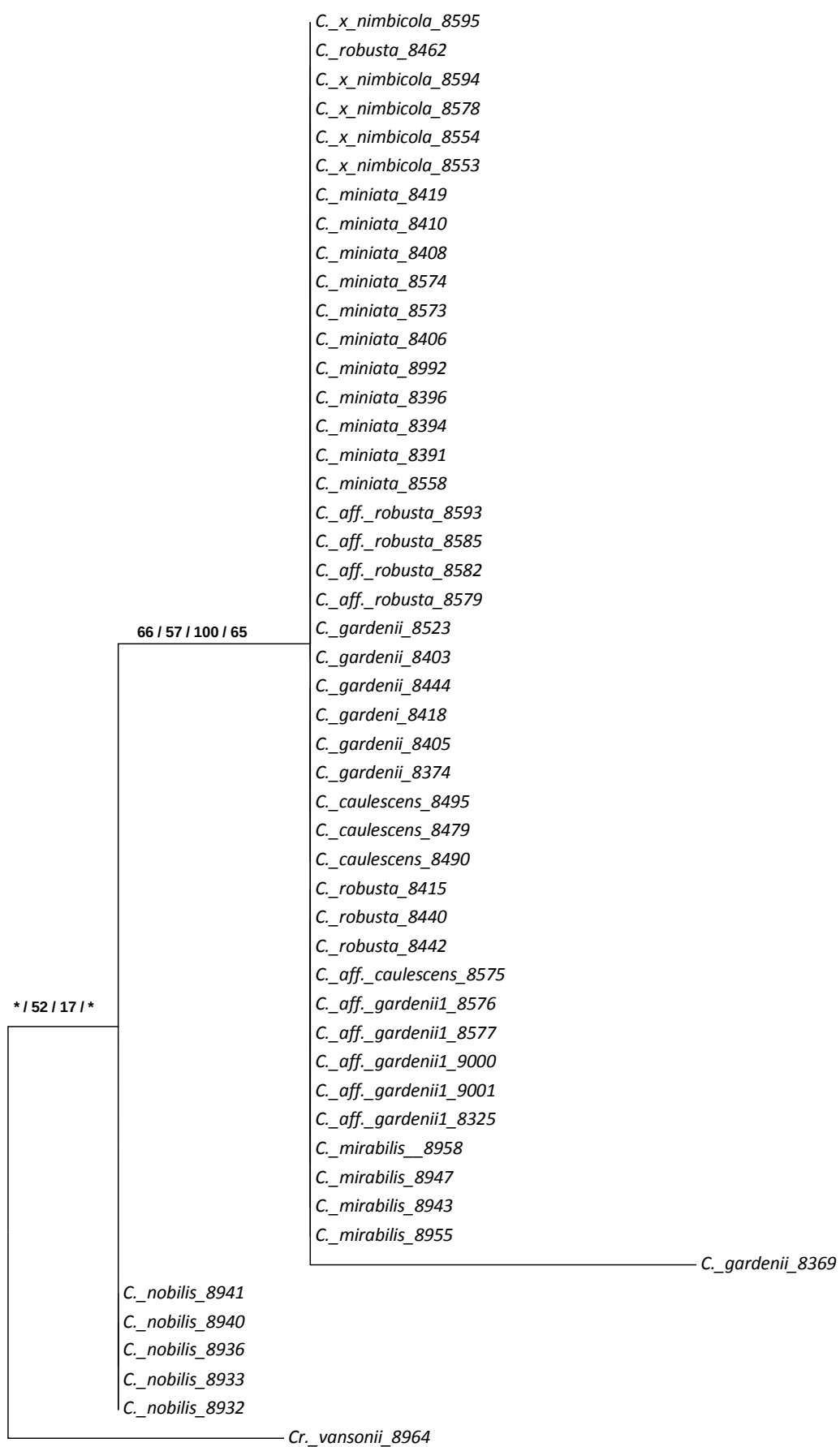
Appendix C4 – *Clivia psbA-trnH*

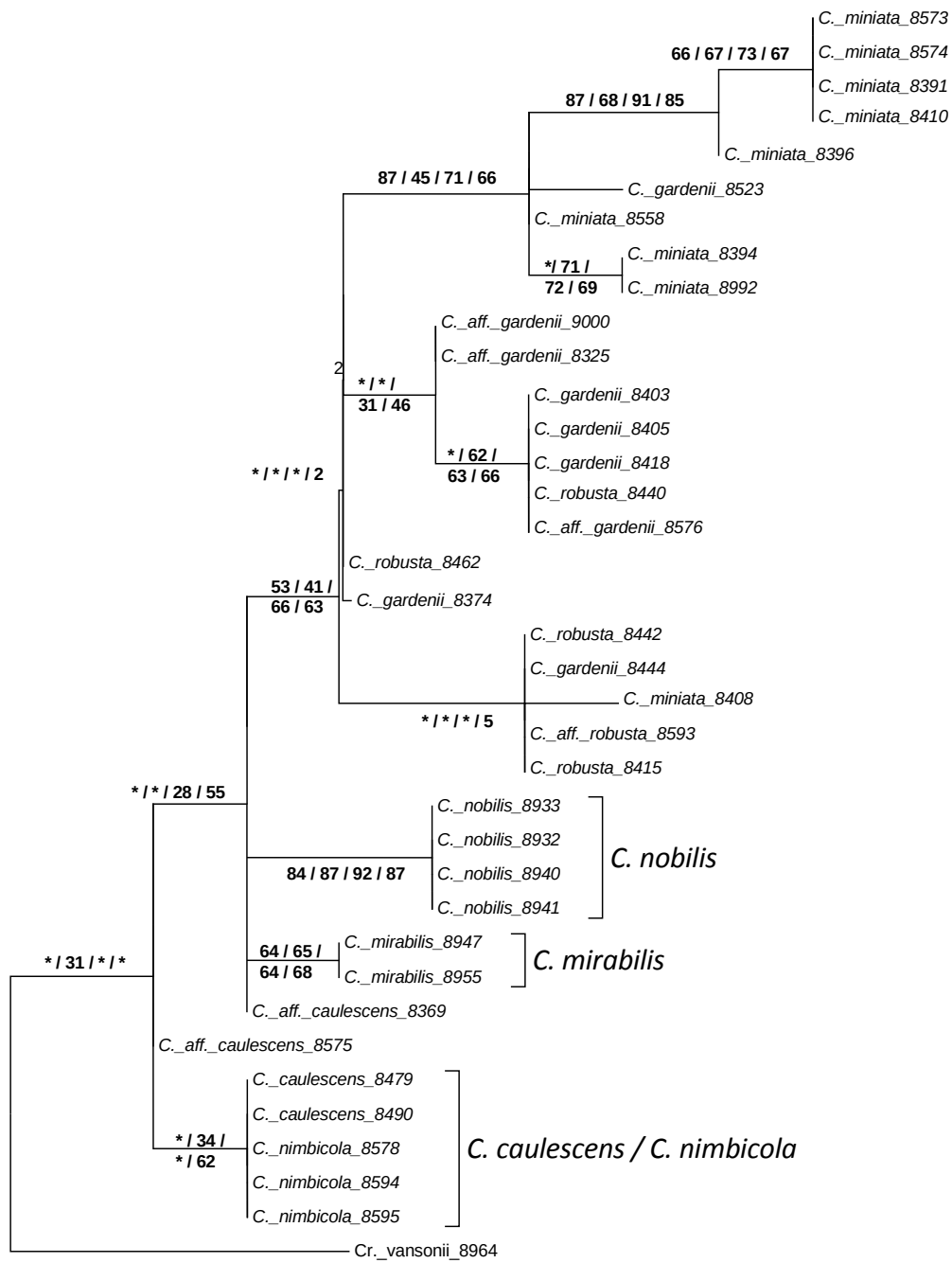


Appendix C5 – *Clivia rbcLa*

Appendix C6 – *Clivia rpl16*

Appendix C7 – *Clivia rpoB*

Appendix C8 – *Clivia rpoC1*

Appendix C9 – *Clivia trnL-F*

# Appendix D

## **Clivia *MOTUs***

---

The software jMOTU calculated the number of Molecular Operational Taxonomic Units (MOTUs) that are created at different cut-off values (measured in bp) from zero to 20. A separate excel spreadsheet was created for all twenty cut-off values for each gene region. These spreadsheets were manually screened. Only the selected data are represented in this appendix in the original output format from jMOTU.

The data is set out as follows:

Column 1: Genus\_region\_cut-off value (bp)\_MOTU cluster number

Column 2: The list of taxa corresponding to a MOTU (or cluster) in column 1

**Appendix D1 – *Clivia atpH-atpI***

62 sequences have been clustered into 10 clusters

|                           |                               |
|---------------------------|-------------------------------|
| Clivia_atpH2_1bp_MOTU0001 | <i>C._aff._gardenii1_8576</i> |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._aff._gardenii1_8577</i> |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._gardenii_8403</i>       |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._gardenii_8405</i>       |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._gardenii_8418</i>       |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._miniata_8406</i>        |
| Clivia_atpH2_1bp_MOTU0001 | <i>C._robusta_8440</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._aff._gardenii1_9000</i> |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._aff._gardenii1_9001</i> |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._aff._robusta_8585</i>   |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._aff._robusta_8593</i>   |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._caulescens_8479</i>     |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._caulescens_8490</i>     |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._gardenii_8369</i>       |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._gardenii_8374</i>       |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._gardenii_8444</i>       |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._gardenii_8523</i>       |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8391</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8394</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8396</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8408</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8410</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8419</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8526</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8558</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8573</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8574</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._miniata_8992</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nimbicola_8553</i>      |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nimbicola_8578</i>      |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nimbicola_8594</i>      |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nimbicola_8595</i>      |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8932</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8933</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8936</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8937</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8938</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8939</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8940</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8941</i>        |
| Clivia_atpH2_1bp_MOTU0002 | <i>C._nobilis_8945</i>        |



Clivia\_atpH2\_1bp\_MOTU0002  
Clivia\_atpH2\_1bp\_MOTU0002  
Clivia\_atpH2\_1bp\_MOTU0003  
Clivia\_atpH2\_1bp\_MOTU0003  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0004  
Clivia\_atpH2\_1bp\_MOTU0005  
Clivia\_atpH2\_1bp\_MOTU0006  
Clivia\_atpH2\_1bp\_MOTU0007  
Clivia\_atpH2\_1bp\_MOTU0008  
Clivia\_atpH2\_1bp\_MOTU0008  
Clivia\_atpH2\_1bp\_MOTU0008  
Clivia\_atpH2\_1bp\_MOTU0009  
Clivia\_atpH2\_1bp\_MOTU0010

*C.\_robusta\_8415*  
*C.\_robusta\_8462*  
*C.\_aff.\_caulescens\_8575*  
*C.\_caulescens\_8495*  
*C.\_mirabilis\_8943*  
*C.\_mirabilis\_8944*  
*C.\_mirabilis\_8947*  
*C.\_mirabilis\_8950*  
*C.\_mirabilis\_8951*  
*C.\_mirabilis\_8954*  
*C.\_mirabilis\_8955*  
*C.\_mirabilis\_8958*  
*C.\_mirabilis\_8963*  
*G.\_lanuginosa\_9051*  
*G.\_lanuginosa\_9055*  
*C.\_nobilis\_8934*  
*C.\_aff.\_robusta\_8579*  
*C.\_aff.\_robusta\_8582*  
*C.\_robusta\_8442*  
*C.\_aff.\_gardenii1\_8325*  
*Cr.\_vansonii\_8964*

**Appendix D2 – *Clivia* ITS2**

21 sequences have been clustered into 10 clusters

|                         |                           |
|-------------------------|---------------------------|
| Clivia_ITS_2bp_MOTU0001 | <i>C._gardenii_8403</i>   |
| Clivia_ITS_2bp_MOTU0001 | <i>C._gardenii_8418</i>   |
| Clivia_ITS_2bp_MOTU0001 | <i>C._miniata_8406</i>    |
| Clivia_ITS_2bp_MOTU0002 | <i>C._mirabilis_8953</i>  |
| Clivia_ITS_2bp_MOTU0002 | <i>C._mirabilis_8963</i>  |
| Clivia_ITS_2bp_MOTU0003 | <i>G._sp._nov_2062</i>    |
| Clivia_ITS_2bp_MOTU0004 | <i>C._gardenii_8523</i>   |
| Clivia_ITS_2bp_MOTU0004 | <i>C._miniata_8391</i>    |
| Clivia_ITS_2bp_MOTU0004 | <i>C._miniata_8394</i>    |
| Clivia_ITS_2bp_MOTU0004 | <i>C._miniata_8419</i>    |
| Clivia_ITS_2bp_MOTU0004 | <i>C._miniata_8992</i>    |
| Clivia_ITS_2bp_MOTU0005 | <i>C._caulescens_9049</i> |
| Clivia_ITS_2bp_MOTU0006 | <i>C._gardenii_8374</i>   |
| Clivia_ITS_2bp_MOTU0006 | <i>C._robusta_8415</i>    |
| Clivia_ITS_2bp_MOTU0007 | <i>C._nobilis_8932</i>    |
| Clivia_ITS_2bp_MOTU0007 | <i>C._nobilis_8933</i>    |
| Clivia_ITS_2bp_MOTU0007 | <i>C._nobilis_8936</i>    |
| Clivia_ITS_2bp_MOTU0007 | <i>C._nobilis_8937</i>    |
| Clivia_ITS_2bp_MOTU0008 | <i>Cr._vansonii_8964</i>  |
| Clivia_ITS_2bp_MOTU0009 | <i>Gethyllis_3834</i>     |
| Clivia_ITS_2bp_MOTU0010 | <i>Gethyllis_4312</i>     |

**Appendix D3 – *Clivia matK***

57 sequences have been clustered into 6 clusters

|                          |                                  |
|--------------------------|----------------------------------|
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8374</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8376</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8403</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8405</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8418</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._gardenii_Spies_8444</i>    |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8391</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8394</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8406</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8408</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8410</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8419</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8558</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._miniata_Spies_8637</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._robusta_Spies_8415</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._robusta_Spies_8440</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._robusta_Spies_8442</i>     |
| Clivia_matK_2bp_MOTU0001 | <i>C._robusta_Spies_8462</i>     |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8417</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8487</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8488</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8494</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8496</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8497</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8498</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8499</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8500</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8501</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8502</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8503</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8504</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8557</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8561</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8562</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8571</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._caulescens_Spies_8609</i>  |
| Clivia_matK_2bp_MOTU0002 | <i>C._x_nimbicola_Spies_8552</i> |
| Clivia_matK_2bp_MOTU0002 | <i>C._x_nimbicola_Spies_8553</i> |
| Clivia_matK_2bp_MOTU0002 | <i>C._x_nimbicola_Spies_8554</i> |
| Clivia_matK_2bp_MOTU0002 | <i>C._x_nimbicola_Spies_8555</i> |
| Clivia_matK_2bp_MOTU0002 | <i>C._x_nimbicola_Spies_8556</i> |

Clivia\_matK\_2bp\_MOTU0002  
Clivia\_matK\_2bp\_MOTU0002  
Clivia\_matK\_2bp\_MOTU0002  
Clivia\_matK\_2bp\_MOTU0003  
Clivia\_matK\_2bp\_MOTU0003  
Clivia\_matK\_2bp\_MOTU0003  
Clivia\_matK\_2bp\_MOTU0004  
Clivia\_matK\_2bp\_MOTU0005  
Clivia\_matK\_2bp\_MOTU0005  
Clivia\_matK\_2bp\_MOTU0005  
Clivia\_matK\_2bp\_MOTU0005  
Clivia\_matK\_2bp\_MOTU0006  
Clivia\_matK\_2bp\_MOTU0006  
Clivia\_matK\_2bp\_MOTU0006  
Clivia\_matK\_2bp\_MOTU0006  
Clivia\_matK\_2bp\_MOTU0006

*C.\_x\_nimbicola\_Spies\_8566*  
*C.\_x\_nimbicola\_Spies\_8570*  
*C.\_x\_nimbicola\_Spies\_8578*  
*C.\_gardenii\_Spies\_8367*  
*C.\_gardenii\_Spies\_8368*  
*C.\_gardenii\_Spies\_8369*  
*C.\_miniata\_Spies\_8396*  
*C.\_mirabilis\_Spies\_8947*  
*C.\_mirabilis\_Spies\_8953*  
*C.\_mirabilis\_Spies\_8955*  
*C.\_mirabilis\_Spies\_8958*  
*C.\_nobilis\_Spies\_8932*  
*C.\_nobilis\_Spies\_8933*  
*C.\_nobilis\_Spies\_8936*  
*C.\_nobilis\_Spies\_8937*  
*C.\_nobilis\_Spies\_8940*

**Appendix D4 – *Clivia psbA-trnH***

17 sequences have been clustered into 7 clusters

|                           |                                  |
|---------------------------|----------------------------------|
| Clivia_trnH_10bp_MOTU0001 | <i>C._gardenii_8374</i>          |
| Clivia_trnH_10bp_MOTU0001 | <i>C._gardenii_8403</i>          |
| Clivia_trnH_10bp_MOTU0001 | <i>C._gardenii_8523</i>          |
| Clivia_trnH_10bp_MOTU0001 | <i>C._miniata_8394</i>           |
| Clivia_trnH_10bp_MOTU0001 | <i>C._miniata_8406</i>           |
| Clivia_trnH_10bp_MOTU0001 | <i>C._miniata_8419</i>           |
| Clivia_trnH_10bp_MOTU0002 | <i>C._robusta_8415</i>           |
| Clivia_trnH_10bp_MOTU0003 | <i>C._mirabilis_8953</i>         |
| Clivia_trnH_10bp_MOTU0004 | <i>C._nobilis_8933</i>           |
| Clivia_trnH_10bp_MOTU0004 | <i>C._nobilis_8936</i>           |
| Clivia_trnH_10bp_MOTU0004 | <i>C._nobilis_8937</i>           |
| Clivia_trnH_10bp_MOTU0005 | <i>Cryptostephanus_8964</i>      |
| Clivia_trnH_10bp_MOTU0006 | <i>C._nimbicola_8595</i>         |
| Clivia_trnH_10bp_MOTU0007 | <i>Gethyllis_4169</i>            |
| Clivia_trnH_10bp_MOTU0007 | <i>Gethyllis_lanigunose_3554</i> |
| Clivia_trnH_10bp_MOTU0007 | <i>Gethyllis_setosa_3227</i>     |
| Clivia_trnH_10bp_MOTU0007 | <i>Gethyllis_sp._nov_2062</i>    |

**Appendix D5 – *Clivia rbcLa***

57 sequences have been clustered into 4 clusters

|                          |                                  |
|--------------------------|----------------------------------|
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8374</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8376</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8403</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8405</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8418</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._gardenii_Spies_8444</i>    |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8391</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8394</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8396</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8406</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8408</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8410</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8419</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8558</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._miniata_Spies_8637</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._robusta_Spies_8415</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._robusta_Spies_8440</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._robusta_Spies_8442</i>     |
| Clivia_rbcL_1bp_MOTU0001 | <i>C._robusta_Spies_8462</i>     |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8417</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8487</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8488</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8494</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8496</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8497</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8498</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8499</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8500</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8501</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8502</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8503</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8504</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8557</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8561</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8562</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8571</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._caulescens_Spies_8609</i>  |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._gardenii_Spies_8367</i>    |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._gardenii_Spies_8368</i>    |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._gardenii_Spies_8369</i>    |
| Clivia_rbcL_1bp_MOTU0002 | <i>C._x_nimbicola_Spies_8552</i> |

Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0002  
Clivia\_rbcL\_1bp\_MOTU0003  
Clivia\_rbcL\_1bp\_MOTU0003  
Clivia\_rbcL\_1bp\_MOTU0003  
Clivia\_rbcL\_1bp\_MOTU0003  
Clivia\_rbcL\_1bp\_MOTU0004  
Clivia\_rbcL\_1bp\_MOTU0004  
Clivia\_rbcL\_1bp\_MOTU0004  
Clivia\_rbcL\_1bp\_MOTU0004  
Clivia\_rbcL\_1bp\_MOTU0004

*C.\_x\_nimbicola\_Spies\_8553*  
*C.\_x\_nimbicola\_Spies\_8554*  
*C.\_x\_nimbicola\_Spies\_8555*  
*C.\_x\_nimbicola\_Spies\_8556*  
*C.\_x\_nimbicola\_Spies\_8566*  
*C.\_x\_nimbicola\_Spies\_8570*  
*C.\_x\_nimbicola\_Spies\_8578*  
*C.\_mirabilis\_Spies\_8947*  
*C.\_mirabilis\_Spies\_8953*  
*C.\_mirabilis\_Spies\_8955*  
*C.\_mirabilis\_Spies\_8958*  
*C.\_nobilis\_Spies\_8932*  
*C.\_nobilis\_Spies\_8933*  
*C.\_nobilis\_Spies\_8936*  
*C.\_nobilis\_Spies\_8937*  
*C.\_nobilis\_Spies\_8940*

**Appendix D6 – *Clivia* rpl16**

38 sequences have been clustered into 6 clusters

|                            |                                |
|----------------------------|--------------------------------|
| Clivia_rpl162_3bp_MOTU0001 | <i>C._miniata_8391</i>         |
| Clivia_rpl162_3bp_MOTU0001 | <i>C._miniata_8408</i>         |
| Clivia_rpl162_3bp_MOTU0001 | <i>C._miniata_8573</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8932</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8933</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8934</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8935</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8936</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8937</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8938</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8939</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8940</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8941</i>         |
| Clivia_rpl162_3bp_MOTU0002 | <i>C._nobilis_8962</i>         |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8943</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8944</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8946</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8947</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8948</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8949</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8950</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8951</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8952</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8953</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8954</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8955</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8956</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8957</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8958</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8959</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8960</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8961</i>       |
| Clivia_rpl162_3bp_MOTU0003 | <i>C._mirabilis_8963</i>       |
| Clivia_rpl162_3bp_MOTU0004 | <i>C._mirabilis_8942</i>       |
| Clivia_rpl162_3bp_MOTU0004 | <i>C._mirabilis_8945</i>       |
| Clivia_rpl162_3bp_MOTU0005 | <i>C._gardenii_8366</i>        |
| Clivia_rpl162_3bp_MOTU0005 | <i>C._gardenii_8369</i>        |
| Clivia_rpl162_3bp_MOTU0006 | <i>C._aff._caulescens_8575</i> |



**Appendix D – *Clivia rpoB***

94 sequences have been clustered into 11 clusters

|                           |                                                 |
|---------------------------|-------------------------------------------------|
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_18</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_21</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_22</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_33</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_34</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_35</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_37</i>                            |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8391</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8394</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8396</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8410</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8419</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8558</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8573</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8574</i>                          |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._miniata_8992</i>                          |
|                           | <i>C._gardenii_x_miniata_Natural_Hybrid_865</i> |
|                           | 8                                               |
| Clivia_rpoB2_0bp_MOTU0001 | <i>C._nobilis_8938</i>                          |
| Clivia_rpoB2_0bp_MOTU0002 | <i>C._nobilis_8940</i>                          |
| Clivia_rpoB2_0bp_MOTU0002 | <i>C._aff._caulescens_8575</i>                  |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8579</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8582</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8593</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8804</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8805</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._aff._robusta_8806</i>                     |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_10</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_13</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_14</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_17</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_20</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_22</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_24</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_25</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_31</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_32</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_38</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_40</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_41</i>                         |
| Clivia_rpoB2_0bp_MOTU0003 | <i>C._caulescens_43</i>                         |

*C. caulescens*\_6  
*C. caulescens*\_8479  
*C. caulescens*\_8490  
*C. caulescens*\_8495  
*C. gardenii*\_8369  
*C. gardenii*\_8403  
*C. gardenii*\_8418  
*C. gardenii*\_8444  
*C. gardenii*\_8777  
*C. gardenii*\_8405  
*C. miniata*\_8406  
*C. nimbicola*\_29  
*C. nimbicola*\_8553  
*C. nimbicola*\_8578  
*C. nimbicola*\_8594  
*C. nimbicola*\_8595  
*C. robusta*\_8415  
*C. robusta*\_8440  
*C. robusta*\_8442  
*C. aff. gardenii*1\_8576  
*C. aff. gardenii*1\_8577  
*C. aff. robusta*\_8585  
*C. gardenii*\_8374  
*C. miniata*\_8616  
*C. robusta*\_8462  
*C. aff. gardenii*1\_8325  
*C. aff. gardenii*1\_9000  
*C. aff. gardenii*1\_9001  
*C. aff. miniata*\_8318  
*C. aff. miniata*\_8469  
*C. aff. miniata*\_8470  
*C. miniata*\_8526  
*C. mirabilis*\_8943  
*C. mirabilis*\_8944  
*C. mirabilis*\_8947  
*C. mirabilis*\_8950  
*C. mirabilis*\_8951  
*C. mirabilis*\_8954  
*C. mirabilis*\_8955  
*C. mirabilis*\_8958  
*C. mirabilis*\_8963  
*C. nobilis*\_8932  
*C. nobilis*\_8933  
*C. nobilis*\_8936  
*C. nobilis*\_8937  
*C. nobilis*\_8939

Clivia\_rpoB2\_0bp\_MOTU0006  
Clivia\_rpoB2\_0bp\_MOTU0007  
Clivia\_rpoB2\_0bp\_MOTU0008  
Clivia\_rpoB2\_0bp\_MOTU0008  
Clivia\_rpoB2\_0bp\_MOTU0009  
Clivia\_rpoB2\_0bp\_MOTU0010  
Clivia\_rpoB2\_0bp\_MOTU0010  
Clivia\_rpoB2\_0bp\_MOTU0011

*C.\_nobilis\_8941*  
*C.\_caulescens\_15*  
*C.\_miniata\_8408*  
*C.\_miniata\_8443*  
*C.\_nimbicola\_23*  
*C.\_gardeni\_8523*  
*C.\_miniata\_42*  
*Cr.\_vansonii\_8964*

**Appendix D8 – *Clivia* rpoC1**

50 sequences have been clustered into 4 clusters

|                           |                                |
|---------------------------|--------------------------------|
| Clivia_rpoC1_0bp_MOTU0001 | <i>C._nobilis_8932</i>         |
| Clivia_rpoC1_0bp_MOTU0001 | <i>C._nobilis_8933</i>         |
| Clivia_rpoC1_0bp_MOTU0001 | <i>C._nobilis_8936</i>         |
| Clivia_rpoC1_0bp_MOTU0001 | <i>C._nobilis_8940</i>         |
| Clivia_rpoC1_0bp_MOTU0001 | <i>C._nobilis_8941</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._caulescens_8575</i> |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._gardenii1_8325</i>  |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._gardenii1_8576</i>  |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._gardenii1_8577</i>  |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._gardenii1_9000</i>  |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._gardenii1_9001</i>  |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._robusta_8579</i>    |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._robusta_8582</i>    |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._robusta_8585</i>    |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._aff._robusta_8593</i>    |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._caulescens_8479</i>      |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._caulescens_8490</i>      |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._caulescens_8495</i>      |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardeni_8418</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardenii_8374</i>        |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardenii_8403</i>        |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardenii_8405</i>        |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardenii_8444</i>        |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._gardenii_8523</i>        |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8391</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8394</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8396</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8406</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8408</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8410</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8419</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8558</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8573</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8574</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._miniata_8992</i>         |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._mirabilis_8943</i>       |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._mirabilis_8947</i>       |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._mirabilis_8955</i>       |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._mirabilis_8958</i>       |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._nimbicola_8553</i>       |
| Clivia_rpoC1_0bp_MOTU0002 | <i>C._nimbicola_8554</i>       |

Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0002  
Clivia\_rpoC1\_0bp\_MOTU0003  
Clivia\_rpoC1\_0bp\_MOTU0004

*C.\_nimbicola\_8578*  
*C.\_nimbicola\_8594*  
*C.\_nimbicola\_8595*  
*C.\_robusta\_8415*  
*C.\_robusta\_8440*  
*C.\_robusta\_8442*  
*C.\_robusta\_8462*  
*C.\_gardenii\_8369*  
*Cr.\_vansonii\_8964*

**Appendix D9 – *Clivia trnL-F***

37 sequences have been clustered into 7 clusters

|                           |                                |
|---------------------------|--------------------------------|
| Clivia_trnLF_4bp_MOTU0001 | <i>C._aff._gardenii_8325</i>   |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._aff._gardenii_8576</i>   |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._aff._gardenii_9000</i>   |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._aff._robusta_8593</i>    |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._gardenii_8374</i>        |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._gardenii_8405</i>        |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._gardenii_8418</i>        |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._gardenii_8444</i>        |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._gardenii_8523</i>        |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._miniata_8394</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._miniata_8408</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._miniata_8558</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._miniata_8992</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._robusta_8415</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._robusta_8440</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._robusta_8442</i>         |
| Clivia_trnLF_4bp_MOTU0001 | <i>C._robusta_8462</i>         |
| Clivia_trnLF_4bp_MOTU0002 | <i>C._gardenii_8403</i>        |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._aff._caulescens_8369</i> |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._aff._caulescens_8575</i> |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._caulescens_8479</i>      |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._caulescens_8490</i>      |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._nimbicola_8578</i>       |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._nimbicola_8594</i>       |
| Clivia_trnLF_4bp_MOTU0003 | <i>C._nimbicola_8595</i>       |
| Clivia_trnLF_4bp_MOTU0004 | <i>C._miniata_8391</i>         |
| Clivia_trnLF_4bp_MOTU0004 | <i>C._miniata_8396</i>         |
| Clivia_trnLF_4bp_MOTU0004 | <i>C._miniata_8410</i>         |
| Clivia_trnLF_4bp_MOTU0004 | <i>C._miniata_8573</i>         |
| Clivia_trnLF_4bp_MOTU0004 | <i>C._miniata_8574</i>         |
| Clivia_trnLF_4bp_MOTU0005 | <i>C._nobilis_8932</i>         |
| Clivia_trnLF_4bp_MOTU0005 | <i>C._nobilis_8933</i>         |
| Clivia_trnLF_4bp_MOTU0005 | <i>C._nobilis_8940</i>         |
| Clivia_trnLF_4bp_MOTU0005 | <i>C._nobilis_8941</i>         |
| Clivia_trnLF_4bp_MOTU0006 | <i>C._mirabilis_8947</i>       |
| Clivia_trnLF_4bp_MOTU0006 | <i>C._mirabilis_8955</i>       |
| Clivia_trnLF_4bp_MOTU0007 | <i>Cr._vansonii_8964</i>       |

# Appendix E

## ***Interspecific and intraspecific distances in Clivia***

---

|                              | <i>C. robusta</i> | <i>C. caulescens</i> | <i>C. miniata</i> | <i>C. nobilis</i> | <i>C. mirabilis</i> | <i>C. × nimbicola</i> | <i>C. gardenii</i> |
|------------------------------|-------------------|----------------------|-------------------|-------------------|---------------------|-----------------------|--------------------|
| <i>C. caulescens</i>         | 0.004             | 0                    | 0.008             | 0.006             | 0.005               | 0                     | 0.004              |
| <i>C. × nimbicola</i>        | 0.003             | 0                    | 0.008             | 0.006             | 0.005               | 0.004                 | 0.004              |
| <i>C. gardenii</i>           | 0.002             | 0.004                | 0.006             | 0.006             | 0.005               | 0.008                 | 0                  |
| <i>C. miniata</i>            | 0.006             | 0.008                | 0                 | 0.01              | 0.009               | 0.006                 | 0.006              |
| <i>C. nobilis</i>            | 0.005             | 0.006                | 0.01              | 0                 | 0.007               | 0.005                 | 0.006              |
| <i>C. mirabilis</i>          | 0.004             | 0.005                | 0.009             | 0.007             | 0                   | 0.007                 | 0.005              |
| <i>C. robusta</i>            | 0                 | 0.004                | 0.006             | 0.005             | 0.004               | 0.003                 | 0.002              |
| Mean Interspecific distances | 0.003429          | 0.005                | 0.006333          | 0.005167          | 0.005833            | 0.004714              | 0.004571           |
| Std. deviation               | 0.001988          | 0.002769             | 0.002875          | 0.002137          | 0.001835            | 0.00269               | 0.001988           |
| Mean Intraspecific distances | 0.0011            | 0.001                | 0.0037            | 0                 | 0                   | n/c                   | 0.0023             |
| Std. deviation               | 0.000968          | 0.000531             | 0.002132          | 0                 | 0                   | n/c                   | 0.00179            |

The inter and intraspecific variation in *Clivia* (data used to compile Figure 4.5). The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances.



# Appendix F

## ***Aligned Lachenalia sequences***

---

**Appendix F1 – *Lachenalia atpH-atpI***

|                             |             |             |            |            |            |     |
|-----------------------------|-------------|-------------|------------|------------|------------|-----|
| <i>L._bifolia_7064</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT | 50  |
| <i>L._bifolia_7322</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8893</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8898</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8900</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8902</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8903</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_8905</i>      | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._sp._9029</i>          | TTGATCACTA  | AAATCTATTG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._sp._9032</i>          | TTGATCACTA  | AAATCTATTG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._mediana_9030</i>      | TTGATCACTA  | AAAT-----TG | AGTTGAAGTA | ACTAAAGCTT | TGAATAA-AT |     |
| <i>L._mediana_9033</i>      | TTGATCACTA  | AAA-----TG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAA-AT |     |
| <i>L._mediana_9034</i>      | TTGATCACTA  | AAAT-----TG | AGTTGAAGTA | ACTAAAGCTT | TGAATAA-AT |     |
| <i>L._punctata_8909</i>     | TTGA-----TA | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._punctata_8910</i>     | TTGA-----TA | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._punctata_8914</i>     | TTGA-----TA | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._punctata_9042</i>     | TTGA-----TA | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._schlechteri_6865</i>  | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6862</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6863</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6869</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6870</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6871</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6873</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6874</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._schlechterii_6880</i> | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6881</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_6902</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._unifolia_9038</i>     | TTGATCACTA  | AAATCTATCG  | AGTTGAAGTA | ACTAAAGCTT | TGAATAAAAT |     |
| <i>L._bifolia_7064</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA | 100 |
| <i>L._bifolia_7322</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8893</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8898</i>      | AA--NTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8900</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8902</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8903</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._bifolia_8905</i>      | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._sp._9029</i>          | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._sp._9032</i>          | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._mediana_9030</i>      | AATATTATGG  | ATGGG-----  | -CCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._mediana_9033</i>      | AATATTATGG  | AT-GG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._mediana_9034</i>      | AATATTATGG  | ATGGG-----  | -CCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._punctata_8909</i>     | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._punctata_8910</i>     | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._punctata_8914</i>     | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._punctata_9042</i>     | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._schlechteri_6865</i>  | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6862</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6863</i>     | AA---TATGG  | ATGGGAATAA  | ----AATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6869</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6870</i>     | AATATTATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6871</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6873</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6874</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._schlechterii_6880</i> | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6881</i>     | AA---TATGG  | ATAGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_6902</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |
| <i>L._unifolia_9038</i>     | AA---TATGG  | ATGGG-----  | GCCCAATAAT | ATATAACTAG | TATATATTTA |     |

|                             |            |            |            |            |             |     |
|-----------------------------|------------|------------|------------|------------|-------------|-----|
| <i>L._bifolia_7064</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  | 150 |
| <i>L._bifolia_7322</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8893</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8898</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8900</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8902</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8903</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_8905</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._sp._9029</i>          | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCGC | CTTTCCTTTAT |     |
| <i>L._sp._9032</i>          | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCGC | CTTTCCTTTAT |     |
| <i>L._mediana_9030</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._mediana_9033</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._mediana_9034</i>      | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._punctata_8909</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._punctata_8910</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._punctata_8914</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._punctata_9042</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._schlechteri_6865</i>  | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6862</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6863</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6869</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6870</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTT--  |     |
| <i>L._unifolia_6871</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6873</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6874</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._schlechterii_6880</i> | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6881</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_6902</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._unifolia_9038</i>     | ATATCACATA | TACATTTGTT | TCTTTCATAA | CGTAAACCAC | ----CTTTAT  |     |
| <i>L._bifolia_7064</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  | 200 |
| <i>L._bifolia_7322</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8893</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8898</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8900</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8902</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8903</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_8905</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._sp._9029</i>          | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._sp._9032</i>          | ATTGAATCG- | ---GATTCTA | GAATGAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._mediana_9030</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._mediana_9033</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._mediana_9034</i>      | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._punctata_8909</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._punctata_8910</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._punctata_8914</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._punctata_9042</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._schlechteri_6865</i>  | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6862</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6863</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6869</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6870</i>     | --TGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6871</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATATATA  |     |
| <i>L._unifolia_6873</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6874</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._schlechterii_6880</i> | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATATATA  |     |
| <i>L._unifolia_6881</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_6902</i>     | ATTGAATCGG | ATTGATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._unifolia_9038</i>     | ATTGAATCG- | ---GATTCTA | ----GAATAA | TTCTTCGAAA | TATATA----  |     |
| <i>L._bifolia_7064</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  | 250 |
| <i>L._bifolia_7322</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8893</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8898</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8900</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8902</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8903</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |
| <i>L._bifolia_8905</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT  |     |

|                             |            |            |            |            |            |     |
|-----------------------------|------------|------------|------------|------------|------------|-----|
| <i>L._sp._9029</i>          | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._sp._9032</i>          | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._mediana_9030</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._mediana_9033</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._mediana_9034</i>      | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._punctata_8909</i>     | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._punctata_8910</i>     | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._punctata_8914</i>     | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._punctata_9042</i>     | CAGGGGCTGT | GACTGGACTT | ATACTTATAG | GCATTCCATA | TATCTAGTAT |     |
| <i>L._schlechteri_6865</i>  | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6862</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6863</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6869</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6870</i>     | CAGGGGCTGT | GACTGGACTT | ATACTTCTAG | GCATTCTATA | TATCTAGTAT |     |
| <i>L._unifolia_6871</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6873</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6874</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._schlechterii_6880</i> | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6881</i>     | CAGGGGTTGT | GACTGGACTT | A-----ATAG | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_6902</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
| <i>L._unifolia_9038</i>     | CAGGGGTTGT | GACTGGACTT | -----ATAG  | GCATTCCATA | TATCTAGTGT |     |
|                             |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC | 300 |
| <i>L._bifolia_7322</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8893</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8898</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8900</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8902</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8903</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._bifolia_8905</i>      | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._sp._9029</i>          | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._sp._9032</i>          | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._mediana_9030</i>      | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._mediana_9033</i>      | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._mediana_9034</i>      | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._punctata_8909</i>     | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._punctata_8910</i>     | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._punctata_8914</i>     | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._punctata_9042</i>     | GACCCTTGTA | TTGTACCCCA | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._schlechteri_6865</i>  | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6862</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6863</i>     | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6869</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6870</i>     | GACCCTTGTA | -----CCCA  | T-----     | -----      | -----      |     |
| <i>L._unifolia_6871</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6873</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6874</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._schlechterii_6880</i> | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6881</i>     | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_6902</i>     | GACCCTTGTA | -----CCCA  | TCAAGCATTT | CGTAATATCG | CCTATCTTTC |     |
| <i>L._unifolia_9038</i>     | GACCCTTGTA | -----CCCA  | TCCAGCATTT | CGTAATATCG | CCTATCTTTC |     |
|                             |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC | 350 |
| <i>L._bifolia_7322</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8893</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8898</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8900</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8902</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8903</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._bifolia_8905</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._sp._9029</i>          | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._sp._9032</i>          | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._mediana_9030</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._mediana_9033</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._mediana_9034</i>      | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._punctata_8909</i>     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._punctata_8910</i>     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |
| <i>L._punctata_8914</i>     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC |     |

|                              |            |            |            |            |             |     |
|------------------------------|------------|------------|------------|------------|-------------|-----|
| <i>L. punctata</i> _9042     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. schlechteri</i> _6865  | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6862     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6863     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6869     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6870     | -----CAGC  | TCTAATCGTA | TATACATATG | TATTTCTAT- | ---TATATTC  |     |
| <i>L. unifolia</i> _6871     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6873     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6874     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. schlechterii</i> _6880 | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6881     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _6902     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
| <i>L. unifolia</i> _9038     | CTCTTACAAC | TCTAATCGTA | TATACATATG | TATTTCTATC | TATTATATTC  |     |
|                              |            |            |            |            |             |     |
| <i>L. bifolia</i> _7064      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  | 400 |
| <i>L. bifolia</i> _7322      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8893      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8898      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8900      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8902      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8903      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. bifolia</i> _8905      | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. sp.</i> _9029          | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. sp.</i> _9032          | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. mediana</i> _9030      | CCCAATATAT | TCCCCAACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. mediana</i> _9033      | CCCAATATAT | TCCCCAACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. mediana</i> _9034      | CCCAATATAT | TCCCCAACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. punctata</i> _8909     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. punctata</i> _8910     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. punctata</i> _8914     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. punctata</i> _9042     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. schlechteri</i> _6865  | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6862     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6863     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6869     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6870     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6871     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6873     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6874     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. schlechterii</i> _6880 | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6881     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _6902     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
| <i>L. unifolia</i> _9038     | CCC-----   | -----AACCA | AGAACCAAGT | AGGTTTCTT  | GAACTATGCA  |     |
|                              |            |            |            |            |             |     |
| <i>L. bifolia</i> _7064      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG | 450 |
| <i>L. bifolia</i> _7322      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8893      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-AAAAATAA | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8898      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8900      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8902      | TTGCCATTGC | CCCAT----- | TGGGATAAGC | TTAAAAAT-A | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8903      | TTGCCATTGC | CCCAT----- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG |     |
| <i>L. bifolia</i> _8905      | TTGCCATTGC | CCCAT----- | TGGGATAAGC | TTAAAAATAA | AATAAAAAAGG |     |
| <i>L. sp.</i> _9029          | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. sp.</i> _9032          | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. mediana</i> _9030      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAA----- | --TAAAAAGG  |     |
| <i>L. mediana</i> _9033      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAA----- | --TAAAAAGG  |     |
| <i>L. mediana</i> _9034      | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAA----- | --TAAAAAGG  |     |
| <i>L. punctata</i> _8909     | TTGCCATTGC | CCCAT----- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. punctata</i> _8910     | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. punctata</i> _8914     | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. punctata</i> _9042     | TTGCCATTGC | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. schlechteri</i> _6865  | -----TTGC  | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6862     | -----TTGC  | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6863     | -----TTGC  | CCCAT----- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6869     | -----TTGC  | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6870     | TTGCCATTGC | CCCATT---- | TGGGATAAGC | TTAAA----- | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6871     | -----TTGC  | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |
| <i>L. unifolia</i> _6873     | -----TTGC  | CCCATT---- | TGGGATAAGC | T-----     | --TAAAAAGG  |     |

```

    L._unifolia_6874 -----TTGC CCCATTTATT TGGGATAAGC T----- --TAAAAAGG
L._schlechterii_6880 -----TTGC CCCATT----- TGGGATAAGC T----- --TAAAAAGG
    L._unifolia_6881 -----TTGC CCCATT----- TGGGATAAGC T----- --TAAAAAGG
    L._unifolia_6902 -----TTGC CCCATT----- TGGGATAAGC T----- --TAAAAAGG
    L._unifolia_9038 -----TTGC CCCATT----- TGGGATAAGC T----- --TAAAAAGG

    L._bifolia_7064 AGAC----TA T 461
    L._bifolia_7322 AGAC----TA T
    L._bifolia_8893 AGAC----TA T
    L._bifolia_8898 AGAC----TA T
    L._bifolia_8900 AGAC----TA T
    L._bifolia_8902 AGAC----TA T
    L._bifolia_8903 AGAC----TA T
    L._bifolia_8905 AGAC----TA T
    L._sp._9029 AGAC----TA T
    L._sp._9032 AGAC----TA T
    L._mediana_9030 AGAC----TA T
    L._mediana_9033 AGAC----TA T
    L._mediana_9034 AGAC----TA T
    L._punctata_8909 AGAC----TA T
    L._punctata_8910 AGAC----TA T
    L._punctata_8914 AGAC----TA T
    L._punctata_9042 AGAC----TA T
L._schlechterii_6865 AGAC----TA T
    L._unifolia_6862 AGAC----TA T
    L._unifolia_6863 AGAC----TA T
    L._unifolia_6869 AGAC----TA T
    L._unifolia_6870 AGAC----TA T
    L._unifolia_6871 AGAC----TA T
    L._unifolia_6873 AGAC----TA T
    L._unifolia_6874 AGAC----TA T
L._schlechterii_6880 AGAC----TA T
    L._unifolia_6881 AGAC----TA T
    L._unifolia_6902 AGAC----TA T
    L._unifolia_9038 AGACAGACTA T

```

Appendix F2 – *Lachenalia* ITS2

|                                 |            |            |             |            |            |     |
|---------------------------------|------------|------------|-------------|------------|------------|-----|
| <i>L._aloides</i> _7059         | GGCTCACGTC | GGAGTGGGTT | CCTTGCAATTC | GGCTCTAGAG | CCGGGGGTCC | 50  |
| <i>L._bifolia</i> _7064         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _7322         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8893         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8898         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8902         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8903         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8904         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8905         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8906         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _8907         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _9115         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _9116         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._bifolia</i> _AB304980.1   | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._carnosa</i> _AB439269.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._carnosa</i> _AB439270.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._carnosa</i> _AB439271.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._flava</i> _7060           | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._hirta</i> _AB304982.1     | GGCTCCCGTC | AGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._hirta</i> _AB439272.1     | GGCTCCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._hirta</i> _AB439273.1     | GGCTCCCGTC | AGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._hirta</i> _AB439274.1     | GGCTCCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB304998.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB304999.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB439277.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAT | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB439278.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAT | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB439279.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAT | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB439280.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAT | CCGGGGGTCC |     |
| <i>L._pallida</i> _AB439281.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAT | CCGGGGGTCC |     |
| <i>L._punctata</i> _8909        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._punctata</i> _8910        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._punctata</i> _8911        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._punctata</i> _8914        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._punctata</i> _9042        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._punctata</i> _AB305001.1  | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._pusilla</i> _AB304996.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAAT-C | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._pusilla</i> _AB304997.1   | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._quadricolor</i> _7062     | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._reflexa</i> _AB439275.1   | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._reflexa</i> _AB439276.1   | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._reflexa</i> _AB439282.1   | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._schlechteri</i> _6865     | GGCTTCCTTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._sp.</i> _6933             | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._splendida</i> _AB305002.1 | GGCTTCCATC | GGAGTGGGTT | CCTTGCAATTC | GGCGCTAGAG | CCGGGGGTCC |     |
| <i>L._unifolia</i> _6863        | GGCTTCCTTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._unifolia</i> _6869        | GGCTTCCTTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._unifolia</i> _6871        | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._unifolia</i> _6873        | GGCTTCCTTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>L._vanzyliae</i> _7063       | GGCTCACGTC | GGAGTGGGTT | CCTTGCAATTC | GGCTCTAGAG | CCGGGGGTCC |     |
| <i>L._zeyheri</i> _7099         | GGCTTCCGTC | GGAGTGGGTT | CCTTGCAATTC | GGCACTAGAG | CCGGGGGTCC |     |
| <i>M._depressa</i> _AB305007.1  | GGCTCCCGTC | AGAATGGGTT | CGTTGCATTA  | GGCAGTTAGA | CCGGGAGTCC |     |
| <i>M._pustulata</i> _AB305008.1 | GGCTCCCGTC | AGAATGGGTT | CGTTGCATTA  | GGCACTTAGA | CCGGGAGTCC |     |
| <i>L._aloides</i> _7059         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC | 100 |
| <i>L._bifolia</i> _7064         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _7322         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8893         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8898         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8902         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8903         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8904         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8905         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8906         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _8907         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |
| <i>L._bifolia</i> _9115         | GCGACGTTTT | -GCGCCGTGC | GTTTCCGTCC  | AACACTCGCC | GCGTCCAACC |     |

*L. bifolia*\_9116 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. bifolia*\_AB304980.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. carnosa*\_AB439269.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. carnosa*\_AB439270.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. carnosa*\_AB439271.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. flava*\_7060 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. hirta*\_AB304982.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. hirta*\_AB439272.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. hirta*\_AB439273.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. hirta*\_AB439274.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. pallida*\_AB304998.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB304999.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB439277.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB439278.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB439279.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB439280.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pallida*\_AB439281.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. punctata*\_8909 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. punctata*\_8910 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. punctata*\_8911 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. punctata*\_8914 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. punctata*\_9042 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. punctata*\_AB305001.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. pusilla*\_AB304996.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. pusilla*\_AB304997.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. quadricolor*\_7062 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. reflexa*\_AB439275.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. reflexa*\_AB439276.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. reflexa*\_AB439282.1 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. schlechteri*\_6865 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. sp.*\_6933 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. splendida*\_AB305002.1 GCGGCGTTTT TGCGCCGTGC GTTTCCTGCC AACACACGCC GCGTCCAACC  
*L. unifolia*\_6863 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. unifolia*\_6869 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. unifolia*\_6871 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. unifolia*\_6873 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. vanzyliae*\_7063 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*L. zeyheri*\_7099 GCGACGTTTT -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCAACC  
*M. depressa*\_AB305007.1 GCGGCGTTTCG -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCGACC  
*M. pustulata*\_AB305008.1 GCGGCGTTTCG -GCGCCGTGC GTTTCCTGCC AACACTCGCC GCGTCCGACC

*L. aloides*\_7059 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC 150  
*L. bifolia*\_7064 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_7322 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8893 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8898 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8902 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8903 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8904 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8905 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8906 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_8907 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_9115 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_9116 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. bifolia*\_AB304980.1 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. carnosa*\_AB439269.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. carnosa*\_AB439270.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. carnosa*\_AB439271.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. flava*\_7060 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. hirta*\_AB304982.1 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. hirta*\_AB439272.1 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. hirta*\_AB439273.1 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. hirta*\_AB439274.1 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB304998.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB304999.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB439277.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB439278.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB439279.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC



*L. pallida*\_AB439280.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. pallida*\_AB439281.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_8909 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_8910 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_8911 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_8914 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_9042 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. punctata*\_AB305001.1 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. pusilla*\_AB304996.1 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGTC  
*L. pusilla*\_AB304997.1 CGGCCGGCGG CCCGCACTTT GACCCGCCGC GCCGCGAGGC ACGGGGGGTC  
*L. quadricolor*\_7062 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. reflexa*\_AB439275.1 CGGCCGGCGG GCCGCACTTC GACCCACCAC GCCGCGAGGC ACGGGGGGCC  
*L. reflexa*\_AB439276.1 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. reflexa*\_AB439282.1 CGGCCGGCGG CCCGCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. schlechteri*\_6865 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. sp.*\_6933 CGGCCGGCGG CCCCACTTC GACCCACCGC GCCGCGAGGC ACGGGGGGCC  
*L. splendida*\_AB305002.1 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. unifolia*\_6863 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. unifolia*\_6869 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. unifolia*\_6871 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. unifolia*\_6873 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. vanzyliae*\_7063 CGGCCGGCGG CCCGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*L. zeyheri*\_7099 CGGCCGGCGG CCAGCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*M. depressa*\_AB305007.1 CGGCCGGCGG CCCCACTTC GACCCGCCGC GCCGCGAGGC ACGGGGGGCC  
*M. pustulata*\_AB305008.1 CGGTCCGGCG CCCCACTTC GACCCGCCGC GCCGCGAGGC ACGGTGGCC

*L. aloides*\_7059 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT 200  
*L. bifolia*\_7064 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_7322 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. bifolia*\_8893 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. bifolia*\_8898 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. bifolia*\_8902 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_8903 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_8904 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_8905 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_8906 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. bifolia*\_8907 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_9115 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. bifolia*\_9116 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. bifolia*\_AB304980.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTAGG CGG-GGACGT  
*L. carnosa*\_AB439269.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. carnosa*\_AB439270.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. carnosa*\_AB439271.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. flava*\_7060 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. hirta*\_AB304982.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCGTTGTG CGG-GGACGT  
*L. hirta*\_AB439272.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCGTTGTG CGG-GGACGT  
*L. hirta*\_AB439273.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCGTTGTG CGG-GGACGT  
*L. hirta*\_AB439274.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCGTTGTG CGG-GGACGT  
*L. pallida*\_AB304998.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB304999.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB439277.1 AATATCCGCG TCCGTGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB439278.1 AATATCCGCA TCCGTGCCCC CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB439279.1 AATATCCGCG TCCGTGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB439280.1 AATATCCGCG TCCGTGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pallida*\_AB439281.1 AATATCCGCG TCCGTGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_8909 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_8910 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_8911 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_8914 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_9042 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. punctata*\_AB305001.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. pusilla*\_AB304996.1 AATATCCGCG TCCGCGTCCG CC---GCCAC -CGCACTGGG CGG-GGACGT  
*L. pusilla*\_AB304997.1 AATATCCGCG TCCGCGTCCG CT---GCCAC CCGCACTGGG CGG-GGACGT  
*L. quadricolor*\_7062 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. reflexa*\_AB439275.1 AATATCCGCG TTCGCGTCTG CCGCCGCCAC CCGCACTGGG CGG-GGACGT  
*L. reflexa*\_AB439276.1 AATATCCGCG TCCGCGTCCG CCGCCACCAC CCGCACTGGG CGG-GGACGT  
*L. reflexa*\_AB439282.1 AATATCCGCG TCCGCGTCCG CCGCCACCAC CCGCACTGGG CGG-GGACGT  
*L. schlechteri*\_6865 AATCTCCGCG TCCGCGYCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT

```

L._sp._6933 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._splendida_AB305002.1 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._unifolia_6863 AATATCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._unifolia_6869 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._unifolia_6871 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._unifolia_6873 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._vanzyliae_7063 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
L._zeyheri_7099 AATCTCCGCG TCCGCGTCCG CCGCCGCCAC CCGCACTGGG CGG-GGACGT
M._depressa_AB305007.1 AATCTCCGCG TCCGCGTCCG CAT-AGCCAC CCACACTAGG CGGAGGACAT
M._pustulata_AB305008.1 AATCTCCGCG TCCGCGTCCG CAT-AGCCAC CCACACTAGG CGGAGGACAT

L._aloides_7059 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN 250
L._bifolia_7064 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_7322 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8893 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8898 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8902 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8903 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8904 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8905 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8906 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_8907 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_9115 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_9116 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._bifolia_AB304980.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._carnosa_AB439269.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._carnosa_AB439270.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._carnosa_AB439271.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._flava_7060 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
L._hirta_AB304982.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._hirta_AB439272.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._hirta_AB439273.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._hirta_AB439274.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB304998.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB304999.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB439277.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB439278.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB439279.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB439280.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pallida_AB439281.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_8909 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_8910 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_8911 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_8914 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_9042 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._punctata_AB305001.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._pusilla_AB304996.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGG---- -GTGCCCTCG
L._pusilla_AB304997.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._quadricolor_7062 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
L._reflexa_AB439275.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._reflexa_AB439276.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._reflexa_AB439282.1 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._schlechteri_6865 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._sp._6933 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
L._splendida_AB305002.1 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._unifolia_6863 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._unifolia_6869 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._unifolia_6871 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
L._unifolia_6873 GGGGTACGCG GAGCGACGCG ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
L._vanzyliae_7063 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
L._zeyheri_7099 GGGGTACGCG AAGCGACGCG ATGCGTGACG CCCAGGCAGG CGNNNNNNNN
M._depressa_AB305007.1 AGGGTACGCG AAGCGACGCA ATGCGTGACG CCCAGGCAGG CGTGCCCTCG
M._pustulata_AB305008.1 AGGGTACGCG AAGCGACACA ATGCGTGACG CCCAGGCAGG CGTGCCCTCG

L._aloides_7059 NNNNNN 256
L._bifolia_7064 GCCGGA
L._bifolia_7322 GCCGGA
L._bifolia_8893 GCCGGA

```

*L.\_bifolia\_8898* GCCGGA  
*L.\_bifolia\_8902* GCCGGA  
*L.\_bifolia\_8903* GCCGGA  
*L.\_bifolia\_8904* GCCGGA  
*L.\_bifolia\_8905* GCCGGA  
*L.\_bifolia\_8906* GCCGGA  
*L.\_bifolia\_8907* GCCGGA  
*L.\_bifolia\_9115* GCCGGA  
*L.\_bifolia\_9116* GCCGGA  
*L.\_bifolia\_AB304980.1* GCCGGA  
*L.\_carnosa\_AB439269.1* GCCGGA  
*L.\_carnosa\_AB439270.1* GCCGGA  
*L.\_carnosa\_AB439271.1* GCCGGA  
*L.\_flava\_7060* NNNNNN  
*L.\_hirta\_AB304982.1* GCCGGA  
*L.\_hirta\_AB439272.1* GCCGGA  
*L.\_hirta\_AB439273.1* GCCGGA  
*L.\_hirta\_AB439274.1* GCCGGA  
*L.\_pallida\_AB304998.1* GCCGGA  
*L.\_pallida\_AB304999.1* GCCGGA  
*L.\_pallida\_AB439277.1* GCCGGA  
*L.\_pallida\_AB439278.1* GCCGGA  
*L.\_pallida\_AB439279.1* GCCGGA  
*L.\_pallida\_AB439280.1* GCCGGA  
*L.\_pallida\_AB439281.1* GCCGGA  
*L.\_punctata\_8909* GCCGGA  
*L.\_punctata\_8910* GCCGGA  
*L.\_punctata\_8911* GCCGGA  
*L.\_punctata\_8914* GCCGGA  
*L.\_punctata\_9042* GCCGGA  
*L.\_punctata\_AB305001.1* GCCGGA  
*L.\_pusilla\_AB304996.1* GCCGGA  
*L.\_pusilla\_AB304997.1* GCCGGA  
*L.\_quadricolor\_7062* NNNNNN  
*L.\_reflexa\_AB439275.1* GCCGGA  
*L.\_reflexa\_AB439276.1* GCCGGA  
*L.\_reflexa\_AB439282.1* GCCGGA  
*L.\_schlechteri\_6865* GCCGGA  
*L.\_sp.\_6933* NNNNNN  
*L.\_splendida\_AB305002.1* GCCGGA  
*L.\_unifolia\_6863* GCCGGA  
*L.\_unifolia\_6869* GCCGGA  
*L.\_unifolia\_6871* NNNNNN  
*L.\_unifolia\_6873* GCCGGA  
*L.\_vanzyliae\_7063* NNNNNN  
*L.\_zeyheri\_7099* NNNNNN  
*M.\_depressa\_AB305007.1* GCCGGA  
*M.\_pustulata\_AB305008.1* GCCGGA

Appendix F3 – *Lachenalia matK*

|                                             |      |            |            |            |            |            |    |
|---------------------------------------------|------|------------|------------|------------|------------|------------|----|
| <i>L._alba</i>                              | 9024 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA | 50 |
| <i>L._alba</i>                              | 9100 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._algoensis</i>                         | 8210 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._aloides</i>                           | 7361 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._ameliae</i>                           | 8215 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._angelica</i>                          | 9109 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._anguinea</i>                          | 9103 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._attenuata</i>                         | 8218 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._aurioliae</i>                         | 8308 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._barkeriana</i>                        | 7715 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 7064 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8893 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8895 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8896 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8898 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8899 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8900 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8901 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8902 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8903 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8904 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8905 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8906 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bifolia</i>                           | 8907 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bolusii</i>                           | 7987 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._bowkeri</i>                           | 9111 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._capensis</i>                          | 8219 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._cernua</i>                            | 8222 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._comptonii</i>                         | 7067 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._concordiana</i>                       | 8310 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._contaminata</i>                       | 7727 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._convallarioides</i>                   | 8228 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._corymbosa</i>                         | 8229 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._dasybotrya</i>                        | 7730 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._dehoopensis</i>                       | 7731 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._doleritica</i>                        | 8230 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._duncanii</i>                          | 9107 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._elegans</i>                           | 7734 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._ensifolia</i>                         | 9101 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 7876 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 9102 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._fistulosa</i>                         | 7357 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._fistulosa</i>                         | 7362 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._flava</i>                             | 7060 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._framesii</i>                          | 8241 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._glauca</i>                            | 8319 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._haarlemensis</i>                      | 9110 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._hirta</i>                             | 6858 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._hirta</i>                             | 6891 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._inconspicua</i>                       | 9095 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._isopetala</i>                         | 8244 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._juncifolia</i>                        | 8245 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._juncifolia</i>                        | 8246 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._karooica</i>                          | 7754 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._karoopoortensis</i>                   | 9108 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._klinghardtiana</i>                    | 7756 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._kliprandensis</i>                     | 8247 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._lactosa</i>                           | 8248 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._latimeriae</i>                        | 7761 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._longibracteata</i>                    | 7768 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._longituba</i>                         | 9105 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._magentea</i>                          | 8313 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._magregoriorum</i>                     | 7772 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._margaretiae</i>                       | 7773 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._marginata</i>                         | 9096 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._marlothii</i>                         | 7776 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._martinae</i>                          | 8254 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._mathewsii</i>                         | 6938 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._maximiliani</i>                       | 8257 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._sp.1</i>                              | 7080 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._sp.1</i>                              | 7082 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._mediana</i> subsp.                    | 9030 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._mediana</i> subsp.                    | 9031 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |
| <i>L._sp.1</i>                              | 9032 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA |    |

|                                                 |            |            |            |            |              |
|-------------------------------------------------|------------|------------|------------|------------|--------------|
| <i>L. mediana</i> subsp. 9033                   | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. mediana</i> subsp. rogersii 7079          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. membranacea</i> 9106                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. moniliformis</i> 7884                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. multifolia</i> 8263                       | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. mutabilis</i> 8264                        | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. namaquensis</i> 7332                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. namibiensis</i> 8266                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. namibiensis</i> 9112                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. nardousbergensis</i> 8267                 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. neilii</i> 7795                           | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. nervosa</i> 9091                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. obscura</i> 9035                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. obscura</i> 9093                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. obscura</i> 9094                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. orchiodoides</i> subsp. orchiodoides 7805 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. orthopetala</i> 7335                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. orthopetala</i> 7358                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. pallida</i> 8284                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. pallida</i> 8312                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. patula</i> 7363                           | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. paucifolia</i> 8277                       | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. peersii</i> 7355                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. peersii</i> 8278                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. perryae</i> 6961                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. polypodantha</i> 8280                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. punctata</i> 7093                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CATGAATATC | ATAATTCGAA   |
| <i>L. punctata</i> 7344                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CATGAATATC | ATAATTCGAA   |
| <i>L. punctata</i> 8909                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CATGAATATC | ATAATTCGAA   |
| <i>L. punctata</i> 8911                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CATGAATATC | ATAATTCGAA   |
| <i>L. punctata</i> 8914                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. purpureo-caerulea</i> 6939                | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. purpureo-caerulea</i> 7074                | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. purpureo-caerulea</i> 7354                | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. purpureo-caerulea</i> 7365                | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. pusilla</i> 9092                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. pusilla</i> 9098                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. quadricolor</i> 7359                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. rosea</i> 7343                            | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. rosea</i> 8286                            | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. salteri</i> 8289                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. schelpei</i> 9089                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. sessiliflora</i> 8314                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. sp.2</i> 8131                             | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. sp.</i> 9090                              | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. stayneri</i> 8316                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. suaveolens</i> 7884                       | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. thomasiae</i> 8292                        | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. trichophylla</i> 8293                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. trichophylla</i> 9097                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. undulata</i> 8317                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6862                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6863                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. schlechteri</i> 6865                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. schlechteri</i> 6866                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6867                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6869                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6870                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6871                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6873                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6874                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. unifolia</i> 6878                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. schlechteri</i> 6880                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. valeriae</i> 9104                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. variegata</i> 7097                        | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. verticillata</i> 7857                     | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. violacea</i> 7101                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. violacea</i> 7102                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. viridiflora</i> 7098                      | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. wrightii</i> 8318                         | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. youngii</i> 7865                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. zebrina</i> 7867                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. zebrina</i> 8305                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. zeyheri</i> 7099                          | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>Massonia</i>                                 | CCCTTTTTCG | ATTCATTGCG | ATTTTTCTCT | CACGAATATC | ATAATTCGAA   |
| <i>L. alba</i> 9024                             | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA100 |

|                                             |      |            |             |            |            |            |
|---------------------------------------------|------|------------|-------------|------------|------------|------------|
| <i>L. alba</i>                              | 9100 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. algoensis</i>                         | 8210 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. aloides</i>                           | 7361 | TAGCCCTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. ameliae</i>                           | 8215 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. angelica</i>                          | 9109 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. anguinea</i>                          | 9103 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. attenuata</i>                         | 8218 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. aurioliae</i>                         | 8308 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. barkeriana</i>                        | 7715 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 7064 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8893 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8895 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8896 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8898 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8899 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8900 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8901 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8902 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8903 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8904 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8905 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8906 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bifolia</i>                           | 8907 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. bolusii</i>                           | 7987 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. bowkeri</i>                           | 9111 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. capensis</i>                          | 8219 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. cernua</i>                            | 8222 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. comptonii</i>                         | 7067 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. concordiana</i>                       | 8310 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. contaminata</i>                       | 7727 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. convallarioides</i>                   | 8228 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | TCAATTTCAA |
| <i>L. corymbosa</i>                         | 8229 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. dasybotrya</i>                        | 7730 | TAGCCCTCTA | GTTATTCTAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. dehoopensis</i>                       | 7731 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. doleritica</i>                        | 8230 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. duncanii</i>                          | 9107 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. elegans</i>                           | 7734 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. ensifolia</i>                         | 9101 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. fistulosa</i>                         | 7357 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. fistulosa</i>                         | 7362 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. flava</i>                             | 7060 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. framesii</i>                          | 8241 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. glauca</i>                            | 8319 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. haarlemensis</i>                      | 9110 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. hirta</i>                             | 6858 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. hirta</i>                             | 6891 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. inconspicua</i>                       | 9095 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. isopetala</i>                         | 8244 | TAGCCTTTCT | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | TC-----AA  |
| <i>L. juncifolia</i>                        | 8245 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. juncifolia</i>                        | 8246 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | TC-----AA  |
| <i>L. karooica</i>                          | 7754 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. karoopoortensis</i>                   | 9108 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. klinghardtiana</i>                    | 7756 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. kliprandensis</i>                     | 8247 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. lactosa</i>                           | 8248 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. latimeriae</i>                        | 7761 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. longibracteata</i>                    | 7768 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. longituba</i>                         | 9105 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. magentea</i>                          | 8313 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. magregoriorum</i>                     | 7772 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. margaretiae</i>                       | 7773 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. marginata</i>                         | 9096 | TAGCCTTCTA | GTTACTCAGA  | AAAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. marlothii</i>                         | 7776 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. martinae</i>                          | 8254 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. mathewsii</i>                         | 6938 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. maximiliani</i>                       | 8257 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. sp.1</i>                              | 7080 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. sp.1</i>                              | 7082 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. mediana</i> subsp.                    | 9030 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. mediana</i> subsp.                    | 9031 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. sp.1</i>                              | 9032 | TAGCCCTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. mediana</i> subsp.                    | 9033 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |
| <i>L. membranacea</i>                       | 9106 | TAGCCTTCTA | GTTACTCAGA  | AGAAATCTAT | TTCTGTTTTT | T-----CAA  |
| <i>L. moniliformis</i>                      | 7884 | TAGCCTTCTC | GTTACTCAGA  | AGAAATCTAT | TTATGTTTTT | T-----CAA  |

|                     |                             |      |            |            |            |            |               |
|---------------------|-----------------------------|------|------------|------------|------------|------------|---------------|
|                     | <i>L. multifolia</i>        | 8263 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. mutabilis</i>         | 8264 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. namaquensis</i>       | 7332 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. namibiensis</i>       | 8266 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. namibiensis</i>       | 9112 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. nardousbergensis</i>  | 8267 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. neilii</i>            | 7795 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. nervosa</i>           | 9091 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. obscura</i>           | 9035 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. obscura</i>           | 9093 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. obscura</i>           | 9094 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
| <i>L. orchiodes</i> | subsp. <i>orchiodes</i>     | 7805 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. orthopetala</i>       | 7335 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. orthopetala</i>       | 7358 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. pallida</i>           | 8284 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. pallida</i>           | 8312 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. patula</i>            | 7363 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. paucifolia</i>        | 8277 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. peersii</i>           | 7355 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. peersii</i>           | 8278 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. perryae</i>           | 6961 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. polypodantha</i>      | 8280 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. punctata</i>          | 7093 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. punctata</i>          | 7344 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. punctata</i>          | 8909 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. punctata</i>          | 8911 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. punctata</i>          | 8914 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. purpureo-caerulea</i> | 6939 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. purpureo-caerulea</i> | 7074 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. purpureo-caerulea</i> | 7354 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. purpureo-caerulea</i> | 7365 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. pusilla</i>           | 9092 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. pusilla</i>           | 9098 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. quadricolor</i>       | 7359 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. rosea</i>             | 7343 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. rosea</i>             | 8286 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. salteri</i>           | 8289 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. schelpei</i>          | 9089 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. sessiliflora</i>      | 8314 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. sp.2</i>              | 8131 | TAGCCCTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. sp</i>                | 9090 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. stayneri</i>          | 8316 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. suaveolens</i>        | 7884 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. thomasiae</i>         | 8292 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. trichophylla</i>      | 8293 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. trichophylla</i>      | 9097 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. undulata</i>          | 8317 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6862 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6863 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. schlechteri</i>       | 6865 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. schlechteri</i>       | 6866 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6867 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6869 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6870 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6871 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6873 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6874 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. unifolia</i>          | 6878 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. schlechteri</i>       | 6880 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. valeriae</i>          | 9104 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. variegata</i>         | 7097 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. verticillata</i>      | 7857 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. violacea</i>          | 7101 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. violacea</i>          | 7102 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. viridiflora</i>       | 7098 | TAGCCTTCTA | GTTACTCAGA | AAAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. wrightii</i>          | 8318 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. youngii</i>           | 7865 | TAGCCTTCTA | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. zebrina</i>           | 7867 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. zebrina</i>           | 8305 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTCTGTTTTT | T-----CAA     |
|                     | <i>L. zeyheri</i>           | 7099 | TAGCCTTCTC | GTTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>Massonia</i>             |      | TAGCCTTCTC | ATTACTCAGA | AGAAATCTAT | TTATGTTTTT | T-----CAA     |
|                     | <i>L. alba</i>              | 9024 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA150 |
|                     | <i>L. alba</i>              | 9100 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
|                     | <i>L. algoensis</i>         | 8210 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
|                     | <i>L. aloides</i>           | 7361 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
|                     | <i>L. ameliae</i>           | 8215 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L. angelica</i>                          | 9109 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. anguinea</i>                          | 9103 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. attenuata</i>                         | 8218 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. aurioliae</i>                         | 8308 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. barkeriana</i>                        | 7715 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 7064 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8893 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8895 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8896 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8898 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8899 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8900 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8901 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8902 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8903 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8904 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8905 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8906 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bifolia</i>                           | 8907 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bolusii</i>                           | 7987 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. bowkeri</i>                           | 9111 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. capensis</i>                          | 8219 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. cernua</i>                            | 8222 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. comptonii</i>                         | 7067 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. concordiana</i>                       | 8310 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. contaminata</i>                       | 7727 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. convallarioides</i>                   | 8228 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. corymbosa</i>                         | 8229 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. dasybotrya</i>                        | 7730 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. dehoopensis</i>                       | 7731 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. doleritica</i>                        | 8230 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. duncanii</i>                          | 9107 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. elegans</i>                           | 7734 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. ensifolia</i>                         | 9101 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. fistulosa</i>                         | 7357 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. fistulosa</i>                         | 7362 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. flava</i>                             | 7060 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. framesii</i>                          | 8241 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. glauca</i>                            | 8319 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. haarlemensis</i>                      | 9110 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. hirta</i>                             | 6858 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. hirta</i>                             | 6891 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. inconspicua</i>                       | 9095 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. isopetala</i>                         | 8244 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. juncifolia</i>                        | 8245 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. juncifolia</i>                        | 8246 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. karooica</i>                          | 7754 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. karoopoortensis</i>                   | 9108 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. klinghardtiana</i>                    | 7756 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. kliprandensis</i>                     | 8247 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. lactosa</i>                           | 8248 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. latimeriae</i>                        | 7761 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. longibracteata</i>                    | 7768 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. longituba</i>                         | 9105 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. magentea</i>                          | 8313 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. magregoriorum</i>                     | 7772 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. margaretae</i>                        | 7773 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. marginata</i>                         | 9096 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. marlothii</i>                         | 7776 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. martinae</i>                          | 8254 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. mathewsii</i>                         | 6938 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. maximiliani</i>                       | 8257 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. sp.1</i>                              | 7080 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. sp.1</i>                              | 7082 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. mediana</i> subsp.                    | 9030 | AAGAAAATAA | AAGAGTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. mediana</i> subsp.                    | 9031 | AAGAAAATAA | AAGAGTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. sp.1</i>                              | 9032 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. mediana</i> subsp.                    | 9033 | AAGAAAATAA | AAGAGTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | AAGAAAATAA | AAGAGTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. membranacea</i>                       | 9106 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. moniliformis</i>                      | 7884 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA |
| <i>L. multifolia</i>                        | 8263 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. mutabilis</i>                         | 8264 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. namaquensis</i>                       | 7332 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |
| <i>L. namibiensis</i>                       | 8266 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA |



|                                               |      |            |            |            |            |               |
|-----------------------------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. namibiensis</i>                         | 9112 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. nardousbergensis</i>                    | 8267 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. neilii</i>                              | 7795 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. nervosa</i>                             | 9091 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. obscura</i>                             | 9035 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. obscura</i>                             | 9093 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. obscura</i>                             | 9094 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. orchioides</i> subsp. <i>orchioides</i> | 7805 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. orthopetala</i>                         | 7335 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. orthopetala</i>                         | 7358 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. pallida</i>                             | 8284 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. pallida</i>                             | 8312 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. patula</i>                              | 7363 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. paucifolia</i>                          | 8277 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. peersii</i>                             | 7355 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. peersii</i>                             | 8278 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. perryae</i>                             | 6961 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. polypodantha</i>                        | 8280 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. punctata</i>                            | 7093 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. punctata</i>                            | 7344 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. punctata</i>                            | 8909 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. punctata</i>                            | 8911 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. punctata</i>                            | 8914 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. purpureo-caerulea</i>                   | 6939 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. purpureo-caerulea</i>                   | 7074 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. purpureo-caerulea</i>                   | 7354 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. purpureo-caerulea</i>                   | 7365 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. pusilla</i>                             | 9092 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. pusilla</i>                             | 9098 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. quadricolor</i>                         | 7359 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. rosea</i>                               | 7343 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. rosea</i>                               | 8286 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. salteri</i>                             | 8289 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. schelpei</i>                            | 9089 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. sessiliflora</i>                        | 8314 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. sp.2</i>                                | 8131 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. sp.</i>                                 | 9090 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. stayneri</i>                            | 8316 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. suaveolens</i>                          | 7884 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. thomasiae</i>                           | 8292 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. trichophylla</i>                        | 8293 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. trichophylla</i>                        | 9097 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. undulata</i>                            | 8317 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6862 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6863 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. schlechteri</i>                         | 6865 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. schlechteri</i>                         | 6866 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6867 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6869 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6870 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6871 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6873 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6874 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. unifolia</i>                            | 6878 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. schlechteri</i>                         | 6880 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. valeriae</i>                            | 9104 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. variegata</i>                           | 7097 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. verticillata</i>                        | 7857 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. violacea</i>                            | 7101 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. violacea</i>                            | 7102 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. viridiflora</i>                         | 7098 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. wrightii</i>                            | 8318 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>L. youngii</i>                             | 7865 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. zebrina</i>                             | 7867 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. zebrina</i>                             | 8305 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. zeyheri</i>                             | 7099 | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTTTAA | TGTATTTGAA    |
| <i>Massonia</i>                               |      | AAGAAAATAA | AAGATTATTT | TGGTTTCTAT | ACAATTCTTA | TGTATTTGAA    |
| <i>L. alba</i>                                | 9024 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT200 |
| <i>L. alba</i>                                | 9100 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. algoensis</i>                           | 8210 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. aloides</i>                             | 7361 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. ameliae</i>                             | 8215 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. angelica</i>                            | 9109 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. anguinea</i>                            | 9103 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. attenuata</i>                           | 8218 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L. aurioliae</i>                           | 8308 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L._barkeriana</i>                        | 7715 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 7064 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8893 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8895 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8896 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8898 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8899 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8900 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8901 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8902 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8903 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8904 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8905 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8906 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bifolia</i>                           | 8907 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bolusii</i>                           | 7987 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._bowkeri</i>                           | 9111 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._capensis</i>                          | 8219 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._cernua</i>                            | 8222 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._comptonii</i>                         | 7067 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._concordiana</i>                       | 8310 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._contaminata</i>                       | 7727 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._convallarioides</i>                   | 8228 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._corymbosa</i>                         | 8229 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._dasybotrya</i>                        | 7730 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._dehoopensis</i>                       | 7731 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._doleritica</i>                        | 8230 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._duncanii</i>                          | 9107 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._elegans</i>                           | 7734 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._ensifolia</i>                         | 9101 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 7876 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 9102 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._fistulosa</i>                         | 7357 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._fistulosa</i>                         | 7362 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._flava</i>                             | 7060 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._framesii</i>                          | 8241 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._glaucia</i>                           | 8319 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._haarlemensis</i>                      | 9110 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._hirta</i>                             | 6858 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._hirta</i>                             | 6891 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._inconspicua</i>                       | 9095 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._isopetala</i>                         | 8244 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._juncifolia</i>                        | 8245 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT |
| <i>L._juncifolia</i>                        | 8246 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT |
| <i>L._karooica</i>                          | 7754 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._karoopoortensis</i>                   | 9108 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._klinghardtiana</i>                    | 7756 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._kliprandensis</i>                     | 8247 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._lactosa</i>                           | 8248 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._latimeriae</i>                        | 7761 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._longibracteata</i>                    | 7768 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._longituba</i>                         | 9105 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._magentea</i>                          | 8313 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT |
| <i>L._magregoriorum</i>                     | 7772 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._margaretiae</i>                       | 7773 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._marginata</i>                         | 9096 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._marlothii</i>                         | 7776 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._martinae</i>                          | 8254 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._mathewsii</i>                         | 6938 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._maximiliani</i>                       | 8257 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._sp.1</i>                              | 7080 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._sp.1</i>                              | 7082 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT |
| <i>L._mediana</i> subsp. <i>9030</i>        |      | TTTGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._mediana</i> subsp. <i>9031</i>        |      | TTTGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._sp.1</i>                              | 9032 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._mediana</i> subsp. <i>9033</i>        |      | TTTGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._mediana</i> subsp. <i>rogersii</i>    | 7079 | TTTGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._membranacea</i>                       | 9106 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._moniliformis</i>                      | 7884 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT |
| <i>L._multifolia</i>                        | 8263 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._mutabilis</i>                         | 8264 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._namaquensis</i>                       | 7332 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._namibiensis</i>                       | 8266 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._namibiensis</i>                       | 9112 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._nardousbergensis</i>                  | 8267 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._neilii</i>                            | 7795 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |
| <i>L._nervosa</i>                           | 9091 | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT |

|                                               |            |            |            |            |               |
|-----------------------------------------------|------------|------------|------------|------------|---------------|
| <i>L._obscura_9035</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._obscura_9093</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._obscura_9094</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._orchiodides_subsp._orchiodides_7805</i> | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._orthopetala_7335</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._orthopetala_7358</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._pallida_8284</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._pallida_8312</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._patula_7363</i>                         | TATGAATTTT | TCTTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._paucifolia_8277</i>                     | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._peersii_7355</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._peersii_8278</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._perryae_6961</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._polypodantha_8280</i>                   | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._punctata_7093</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._punctata_7344</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._punctata_8909</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._punctata_8911</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._punctata_8914</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._purpureo-caerulea_6939</i>              | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._purpureo-caerulea_7074</i>              | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._purpureo-caerulea_7354</i>              | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._purpureo-caerulea_7365</i>              | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._pusilla_9092</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._pusilla_9098</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._quadricolor_7359</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._rosea_7343</i>                          | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._rosea_8286</i>                          | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._salteri_8289</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._schelpei_9089</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._sessiliflora_8314</i>                   | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._sp.2_8131</i>                           | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._sp_9090</i>                             | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._stayneri_8316</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._suaveolens_7884</i>                     | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._thomasiae_8292</i>                      | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._trichophylla_8293</i>                   | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._trichophylla_9097</i>                   | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._undulata_8317</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._unifolia_6862</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6863</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._schlechteri_6865</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._schlechteri_6866</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._unifolia_6867</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6869</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6870</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._unifolia_6871</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6873</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6874</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._unifolia_6878</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._schlechteri_6880</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._valeriae_9104</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._variegata_7097</i>                      | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._verticillata_7857</i>                   | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._violacea_7101</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._violacea_7102</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._viridiflora_7098</i>                    | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._wrightii_8318</i>                       | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>L._youngii_7865</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._zebrina_7867</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._zebrina_8305</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._zeyheri_7099</i>                        | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGAAT    |
| <i>Massonia</i>                               | TATGAATTTT | TATTAGTGTT | TATTCGTAAA | CAATCTTCTT | ATTTACGATT    |
| <i>L._alba_9024</i>                           | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAAAA250 |
| <i>L._alba_9100</i>                           | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L._algoensis_8210</i>                      | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._aloides_7361</i>                        | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._ameliae_8215</i>                        | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACATTTTTC | TATGGAAA---   |
| <i>L._angelica_9109</i>                       | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACATTTTTC | TATGGAAAAA    |
| <i>L._anguinea_9103</i>                       | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._attenuata_8218</i>                      | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L._aurioliae_8308</i>                      | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L._barkeriana_7715</i>                     | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._bifolia_7064</i>                        | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._bifolia_8893</i>                        | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |
| <i>L._bifolia_8895</i>                        | AACA---TCT | TCTGGAACCT | TTCTTGAACG | AACACTTTTC | TATGGAAA---   |

|                                             |      |            |          |            |            |            |
|---------------------------------------------|------|------------|----------|------------|------------|------------|
| <i>L. bifolia</i>                           | 8896 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8898 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8899 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8900 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8901 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8902 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8903 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8904 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8905 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8906 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bifolia</i>                           | 8907 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. bolusii</i>                           | 7987 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. bowkeri</i>                           | 9111 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. capensis</i>                          | 8219 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. cernua</i>                            | 8222 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. comptonii</i>                         | 7067 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. concordiana</i>                       | 8310 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. contaminata</i>                       | 7727 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. convallarioides</i>                   | 8228 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AATACTTTTC | TATGGAAAAA |
| <i>L. corymbosa</i>                         | 8229 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. dasybotrya</i>                        | 7730 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. dehoopensis</i>                       | 7731 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. doleritica</i>                        | 8230 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. duncanii</i>                          | 9107 | AACAACATCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. elegans</i>                           | 7734 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. ensifolia</i>                         | 9101 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. fistulosa</i>                         | 7357 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. fistulosa</i>                         | 7362 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. flava</i>                             | 7060 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. framesii</i>                          | 8241 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. glauca</i>                            | 8319 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. haarlemensis</i>                      | 9110 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. hirta</i>                             | 6858 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. hirta</i>                             | 6891 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. inconspicua</i>                       | 9095 | AACAACATCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. isopetala</i>                         | 8244 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. juncifolia</i>                        | 8245 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. juncifolia</i>                        | 8246 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. karooica</i>                          | 7754 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. karoopoortensis</i>                   | 9108 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. klinghardtiana</i>                    | 7756 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. kliprandensis</i>                     | 8247 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. lactosa</i>                           | 8248 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. latimeriae</i>                        | 7761 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. longibracteata</i>                    | 7768 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. longituba</i>                         | 9105 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. magentea</i>                          | 8313 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. magregoriorum</i>                     | 7772 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. margaretiae</i>                       | 7773 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. marginata</i>                         | 9096 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. marlothii</i>                         | 7776 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. martinae</i>                          | 8254 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. mathewsii</i>                         | 6938 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. maximiliani</i>                       | 8257 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. sp.1</i>                              | 7080 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. sp.1</i>                              | 7082 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. mediana</i> subsp.                    | 9030 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. mediana</i> subsp.                    | 9031 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. sp.1</i>                              | 9032 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. mediana</i> subsp.                    | 9033 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. membranacea</i>                       | 9106 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. moniliformis</i>                      | 7884 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. multifolia</i>                        | 8263 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. mutabilis</i>                         | 8264 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. namaquensis</i>                       | 7332 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. namibiensis</i>                       | 8266 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. namibiensis</i>                       | 9112 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. nardousbergensis</i>                  | 8267 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. neilii</i>                            | 7795 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. nervosa</i>                           | 9091 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |
| <i>L. obscura</i>                           | 9035 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. obscura</i>                           | 9093 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. obscura</i>                           | 9094 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAAAAA |
| <i>L. orchiodes</i> subsp. <i>orchiodes</i> | 7805 | AACA---TCT | TCTGGAAC | TTCTTGAACG | AACACTTTTC | TATGGAA--- |

|                                   |            |             |             |            |               |
|-----------------------------------|------------|-------------|-------------|------------|---------------|
| <i>L. orthopetala</i> _7335       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. orthopetala</i> _7358       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. pallida</i> _8284           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. pallida</i> _8312           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. patula</i> _7363            | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. paucifolia</i> _8277        | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. peersii</i> _7355           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. peersii</i> _8278           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. perryae</i> _6961           | AACATCATCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. polypodantha</i> _8280      | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACATTTTTC | TATGGAA---    |
| <i>L. punctata</i> _7093          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. punctata</i> _7344          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. punctata</i> _8909          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. punctata</i> _8911          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. punctata</i> _8914          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. purpureo-caerulea</i> _6939 | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. purpureo-caerulea</i> _7074 | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. purpureo-caerulea</i> _7354 | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. purpureo-caerulea</i> _7365 | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. pusilla</i> _9092           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. pusilla</i> _9098           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. quadricolor</i> _7359       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. rosea</i> _7343             | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. rosea</i> _8286             | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. salteri</i> _8289           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. schelpei</i> _9089          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. sessiliflora</i> _8314      | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. sp.</i> _2_8131             | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. sp.</i> _9090               | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. stayneri</i> _8316          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. suaveolens</i> _7884        | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. thomasiae</i> _8292         | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. trichophylla</i> _8293      | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACATTTTTC | TATGGAAAAA    |
| <i>L. trichophylla</i> _9097      | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACATTTTTC | TATGGAAAAA    |
| <i>L. undulata</i> _8317          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. unifolia</i> _6862          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6863          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. schlechteri</i> _6865       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. schlechteri</i> _6866       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. unifolia</i> _6867          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6869          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6870          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. unifolia</i> _6871          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6873          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6874          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. unifolia</i> _6878          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. schlechteri</i> _6880       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. valeriae</i> _9104          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. variegata</i> _7097         | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. verticillata</i> _7857      | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. violacea</i> _7101          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. violacea</i> _7102          | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. viridiflora</i> _7098       | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. wrightii</i> _8318          | AATA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. youngii</i> _7865           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAA---    |
| <i>L. zebrina</i> _7867           | AATA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. zebrina</i> _8305           | AATA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. zeyheri</i> _7099           | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>Massonia</i>                   | AACA---TCT | TCTGGAACCTT | TTCTTTGAACG | AACACTTTTC | TATGGAAAAA    |
| <i>L. alba</i> _9024              | TAGAACATCT | TCAAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC300 |
| <i>L. alba</i> _9100              | TAGAACATCT | TCAAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. algoensis</i> _8210         | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. aloides</i> _7361           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. ameliae</i> _8215           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. angelica</i> _9109          | TAGAACATCT | TCAAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. anguinea</i> _9103          | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAT  | ATGTCGTAAC    |
| <i>L. attenuata</i> _8218         | TAGAACATCT | TCAAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. aurioliae</i> _8308         | TAGAACATCT | TCAAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. barkeriana</i> _7715        | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _7064           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8893           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8895           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8896           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8898           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8899           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |
| <i>L. bifolia</i> _8900           | -----      | --AAATAGAA  | CATTTTATA-  | -----GTAGT | ATGTCGTAAC    |

|                                             |      |            |            |            |            |       |            |
|---------------------------------------------|------|------------|------------|------------|------------|-------|------------|
| <i>L. bifolia</i>                           | 8901 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8902 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8903 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8904 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8905 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8906 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bifolia</i>                           | 8907 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bolusii</i>                           | 7987 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. bowkeri</i>                           | 9111 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. capensis</i>                          | 8219 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. cernua</i>                            | 8222 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. comptonii</i>                         | 7067 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. concordiana</i>                       | 8310 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. contaminata</i>                       | 7727 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. convallarioides</i>                   | 8228 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGCCGTAAC |
| <i>L. corymbosa</i>                         | 8229 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. dasybotrya</i>                        | 7730 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. dehoopensis</i>                       | 7731 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. doleritica</i>                        | 8230 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. duncanii</i>                          | 9107 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. elegans</i>                           | 7734 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. ensifolia</i>                         | 9101 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. fistulosa</i>                         | 7357 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. fistulosa</i>                         | 7362 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. flava</i>                             | 7060 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. framesii</i>                          | 8241 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. glauca</i>                            | 8319 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. haarlemensis</i>                      | 9110 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. hirta</i>                             | 6858 | TAGAA---CT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. hirta</i>                             | 6891 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. inconspicua</i>                       | 9095 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. isopetala</i>                         | 8244 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. juncifolia</i>                        | 8245 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. juncifolia</i>                        | 8246 | TAGAACATCT | TCAAATAGAA | CATTTTATAT | TTATAGTAGT |       | ATGTCGTAAC |
| <i>L. karooica</i>                          | 7754 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. karoopoortensis</i>                   | 9108 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. klinghardtiana</i>                    | 7756 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. kliprandensis</i>                     | 8247 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTTGTAAC |
| <i>L. lactosa</i>                           | 8248 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. latimeriae</i>                        | 7761 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. longibracteata</i>                    | 7768 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. longituba</i>                         | 9105 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. magentea</i>                          | 8313 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. magregorium</i>                       | 7772 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. margaretae</i>                        | 7773 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. marginata</i>                         | 9096 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. marlothii</i>                         | 7776 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. martinae</i>                          | 8254 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mathewsii</i>                         | 6938 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. maximiliani</i>                       | 8257 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. sp.1</i>                              | 7080 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. sp.1</i>                              | 7082 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mediana</i> subsp. <i>9030</i>        |      | TAGAACATCT | TCAAATAGAA | CATTTTCTA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mediana</i> subsp. <i>9031</i>        |      | TAGAACATCT | TCAAATAGAA | CATTTTCTA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. sp.1</i>                              | 9032 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mediana</i> subsp. <i>9033</i>        |      | TAGAACATCT | TCAAATAGAA | CATTTTCTA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | TAGAACATCT | TCAAATAGAA | CATTTTCTA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. membranacea</i>                       | 9106 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. moniliformis</i>                      | 7884 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. multifolia</i>                        | 8263 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. mutabilis</i>                         | 8264 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. namaquensis</i>                       | 7332 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. namibiensis</i>                       | 8266 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTTGTAAC |
| <i>L. namibiensis</i>                       | 9112 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. nardousbergensis</i>                  | 8267 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. neilii</i>                            | 7795 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. nervosa</i>                           | 9091 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. obscura</i>                           | 9035 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. obscura</i>                           | 9093 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. obscura</i>                           | 9094 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. orchiodes</i> subsp. <i>orchiodes</i> | 7805 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. orthopetala</i>                       | 7335 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. orthopetala</i>                       | 7358 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. pallida</i>                           | 8284 | -----      | --AAATAGAA | CATTTTATA- | -----      | GTAGT | ATGTCGTAAC |
| <i>L. pallida</i>                           | 8312 | TAGAACATCT | TCAAATAG-- | ----TTATA- | -----      | GTAGT | ATGTCGTAAC |

|                             |      |            |            |            |            |               |
|-----------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. patula</i>            | 7363 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. paucifolia</i>        | 8277 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATCTCGTAAC    |
| <i>L. peersii</i>           | 7355 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. peersii</i>           | 8278 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. perryae</i>           | 6961 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. polypodantha</i>      | 8280 | -----      | --AAATAGAA | CATTTTATA- | -----ATAGT | ATGTCGTAAC    |
| <i>L. punctata</i>          | 7093 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. punctata</i>          | 7344 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. punctata</i>          | 8909 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. punctata</i>          | 8911 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. punctata</i>          | 8914 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. purpureo-caerulea</i> | 6939 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. purpureo-caerulea</i> | 7074 | TAGAA---CT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. purpureo-caerulea</i> | 7354 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. purpureo-caerulea</i> | 7365 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. pusilla</i>           | 9092 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. pusilla</i>           | 9098 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. quadricolor</i>       | 7359 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. rosea</i>             | 7343 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. rosea</i>             | 8286 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. salteri</i>           | 8289 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. schelpei</i>          | 9089 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. sessiliflora</i>      | 8314 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. sp.</i>               | 8131 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. sp.</i>               | 9090 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. stayneri</i>          | 8316 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. suaveolens</i>        | 7884 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. thomasiae</i>         | 8292 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. trichophylla</i>      | 8293 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. trichophylla</i>      | 9097 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. undulata</i>          | 8317 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6862 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6863 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. schlechteri</i>       | 6865 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. schlechteri</i>       | 6866 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6867 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6869 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6870 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6871 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6873 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6874 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. unifolia</i>          | 6878 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. schlechteri</i>       | 6880 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. valeriae</i>          | 9104 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. variegata</i>         | 7097 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. verticillata</i>      | 7857 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. violacea</i>          | 7101 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. violacea</i>          | 7102 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. viridiflora</i>       | 7098 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. wrightii</i>          | 8318 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. youngii</i>           | 7865 | -----      | --AAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. zebrina</i>           | 7867 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. zebrina</i>           | 8305 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. zeyheri</i>           | 7099 | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>Massonia</i>             |      | TAGAACATCT | TCAAATAGAA | CATTTTATA- | -----GTAGT | ATGTCGTAAC    |
| <i>L. alba</i>              | 9024 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT350 |
| <i>L. alba</i>              | 9100 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. algoensis</i>         | 8210 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. aloides</i>           | 7361 | TATTCTCATA | TAACTTTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. ameliae</i>           | 8215 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. angelica</i>          | 9109 | TATTCTCATA | TAACTCTATG | GCTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. anguinea</i>          | 9103 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. attenuata</i>         | 8218 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. aurioliae</i>         | 8308 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. barkeriana</i>        | 7715 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 7064 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8893 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8895 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8896 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8898 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8899 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8900 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8901 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8902 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8903 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. bifolia</i>           | 8904 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |

|                                                     |            |            |            |            |            |
|-----------------------------------------------------|------------|------------|------------|------------|------------|
| <i>L. bifolia</i> _8905                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. bifolia</i> _8906                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. bifolia</i> _8907                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. bolusii</i> _7987                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. bowkeri</i> _9111                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. capensis</i> _8219                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. cernua</i> _8222                              | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. comptonii</i> _7067                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. concordiana</i> _8310                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. contaminata</i> _7727                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. convallarioides</i> _8228                     | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. corymbosa</i> _8229                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. dasybotrya</i> _7730                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. dehoopensis</i> _7731                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. doleritica</i> _8230                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. duncanii</i> _9107                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. elegans</i> _7734                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. ensifolia</i> _9101                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. ensifolia</i> _subsp._ <i>maughanii</i> _7876 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. ensifolia</i> _subsp._ <i>maughanii</i> _9102 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. fistulosa</i> _7357                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. fistulosa</i> _7362                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. flava</i> _7060                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. framesii</i> _8241                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. glauca</i> _8319                              | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. haarlemensis</i> _9110                        | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. hirta</i> _6858                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. hirta</i> _6891                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. inconspicua</i> _9095                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. isopetala</i> _8244                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. juncifolia</i> _8245                          | TATTCTCATA | TAACTCTACG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. juncifolia</i> _8246                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. karooica</i> _7754                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. karoopoortensis</i> _9108                     | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. klinghardtiana</i> _7756                      | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. kliprandensis</i> _8247                       | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. lactosa</i> _8248                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. latimeriae</i> _7761                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. longibracteata</i> _7768                      | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. longituba</i> _9105                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. magentea</i> _8313                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. magregorium</i> _7772                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. margaretae</i> _7773                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. marginata</i> _9096                           | TATTCTCATA | TAACTTATG  | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. marlothii</i> _7776                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. martinae</i> _8254                            | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mathewsii</i> _6938                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. maximiliani</i> _8257                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. sp.</i> _1_7080                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. sp.</i> _1_7082                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mediana</i> _subsp._9030                      | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mediana</i> _subsp._9031                      | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. sp.</i> _1_9032                               | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mediana</i> _subsp._9033                      | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079    | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. membranacea</i> _9106                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. moniliformis</i> _7884                        | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. multifolia</i> _8263                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. mutabilis</i> _8264                           | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. namaquensis</i> _7332                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. namibiensis</i> _8266                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. namibiensis</i> _9112                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. nardousbergensis</i> _8267                    | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. neilii</i> _7795                              | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. nervosa</i> _9091                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. obscura</i> _9035                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. obscura</i> _9093                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. obscura</i> _9094                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. orchoides</i> _subsp._ <i>orchoides</i> _7805 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. orthopetala</i> _7335                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. orthopetala</i> _7358                         | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. pallida</i> _8284                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. pallida</i> _8312                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. patula</i> _7363                              | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. paucifolia</i> _8277                          | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. peersii</i> _7355                             | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |
| <i>L. peersii</i> _8278                             | TATTCTCATA | TAACTTATG  | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT |



|                             |      |            |            |            |            |               |
|-----------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. perryae</i>           | 6961 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. polypodantha</i>      | 8280 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. punctata</i>          | 7093 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. punctata</i>          | 7344 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. punctata</i>          | 8909 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. punctata</i>          | 8911 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. punctata</i>          | 8914 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. purpureo-caerulea</i> | 6939 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. purpureo-caerulea</i> | 7074 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. purpureo-caerulea</i> | 7354 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. purpureo-caerulea</i> | 7365 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. pusilla</i>           | 9092 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. pusilla</i>           | 9098 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. quadricolor</i>       | 7359 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. rosea</i>             | 7343 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. rosea</i>             | 8286 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. salteri</i>           | 8289 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. schelpei</i>          | 9089 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. sessiliflora</i>      | 8314 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. sp.</i>               | 8131 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. sp.</i>               | 9090 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. stayneri</i>          | 8316 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. suaveolens</i>        | 7884 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. thomasiae</i>         | 8292 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. trichophylla</i>      | 8293 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. trichophylla</i>      | 9097 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. undulata</i>          | 8317 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6862 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6863 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. schlechteri</i>       | 6865 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. schlechteri</i>       | 6866 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6867 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6869 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6870 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6871 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6873 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6874 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. unifolia</i>          | 6878 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. schlechteri</i>       | 6880 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. valeriae</i>          | 9104 | TATTATCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. variegata</i>         | 7097 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. verticillata</i>      | 7857 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. violacea</i>          | 7101 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. violacea</i>          | 7102 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. viridiflora</i>       | 7098 | TATTCTCATA | TAACTTTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. wrightii</i>          | 8318 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. youngii</i>           | 7865 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. zebrina</i>           | 7867 | TATTCTCATA | GAACCTTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. zebrina</i>           | 8305 | TATTCTCATA | GAACCTTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. zeyheri</i>           | 7099 | TATTCTCATA | TAACTCTATG | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>Massonia</i>             |      | TATTCTCATA | GAACCTATG  | GTTCTTCAAA | GAGCCTTTCA | TGCATTATGT    |
| <i>L. alba</i>              | 9024 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA400 |
| <i>L. alba</i>              | 9100 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. algoensis</i>         | 8210 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. aloides</i>           | 7361 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. ameliae</i>           | 8215 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. angelica</i>          | 9109 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. anguinea</i>          | 9103 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. attenuata</i>         | 8218 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. aurioliae</i>         | 8308 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. barkeriana</i>        | 7715 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 7064 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8893 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8895 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8896 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8898 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8899 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8900 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8901 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8902 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8903 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8904 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8905 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8906 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bifolia</i>           | 8907 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. bolusii</i>           | 7987 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L. bowkeri</i>                           | 9111 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. capensis</i>                          | 8219 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. cernua</i>                            | 8222 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. comptonii</i>                         | 7067 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. concordiana</i>                       | 8310 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. contaminata</i>                       | 7727 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. convallarioides</i>                   | 8228 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. corymbosa</i>                         | 8229 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. dasybotrya</i>                        | 7730 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. dehoopensis</i>                       | 7731 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. doleritica</i>                        | 8230 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. duncanii</i>                          | 9107 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. elegans</i>                           | 7734 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. ensifolia</i>                         | 9101 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. fistulosa</i>                         | 7357 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. fistulosa</i>                         | 7362 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. flava</i>                             | 7060 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. framesii</i>                          | 8241 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. glauca</i>                            | 8319 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. haarlemensis</i>                      | 9110 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. hirta</i>                             | 6858 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. hirta</i>                             | 6891 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. inconspicua</i>                       | 9095 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. isopetala</i>                         | 8244 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. juncifolia</i>                        | 8245 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. juncifolia</i>                        | 8246 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. karooica</i>                          | 7754 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. karoopoortensis</i>                   | 9108 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. klinghardtiana</i>                    | 7756 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. kliprandensis</i>                     | 8247 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. lactosa</i>                           | 8248 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. latimeriae</i>                        | 7761 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. longibracteata</i>                    | 7768 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. longituba</i>                         | 9105 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. magentea</i>                          | 8313 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. magregoriorum</i>                     | 7772 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. margaretiae</i>                       | 7773 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. marginata</i>                         | 9096 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. marlothii</i>                         | 7776 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. martinae</i>                          | 8254 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mathewsii</i>                         | 6938 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. maximiliani</i>                       | 8257 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. sp.1</i>                              | 7080 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. sp.1</i>                              | 7082 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mediana</i> subsp. <i>9030</i>        |      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mediana</i> subsp. <i>9031</i>        |      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. sp.1</i>                              | 9032 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mediana</i> subsp. <i>9033</i>        |      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. membranacea</i>                       | 9106 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. moniliformis</i>                      | 7884 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. multifolia</i>                        | 8263 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. mutabilis</i>                         | 8264 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. namaquensis</i>                       | 7332 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. namibiensis</i>                       | 8266 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. namibiensis</i>                       | 9112 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. nardousbergensis</i>                  | 8267 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. neilii</i>                            | 7795 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. nervosa</i>                           | 9091 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. obscura</i>                           | 9035 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. obscura</i>                           | 9093 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. obscura</i>                           | 9094 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. orchoides</i> subsp. <i>orchoides</i> | 7805 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. orthopetala</i>                       | 7335 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. orthopetala</i>                       | 7358 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. pallida</i>                           | 8284 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. pallida</i>                           | 8312 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGAAC  | CATTTTCTGA |
| <i>L. patula</i>                            | 7363 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. paucifolia</i>                        | 8277 | TCGATATCAA | GGAAAAGCAA | TTTTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. peersii</i>                           | 7355 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. peersii</i>                           | 8278 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA |
| <i>L. perryae</i>                           | 6961 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. polypodantha</i>                      | 8280 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. punctata</i>                          | 7093 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |
| <i>L. punctata</i>                          | 7344 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA |

|                                   |            |            |            |            |               |
|-----------------------------------|------------|------------|------------|------------|---------------|
| <i>L. punctata</i> _8909          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. punctata</i> _8911          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. punctata</i> _8914          | TCGATATCAG | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. purpureo-caerulea</i> _6939 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. purpureo-caerulea</i> _7074 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. purpureo-caerulea</i> _7354 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. purpureo-caerulea</i> _7365 | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. pusilla</i> _9092           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. pusilla</i> _9098           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. quadricolor</i> _7359       | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA    |
| <i>L. rosea</i> _7343             | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. rosea</i> _8286             | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. salteri</i> _8289           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. schelpei</i> _9089          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. sessiliflora</i> _8314      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA    |
| <i>L. sp.</i> _2_8131             | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. sp.</i> _9090               | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. stayneri</i> _8316          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. suaveolens</i> _7884        | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. thomasiae</i> _8292         | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. trichophylla</i> _8293      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. trichophylla</i> _9097      | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. undulata</i> _8317          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6862          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6863          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. schlechteri</i> _6865       | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. schlechteri</i> _6866       | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6867          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6869          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6870          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6871          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6873          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6874          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. unifolia</i> _6878          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. schlechteri</i> _6880       | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. valeriae</i> _9104          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. variegata</i> _7097         | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. verticillata</i> _7857      | TCGATATCAG | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. violacea</i> _7101          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. violacea</i> _7102          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. viridiflora</i> _7098       | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGATT | CATTTTCTGA    |
| <i>L. wrightii</i> _8318          | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. youngii</i> _7865           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. zebrina</i> _7867           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. zebrina</i> _8305           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. zeyheri</i> _7099           | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>Massonia</i>                   | TCGATATCAA | GGAAAAGCAA | TTCTTGCTTC | AAAAGGGACT | CATTTTCTGA    |
| <i>L. alba</i> _9024              | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC450 |
| <i>L. alba</i> _9100              | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. algoensis</i> _8210         | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. aloides</i> _7361           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. ameliae</i> _8215           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. angelica</i> _9109          | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. anguinea</i> _9103          | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. attenuata</i> _8218         | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. aurioliae</i> _8308         | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. barkeriana</i> _7715        | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _7064           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8893           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8895           | CGAAGAAATG | GAAATATCAT | TTTCTCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8896           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8898           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8899           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8900           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8901           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8902           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8903           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8904           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8905           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8906           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bifolia</i> _8907           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bolusii</i> _7987           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. bowkeri</i> _9111           | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. capensis</i> _8219          | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. cernua</i> _8222            | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC    |
| <i>L. comptonii</i> _7067         | CGAAGAAATG | GAAATCTCAT | TTTCTCAATT | TCTGGCAATA | TTATTTTCAC    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L._concordiana</i>                       | 8310 | CGAAGAAATG | GAAATATCAT | TTTCTCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._contaminata</i>                       | 7727 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._convallarioides</i>                   | 8228 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._corymbosa</i>                         | 8229 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._dasybotrya</i>                        | 7730 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._dehoopensis</i>                       | 7731 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._doleritica</i>                        | 8230 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._duncanii</i>                          | 9107 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._elegans</i>                           | 7734 | CGAAGAAATG | GAAATATCAT | TTTCTCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._ensifolia</i>                         | 9101 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 7876 | CGAAGAAATG | GAAATCTCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._ensifolia</i> subsp. <i>maughanii</i> | 9102 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._fistulosa</i>                         | 7357 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._fistulosa</i>                         | 7362 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._flava</i>                             | 7060 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._framesii</i>                          | 8241 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._glauca</i>                            | 8319 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._haarlemensis</i>                      | 9110 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._hirta</i>                             | 6858 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._hirta</i>                             | 6891 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._inconspicua</i>                       | 9095 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._isopetala</i>                         | 8244 | CGAAGAAATG | GAAATATTAT | TTTATTAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._juncifolia</i>                        | 8245 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._juncifolia</i>                        | 8246 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._karooica</i>                          | 7754 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._karoopoortensis</i>                   | 9108 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._klinghardtiana</i>                    | 7756 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._kliprandensis</i>                     | 8247 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._lactosa</i>                           | 8248 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._latimeriae</i>                        | 7761 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._longibracteata</i>                    | 7768 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._longituba</i>                         | 9105 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._magentea</i>                          | 8313 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._magregoriorum</i>                     | 7772 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._margaretiae</i>                       | 7773 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._marginata</i>                         | 9096 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._marlothii</i>                         | 7776 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._martinae</i>                          | 8254 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mathewsii</i>                         | 6938 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._maximiliani</i>                       | 8257 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._sp.1</i>                              | 7080 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._sp.1</i>                              | 7082 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mediana</i> subsp.                    | 9030 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mediana</i> subsp.                    | 9031 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._sp.1</i>                              | 9032 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mediana</i> subsp.                    | 9033 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mediana</i> subsp. <i>rogersii</i>    | 7079 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._membranacea</i>                       | 9106 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._moniliformis</i>                      | 7884 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._multifolia</i>                        | 8263 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._mutabilis</i>                         | 8264 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._namaquensis</i>                       | 7332 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._namibiensis</i>                       | 8266 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._namibiensis</i>                       | 9112 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._nardousbergensis</i>                  | 8267 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._neilii</i>                            | 7795 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._nervosa</i>                           | 9091 | CAAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._obscura</i>                           | 9035 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._obscura</i>                           | 9093 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._obscura</i>                           | 9094 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._orchiodes</i> subsp. <i>orchiodes</i> | 7805 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._orthopetala</i>                       | 7335 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._orthopetala</i>                       | 7358 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._pallida</i>                           | 8284 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._pallida</i>                           | 8312 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._patula</i>                            | 7363 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._paucifolia</i>                        | 8277 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TATGGCAATA | TTATTTTCAC |
| <i>L._peersii</i>                           | 7355 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._peersii</i>                           | 8278 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._perryae</i>                           | 6961 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._polypodantha</i>                      | 8280 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._punctata</i>                          | 7093 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._punctata</i>                          | 7344 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._punctata</i>                          | 8909 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._punctata</i>                          | 8911 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._punctata</i>                          | 8914 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |
| <i>L._purpureo-caerulea</i>                 | 6939 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC |

|                             |      |            |            |            |            |                |
|-----------------------------|------|------------|------------|------------|------------|----------------|
| <i>L._purpureo-caerulea</i> | 7074 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._purpureo-caerulea</i> | 7354 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._purpureo-caerulea</i> | 7365 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._pusilla</i>           | 9092 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._pusilla</i>           | 9098 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._quadricolor</i>       | 7359 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._rosea</i>             | 7343 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._rosea</i>             | 8286 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._salteri</i>           | 8289 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._schelpei</i>          | 9089 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._sessiliflora</i>      | 8314 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._sp.2</i>              | 8131 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._sp</i>                | 9090 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._stayneri</i>          | 8316 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._suaveolens</i>        | 7884 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._thomasiae</i>         | 8292 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._trichophylla</i>      | 8293 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._trichophylla</i>      | 9097 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._undulata</i>          | 8317 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6862 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6863 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._schlechteri</i>       | 6865 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._schlechteri</i>       | 6866 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6867 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6869 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6870 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6871 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6873 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6874 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._unifolia</i>          | 6878 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._schlechteri</i>       | 6880 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._valeriae</i>          | 9104 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._variegata</i>         | 7097 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._verticillata</i>      | 7857 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._violacea</i>          | 7101 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._violacea</i>          | 7102 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._viridiflora</i>       | 7098 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._wrightii</i>          | 8318 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._youngii</i>           | 7865 | CGAAGAAATG | GAAATCTCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._zebrina</i>           | 7867 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._zebrina</i>           | 8305 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>L._zeyheri</i>           | 7099 | CGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
| <i>Massonia</i>             |      | TGAAGAAATG | GAAATATCAT | TTTATCAATT | TCTGGCAATA | TTATTTTCAC     |
|                             |      |            |            |            |            |                |
| <i>L._alba</i>              | 9024 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA500 |
| <i>L._alba</i>              | 9100 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._algoensis</i>         | 8210 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._aloides</i>           | 7361 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._ameliae</i>           | 8215 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._angelica</i>          | 9109 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._anguinea</i>          | 9103 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._attenuata</i>         | 8218 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._aurioliae</i>         | 8308 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._barkeriana</i>        | 7715 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 7064 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8893 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8895 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8896 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8898 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8899 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8900 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8901 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8902 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8903 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8904 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8905 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8906 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bifolia</i>           | 8907 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bolusii</i>           | 7987 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._bowkeri</i>           | 9111 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._capensis</i>          | 8219 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._cernua</i>            | 8222 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._comptonii</i>         | 7067 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._concordiana</i>       | 8310 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._contaminata</i>       | 7727 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._convallarioides</i>   | 8228 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |
| <i>L._corymbosa</i>         | 8229 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACATA    |

|                                             |      |            |            |            |            |             |
|---------------------------------------------|------|------------|------------|------------|------------|-------------|
| <i>L. dasybotrya</i>                        | 7730 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. dehoopensis</i>                       | 7731 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. doleritica</i>                        | 8230 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. duncanii</i>                          | 9107 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. elegans</i>                           | 7734 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. ensifolia</i>                         | 9101 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. fistulosa</i>                         | 7357 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. fistulosa</i>                         | 7362 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. flava</i>                             | 7060 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. framesii</i>                          | 8241 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. glauca</i>                            | 8319 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. haarlemensis</i>                      | 9110 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. hirta</i>                             | 6858 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. hirta</i>                             | 6891 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. inconspicua</i>                       | 9095 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. isopetala</i>                         | 8244 | TTTTGGTCTC | AACCGTACAG | GATCCATAGA | AACAACCAAT | TATCAAACCTA |
| <i>L. juncifolia</i>                        | 8245 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. juncifolia</i>                        | 8246 | TTTTGGTCTC | AATCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. karooica</i>                          | 7754 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. karoopoortensis</i>                   | 9108 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. klinghardtiana</i>                    | 7756 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. kliprandensis</i>                     | 8247 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. lactosa</i>                           | 8248 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. latimeriae</i>                        | 7761 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. longibracteata</i>                    | 7768 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. longituba</i>                         | 9105 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. magentea</i>                          | 8313 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. magregoriorum</i>                     | 7772 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. margaretae</i>                        | 7773 | TTTTGGTCTC | AACCGTACAG | GATCCATAGA | A---ATCAAT | TATCAAACCTA |
| <i>L. marginata</i>                         | 9096 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. marlothii</i>                         | 7776 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. martinae</i>                          | 8254 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mathewsii</i>                         | 6938 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. maximiliani</i>                       | 8257 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. sp.1</i>                              | 7080 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. sp.1</i>                              | 7082 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mediana</i> subsp. <i>9030</i>        |      | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mediana</i> subsp. <i>9031</i>        |      | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. sp.1</i>                              | 9032 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mediana</i> subsp. <i>9033</i>        |      | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. membranacea</i>                       | 9106 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. moniliformis</i>                      | 7884 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. multifolia</i>                        | 8263 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. mutabilis</i>                         | 8264 | TTTTGGTCTC | AACCGTACAG | GATCCATAGA | A---ATCAAT | TATCAAACCTA |
| <i>L. namaquensis</i>                       | 7332 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. namibiensis</i>                       | 8266 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. namibiensis</i>                       | 9112 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. nardousbergensis</i>                  | 8267 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. neilii</i>                            | 7795 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. nervosa</i>                           | 9091 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. obscura</i>                           | 9035 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. obscura</i>                           | 9093 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. obscura</i>                           | 9094 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. orchiodes</i> subsp. <i>orchiodes</i> | 7805 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. orthopetala</i>                       | 7335 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. orthopetala</i>                       | 7358 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. pallida</i>                           | 8284 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. pallida</i>                           | 8312 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. patula</i>                            | 7363 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. paucifolia</i>                        | 8277 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. peersii</i>                           | 7355 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. peersii</i>                           | 8278 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. perryae</i>                           | 6961 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. polypodantha</i>                      | 8280 | TTTTGGTCTC | AACCGTATAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. punctata</i>                          | 7093 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. punctata</i>                          | 7344 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. punctata</i>                          | 8909 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. punctata</i>                          | 8911 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. punctata</i>                          | 8914 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. purpureo-caerulea</i>                 | 6939 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. purpureo-caerulea</i>                 | 7074 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. purpureo-caerulea</i>                 | 7354 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. purpureo-caerulea</i>                 | 7365 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |
| <i>L. pusilla</i>                           | 9092 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA |

|                           |      |            |            |            |            |               |
|---------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. pusilla</i>         | 9098 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. quadricolor</i>     | 7359 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. rosea</i>           | 7343 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. rosea</i>           | 8286 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. salteri</i>         | 8289 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. schelpei</i>        | 9089 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. sessiliflora</i>    | 8314 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. sp.</i>             | 8131 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. sp.</i>             | 9090 | TTTTGGTTTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. stayneri</i>        | 8316 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. suaveolens</i>      | 7884 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. thomasiae</i>       | 8292 | TTTTGGTCTC | AACCGTACAG | GATCCATAGA | A---ATCAAT | TATCAAACCTA   |
| <i>L. trichophylla</i>    | 8293 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. trichophylla</i>    | 9097 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. undulata</i>        | 8317 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6862 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6863 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. schlechteri</i>     | 6865 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. schlechteri</i>     | 6866 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6867 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6869 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6870 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6871 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6873 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6874 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. unifolia</i>        | 6878 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. schlechteri</i>     | 6880 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. valeriae</i>        | 9104 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. variegata</i>       | 7097 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. verticillata</i>    | 7857 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. violacea</i>        | 7101 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. violacea</i>        | 7102 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. viridiflora</i>     | 7098 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. wrightii</i>        | 8318 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. youngii</i>         | 7865 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. zebrina</i>         | 7867 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. zebrina</i>         | 8305 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>L. zeyheri</i>         | 7099 | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ATCAAT | TATCAAACCTA   |
| <i>Massonia</i>           |      | TTTTGGTCTC | AACCGTACAG | GATCCATATA | A---ACCAAT | TATCAAACCTA   |
| <i>L. alba</i>            | 9024 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG550 |
| <i>L. alba</i>            | 9100 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. algoensis</i>       | 8210 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. aloides</i>         | 7361 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. ameliae</i>         | 8215 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. angelica</i>        | 9109 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. anguinea</i>        | 9103 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. attenuata</i>       | 8218 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. aurioliae</i>       | 8308 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. barkeriana</i>      | 7715 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 7064 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8893 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8895 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8896 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8898 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8899 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8900 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8901 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8902 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8903 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8904 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8905 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8906 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bifolia</i>         | 8907 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bolusii</i>         | 7987 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. bowkeri</i>         | 9111 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. capensis</i>        | 8219 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. cernua</i>          | 8222 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. comptonii</i>       | 7067 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. concordiana</i>     | 8310 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. contaminata</i>     | 7727 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. convallarioides</i> | 8228 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. corymbosa</i>       | 8229 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. dasybotrya</i>      | 7730 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. dehoopensis</i>     | 7731 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. doleritica</i>      | 8230 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. duncanii</i>        | 9107 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |

|                                               |      |            |            |            |            |            |
|-----------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L. elegans</i>                             | 7734 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. ensifolia</i>                           | 9101 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. ensifolia</i> subsp. <i>maughanii</i>   | 7876 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. ensifolia</i> subsp. <i>maughanii</i>   | 9102 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. fistulosa</i>                           | 7357 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. fistulosa</i>                           | 7362 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. flava</i>                               | 7060 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. framesii</i>                            | 8241 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. glauca</i>                              | 8319 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. haarlemensis</i>                        | 9110 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. hirta</i>                               | 6858 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. hirta</i>                               | 6891 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. inconspicua</i>                         | 9095 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. isopetala</i>                           | 8244 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. juncifolia</i>                          | 8245 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. juncifolia</i>                          | 8246 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. karooica</i>                            | 7754 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. karoopoortensis</i>                     | 9108 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. klinghardtiana</i>                      | 7756 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. kliprandensis</i>                       | 8247 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. lactosa</i>                             | 8248 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. latimeriae</i>                          | 7761 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. longibracteata</i>                      | 7768 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. longituba</i>                           | 9105 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. magentea</i>                            | 8313 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. magregoriorum</i>                       | 7772 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. margaretae</i>                          | 7773 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. marginata</i>                           | 9096 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. marlothii</i>                           | 7776 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. martinae</i>                            | 8254 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mathewsii</i>                           | 6938 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. maximiliani</i>                         | 8257 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. sp.1</i>                                | 7080 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. sp.1</i>                                | 7082 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mediana</i> subsp.                      | 9030 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mediana</i> subsp.                      | 9031 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. sp.1</i>                                | 9032 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mediana</i> subsp.                      | 9033 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mediana</i> subsp. <i>rogersii</i>      | 7079 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. membranacea</i>                         | 9106 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. moniliformis</i>                        | 7884 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. multifolia</i>                          | 8263 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. mutabilis</i>                           | 8264 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. namaquensis</i>                         | 7332 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. namibiensis</i>                         | 8266 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. namibiensis</i>                         | 9112 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. nardousbergensis</i>                    | 8267 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. neilii</i>                              | 7795 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. nervosa</i>                             | 9091 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. obscura</i>                             | 9035 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. obscura</i>                             | 9093 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. obscura</i>                             | 9094 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. orchioides</i> subsp. <i>orchioides</i> | 7805 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. orthopetala</i>                         | 7335 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. orthopetala</i>                         | 7358 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. pallida</i>                             | 8284 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. pallida</i>                             | 8312 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. patula</i>                              | 7363 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. paucifolia</i>                          | 8277 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. peersii</i>                             | 7355 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. peersii</i>                             | 8278 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. perryae</i>                             | 6961 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. polypodantha</i>                        | 8280 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. punctata</i>                            | 7093 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. punctata</i>                            | 7344 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. punctata</i>                            | 8909 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. punctata</i>                            | 8911 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. punctata</i>                            | 8914 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. purpureo-caerulea</i>                   | 6939 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. purpureo-caerulea</i>                   | 7074 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. purpureo-caerulea</i>                   | 7354 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. purpureo-caerulea</i>                   | 7365 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. pusilla</i>                             | 9092 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. pusilla</i>                             | 9098 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. quadricolor</i>                         | 7359 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. rosea</i>                               | 7343 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |
| <i>L. rosea</i>                               | 8286 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG |



|                                             |      |            |            |            |            |               |
|---------------------------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. salteri</i>                           | 8289 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. schelpei</i>                          | 9089 | CTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. sessiliflora</i>                      | 8314 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. sp.2</i>                              | 8131 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. sp.</i>                               | 9090 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. stayneri</i>                          | 8316 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. suaveolens</i>                        | 7884 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. thomasiae</i>                         | 8292 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. trichophylla</i>                      | 8293 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. trichophylla</i>                      | 9097 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. undulata</i>                          | 8317 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6862 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6863 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. schlechteri</i>                       | 6865 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. schlechteri</i>                       | 6866 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6867 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6869 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6870 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6871 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6873 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6874 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. unifolia</i>                          | 6878 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. schlechteri</i>                       | 6880 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. valeriae</i>                          | 9104 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. variegata</i>                         | 7097 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. verticillata</i>                      | 7857 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. violacea</i>                          | 7101 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. violacea</i>                          | 7102 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. viridiflora</i>                       | 7098 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. wrightii</i>                          | 8318 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. youngii</i>                           | 7865 | TTCTTTCTAT | TTTATGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. zebrina</i>                           | 7867 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. zebrina</i>                           | 8305 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>L. zeyheri</i>                           | 7099 | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
| <i>Massonia</i>                             |      | TTCTTTCTAT | TTTCTGGGTT | ATCTTTCAAG | TCTATTAAGA | AATTCTTCGG    |
|                                             |      |            |            |            |            |               |
| <i>L. alba</i>                              | 9024 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT600 |
| <i>L. alba</i>                              | 9100 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. algoensis</i>                         | 8210 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. aloides</i>                           | 7361 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. ameliae</i>                           | 8215 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. angelica</i>                          | 9109 | CGGCAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. anguinea</i>                          | 9103 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. attenuata</i>                         | 8218 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. aurioliae</i>                         | 8308 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. barkeriana</i>                        | 7715 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 7064 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8893 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8895 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8896 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8898 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8899 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8900 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8901 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8902 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8903 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8904 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8905 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8906 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bifolia</i>                           | 8907 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bolusii</i>                           | 7987 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. bowkeri</i>                           | 9111 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. capensis</i>                          | 8219 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. cernua</i>                            | 8222 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. comptonii</i>                         | 7067 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. concordiana</i>                       | 8310 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. contaminata</i>                       | 7727 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. convallarioides</i>                   | 8228 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. corymbosa</i>                         | 8229 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. dasybotrya</i>                        | 7730 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. dehoopensis</i>                       | 7731 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. doleritica</i>                        | 8230 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. duncanii</i>                          | 9107 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. elegans</i>                           | 7734 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. ensifolia</i>                         | 9101 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L._fistulosa</i>                         | 7357 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._fistulosa</i>                         | 7362 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._flava</i>                             | 7060 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._framesii</i>                          | 8241 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._glauca</i>                            | 8319 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._haarlemensis</i>                      | 9110 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._hirta</i>                             | 6858 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._hirta</i>                             | 6891 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._inconspicua</i>                       | 9095 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._isopetala</i>                         | 8244 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._juncifolia</i>                        | 8245 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._juncifolia</i>                        | 8246 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._karooica</i>                          | 7754 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._karoopoortensis</i>                   | 9108 | TGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._klinghardtiana</i>                    | 7756 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._kliprandensis</i>                     | 8247 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._lactosa</i>                           | 8248 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._latimeriae</i>                        | 7761 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._longibracteata</i>                    | 7768 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._longituba</i>                         | 9105 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._magentea</i>                          | 8313 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._magregoriorum</i>                     | 7772 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._margaretiae</i>                       | 7773 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._marginata</i>                         | 9096 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._marlothii</i>                         | 7776 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._martinae</i>                          | 8254 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mathewsii</i>                         | 6938 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._maximiliani</i>                       | 8257 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._sp.1</i>                              | 7080 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._sp.1</i>                              | 7082 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mediana</i> subsp.                    | 9030 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mediana</i> subsp.                    | 9031 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._sp.1</i>                              | 9032 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mediana</i> subsp.                    | 9033 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mediana</i> subsp. <i>rogersii</i>    | 7079 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._membranacea</i>                       | 9106 | TGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._moniliformis</i>                      | 7884 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._multifolia</i>                        | 8263 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._mutabilis</i>                         | 8264 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._namaquensis</i>                       | 7332 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._namibiensis</i>                       | 8266 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._namibiensis</i>                       | 9112 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._nardousbergensis</i>                  | 8267 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._neilii</i>                            | 7795 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._nervosa</i>                           | 9091 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._obscura</i>                           | 9035 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._obscura</i>                           | 9093 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._obscura</i>                           | 9094 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._orchiodes</i> subsp. <i>orchiodes</i> | 7805 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._orthopetala</i>                       | 7335 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._orthopetala</i>                       | 7358 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._pallida</i>                           | 8284 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._pallida</i>                           | 8312 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._patula</i>                            | 7363 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._paucifolia</i>                        | 8277 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._peersii</i>                           | 7355 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._peersii</i>                           | 8278 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._perryae</i>                           | 6961 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._polypodantha</i>                      | 8280 | CGGCAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._punctata</i>                          | 7093 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._punctata</i>                          | 7344 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._punctata</i>                          | 8909 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._punctata</i>                          | 8911 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._punctata</i>                          | 8914 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._purpureo-caerulea</i>                 | 6939 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._purpureo-caerulea</i>                 | 7074 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._purpureo-caerulea</i>                 | 7354 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._purpureo-caerulea</i>                 | 7365 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._pusilla</i>                           | 9092 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._pusilla</i>                           | 9098 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._quadricolor</i>                       | 7359 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._rosea</i>                             | 7343 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._rosea</i>                             | 8286 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._salteri</i>                           | 8289 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._schelpei</i>                          | 9089 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._sessiliflora</i>                      | 8314 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |
| <i>L._sp.2</i>                              | 8131 | CGGTAAGGAA | TCAAATGTGA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT |

|                                           |            |            |            |            |               |
|-------------------------------------------|------------|------------|------------|------------|---------------|
| <i>L._sp_9090</i>                         | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._stayneri_8316</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._suaveolens_7884</i>                 | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._thomasiae_8292</i>                  | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._trichophylla_8293</i>               | CGGCAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._trichophylla_9097</i>               | CGGCAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._undulata_8317</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6862</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6863</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._schlechteri_6865</i>                | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._schlechteri_6866</i>                | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6867</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6869</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6870</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6871</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6873</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6874</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._unifolia_6878</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._schlechteri_6880</i>                | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._valeriae_9104</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._variegata_7097</i>                  | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._verticillata_7857</i>               | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._violacea_7101</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._violacea_7102</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._viridiflora_7098</i>                | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._wrightii_8318</i>                   | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._youngii_7865</i>                    | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._zebrina_7867</i>                    | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._zebrina_8305</i>                    | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._zeyheri_7099</i>                    | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>Massonia</i>                           | CGGTAAGGAA | TCAAATGTTA | GAGAATTCAT | TTCTAATAGA | TACTGTTACT    |
| <i>L._alba_9024</i>                       | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT650 |
| <i>L._alba_9100</i>                       | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._algoensis_8210</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._aloides_7361</i>                    | AATAAATTG  | ATACCGCAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._ameliae_8215</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._angelica_9109</i>                   | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._anguinea_9103</i>                   | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._attenuata_8218</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._aurioliae_8308</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._barkeriana_7715</i>                 | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_7064</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8893</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8895</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8896</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8898</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8899</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8900</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8901</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8902</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8903</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8904</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8905</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8906</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bifolia_8907</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bolusii_7987</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._bowkeri_9111</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._capensis_8219</i>                   | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._cernua_8222</i>                     | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._comptonii_7067</i>                  | AACAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._concordiana_8310</i>                | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._contaminata_7727</i>                | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._convallarioides_8228</i>            | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._corymbosa_8229</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._dasybotrya_7730</i>                 | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._dehoopensis_7731</i>                | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._doleritica_8230</i>                 | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._duncanii_9107</i>                   | AATAAATTG  | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._elegans_7734</i>                    | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._ensifolia_9101</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | ATTCTTATTG | GATCCTTGTT    |
| <i>L._ensifolia_subsp._maughanii_7876</i> | AATCAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._ensifolia_subsp._maughanii_9102</i> | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._fistulosa_7357</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._fistulosa_7362</i>                  | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._flava_7060</i>                      | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._framesii_8241</i>                   | AATAAATTG  | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |

|                                             |      |            |            |            |            |            |
|---------------------------------------------|------|------------|------------|------------|------------|------------|
| <i>L. glauca</i>                            | 8319 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. haarlemensis</i>                      | 9110 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. hirta</i>                             | 6858 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. hirta</i>                             | 6891 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. inconspicua</i>                       | 9095 | AATAAAATTG | ATACCACAGT | CCCGGCTATT | CTT---ATTG | GATCCTTGTT |
| <i>L. isopetala</i>                         | 8244 | AATAAAATTG | ATACCATAGT | CCCTATTCTT | ATT-----G  | GATCCTTGTC |
| <i>L. juncifolia</i>                        | 8245 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. juncifolia</i>                        | 8246 | AATAAAATTG | ATACCACAGT | TCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. karooica</i>                          | 7754 | AATAAAATTG | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. karoopoortensis</i>                   | 9108 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. klinghardtiana</i>                    | 7756 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. kliprandensis</i>                     | 8247 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. lactosa</i>                           | 8248 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. latimeriae</i>                        | 7761 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. longibracteata</i>                    | 7768 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. longituba</i>                         | 9105 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. magentea</i>                          | 8313 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. magregoriorum</i>                     | 7772 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. margaretae</i>                        | 7773 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. marginata</i>                         | 9096 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. marlothii</i>                         | 7776 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. martinae</i>                          | 8254 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mathewsii</i>                         | 6938 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. maxmilianii</i>                       | 8257 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sp.1</i>                              | 7080 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sp.1</i>                              | 7082 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mediana</i> subsp.                    | 9030 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mediana</i> subsp.                    | 9031 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sp.1</i>                              | 9032 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mediana</i> subsp.                    | 9033 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. membranacea</i>                       | 9106 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. moniliformis</i>                      | 7884 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. multifolia</i>                        | 8263 | AATAAAATTG | ATACCACAGT | TCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. mutabilis</i>                         | 8264 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. namaquensis</i>                       | 7332 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. namibiensis</i>                       | 8266 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. namibiensis</i>                       | 9112 | AATAAAATTG | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. nardousbergensis</i>                  | 8267 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. neilii</i>                            | 7795 | AATAAAATTG | ATACCACAGT | CCCGGCTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. nervosa</i>                           | 9091 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. obscura</i>                           | 9035 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. obscura</i>                           | 9093 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. obscura</i>                           | 9094 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. orchiodes</i> subsp. <i>orchiodes</i> | 7805 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. orthopetala</i>                       | 7335 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. orthopetala</i>                       | 7358 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. pallida</i>                           | 8284 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. pallida</i>                           | 8312 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. patula</i>                            | 7363 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. paucifolia</i>                        | 8277 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. peersii</i>                           | 7355 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. peersii</i>                           | 8278 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. perryae</i>                           | 6961 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. polypodantha</i>                      | 8280 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. punctata</i>                          | 7093 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. punctata</i>                          | 7344 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. punctata</i>                          | 8909 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. punctata</i>                          | 8911 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. punctata</i>                          | 8914 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. purpureo-caerulea</i>                 | 6939 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. purpureo-caerulea</i>                 | 7074 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. purpureo-caerulea</i>                 | 7354 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. purpureo-caerulea</i>                 | 7365 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. pusilla</i>                           | 9092 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. pusilla</i>                           | 9098 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. quadricolor</i>                       | 7359 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | TTTCTTATTG | GATCCTTGTT |
| <i>L. rosea</i>                             | 7343 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. rosea</i>                             | 8286 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. salteri</i>                           | 8289 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. schelpei</i>                          | 9089 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sessiliflora</i>                      | 8314 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sp.2</i>                              | 8131 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. sp.</i>                               | 9090 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. stayneri</i>                          | 8316 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. suaveolens</i>                        | 7884 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |
| <i>L. thomasiae</i>                         | 8292 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT |

|                                      |      |            |            |            |            |               |
|--------------------------------------|------|------------|------------|------------|------------|---------------|
| <i>L._trichophylla</i>               | 8293 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._trichophylla</i>               | 9097 | AATAAAATTG | ATACCGCAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._undulata</i>                   | 8317 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6862 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6863 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._schlechteri</i>                | 6865 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._schlechteri</i>                | 6866 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6867 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6869 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6870 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6871 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6873 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6874 | AATAAAATTG | ATACCACAGT | CTCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._unifolia</i>                   | 6878 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._schlechteri</i>                | 6880 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._valeriae</i>                   | 9104 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._variegata</i>                  | 7097 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._verticillata</i>               | 7857 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._violacea</i>                   | 7101 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._violacea</i>                   | 7102 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._viridiflora</i>                | 7098 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._wrightii</i>                   | 8318 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._youngii</i>                    | 7865 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._zebrina</i>                    | 7867 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._zebrina</i>                    | 8305 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._zeyheri</i>                    | 7099 | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>Massonia</i>                      |      | AATAAAATTG | ATACCACAGT | CCCGGTTATT | CTTCTTATTG | GATCCTTGTT    |
| <i>L._alba</i>                       | 9024 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT700 |
| <i>L._alba</i>                       | 9100 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._algoensis</i>                  | 8210 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._aloides</i>                    | 7361 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._ameliae</i>                    | 8215 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._angelica</i>                   | 9109 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._anguinea</i>                   | 9103 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._attenuata</i>                  | 8218 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._aurioliae</i>                  | 8308 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._barkeriana</i>                 | 7715 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 7064 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8893 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8895 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8896 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8898 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8899 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8900 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8901 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8902 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8903 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8904 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8905 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8906 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bifolia</i>                    | 8907 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bolusii</i>                    | 7987 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._bowkeri</i>                    | 9111 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._capensis</i>                   | 8219 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._cernua</i>                     | 8222 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._comptonii</i>                  | 7067 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._concordiana</i>                | 8310 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._contaminata</i>                | 7727 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._convallarioides</i>            | 8228 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._corymbosa</i>                  | 8229 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._dasybotrya</i>                 | 7730 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._dehoopensis</i>                | 7731 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._doleritica</i>                 | 8230 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._duncanii</i>                   | 9107 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._elegans</i>                    | 7734 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._ensifolia</i>                  | 9101 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._ensifolia_subsp._maughanii</i> | 7876 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._ensifolia_subsp._maughanii</i> | 9102 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._fistulosa</i>                  | 7357 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._fistulosa</i>                  | 7362 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._flava</i>                      | 7060 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._framesii</i>                   | 8241 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._glauca</i>                     | 8319 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._haarlemensis</i>               | 9110 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._hirta</i>                      | 6858 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L._hirta</i>                      | 6891 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |

|                                      |      |            |            |            |            |             |
|--------------------------------------|------|------------|------------|------------|------------|-------------|
| <i>L. inconspicua</i>                | 9095 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. isopetala</i>                  | 8244 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCATATCT |
| <i>L. juncifolia</i>                 | 8245 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. juncifolia</i>                 | 8246 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. karooica</i>                   | 7754 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. karooipoortensis</i>           | 9108 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. klinghardtiana</i>             | 7756 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. kliprandensis</i>              | 8247 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. lactosa</i>                    | 8248 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. latimeriae</i>                 | 7761 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. longibracteata</i>             | 7768 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. longituba</i>                  | 9105 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. magentea</i>                   | 8313 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. magregriorum</i>               | 7772 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. margaretae</i>                 | 7773 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. marginata</i>                  | 9096 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. marlothii</i>                  | 7776 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. martinae</i>                   | 8254 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGACCT  |
| <i>L. mathewsii</i>                  | 6938 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. maximiliani</i>                | 8257 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sp.1</i>                       | 7080 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sp.1</i>                       | 7082 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. mediana</i> subsp.             | 9030 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. mediana</i> subsp.             | 9031 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sp.1</i>                       | 9032 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. mediana</i> subsp.             | 9033 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. mediana</i> subsp. rogersii    | 7079 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. membranacea</i>                | 9106 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. moniliformis</i>               | 7884 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. multifolia</i>                 | 8263 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. mutabilis</i>                  | 8264 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. namaquensis</i>                | 7332 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. namibiensis</i>                | 8266 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. namibiensis</i>                | 9112 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. nardousbergensis</i>           | 8267 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. neilii</i>                     | 7795 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. nervosa</i>                    | 9091 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. obscura</i>                    | 9035 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. obscura</i>                    | 9093 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. obscura</i>                    | 9094 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. orchoides</i> subsp. orchoides | 7805 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. orthopetala</i>                | 7335 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. orthopetala</i>                | 7358 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. pallida</i>                    | 8284 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. pallida</i>                    | 8312 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. patula</i>                     | 7363 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. paucifolia</i>                 | 8277 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. peersii</i>                    | 7355 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. peersii</i>                    | 8278 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. perryae</i>                    | 6961 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. polypodantha</i>               | 8280 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. punctata</i>                   | 7093 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. punctata</i>                   | 7344 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. punctata</i>                   | 8909 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. punctata</i>                   | 8911 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. punctata</i>                   | 8914 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. purpureo-caerulea</i>          | 6939 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. purpureo-caerulea</i>          | 7074 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. purpureo-caerulea</i>          | 7354 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. purpureo-caerulea</i>          | 7365 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. pusilla</i>                    | 9092 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. pusilla</i>                    | 9098 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. quadricolor</i>                | 7359 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. rosea</i>                      | 7343 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. rosea</i>                      | 8286 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. salteri</i>                    | 8289 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. schelpei</i>                   | 9089 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sessiliflora</i>               | 8314 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sp.2</i>                       | 8131 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. sp.</i>                        | 9090 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. stayneri</i>                   | 8316 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. suaveolens</i>                 | 7884 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. thomasiae</i>                  | 8292 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. trichophylla</i>               | 8293 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. trichophylla</i>               | 9097 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. undulata</i>                   | 8317 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |
| <i>L. unifolia</i>                   | 6862 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT  |

|                                             |      |            |            |            |            |               |
|---------------------------------------------|------|------------|------------|------------|------------|---------------|
| <i>L. unifolia</i>                          | 6863 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. schlechteri</i>                       | 6865 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. schlechteri</i>                       | 6866 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6867 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6869 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6870 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6871 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6873 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6874 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. unifolia</i>                          | 6878 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. schlechteri</i>                       | 6880 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. valeriae</i>                          | 9104 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. variegata</i>                         | 7097 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. verticillata</i>                      | 7857 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. violacea</i>                          | 7101 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. violacea</i>                          | 7102 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. viridiflora</i>                       | 7098 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. wrightii</i>                          | 8318 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. youngii</i>                           | 7865 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. zebrina</i>                           | 7867 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. zebrina</i>                           | 8305 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. zeyheri</i>                           | 7099 | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>Massonia</i>                             |      | TAAAGCTAAA | TTTTGTACCG | TATCAGGTCA | TCCTATTAGT | AAGCCGATCT    |
| <i>L. alba</i>                              | 9024 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG 749 |
| <i>L. alba</i>                              | 9100 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. algoensis</i>                         | 8210 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. aloides</i>                           | 7361 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. ameliae</i>                           | 8215 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. angelica</i>                          | 9109 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. anguinea</i>                          | 9103 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. attenuata</i>                         | 8218 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. aurioliae</i>                         | 8308 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. barkeriana</i>                        | 7715 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 7064 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATN     |
| <i>L. bifolia</i>                           | 8893 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8895 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8896 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8898 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8899 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8900 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8901 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATN     |
| <i>L. bifolia</i>                           | 8902 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8903 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8904 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8905 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8906 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bifolia</i>                           | 8907 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bolusii</i>                           | 7987 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. bowkeri</i>                           | 9111 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. capensis</i>                          | 8219 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. cernua</i>                            | 8222 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. comptonii</i>                         | 7067 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. concordiana</i>                       | 8310 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. contaminata</i>                       | 7727 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. convallarioides</i>                   | 8228 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. corymbosa</i>                         | 8229 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. dasybotrya</i>                        | 7730 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. dehoopensis</i>                       | 7731 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. doleritica</i>                        | 8230 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. duncanii</i>                          | 9107 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. elegans</i>                           | 7734 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. ensifolia</i>                         | 9101 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 7876 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. ensifolia</i> subsp. <i>maughanii</i> | 9102 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. fistulosa</i>                         | 7357 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. fistulosa</i>                         | 7362 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. flava</i>                             | 7060 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. framesii</i>                          | 8241 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. glauca</i>                            | 8319 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. haarlemensis</i>                      | 9110 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. hirta</i>                             | 6858 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. hirta</i>                             | 6891 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. inconspicua</i>                       | 9095 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. isopetala</i>                         | 8244 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. juncifolia</i>                        | 8245 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |
| <i>L. juncifolia</i>                        | 8246 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG     |

|                                             |      |            |            |            |            |           |
|---------------------------------------------|------|------------|------------|------------|------------|-----------|
| <i>L. karooica</i>                          | 7754 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. karoopoortensis</i>                   | 9108 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. klinghardtiana</i>                    | 7756 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. kliprandensis</i>                     | 8247 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. lactosa</i>                           | 8248 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. latimeriae</i>                        | 7761 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. longibracteata</i>                    | 7768 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. longituba</i>                         | 9105 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. magentea</i>                          | 8313 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. magregoriorum</i>                     | 7772 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. margaretae</i>                        | 7773 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. marginata</i>                         | 9096 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. marlothii</i>                         | 7776 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. martinae</i>                          | 8254 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. mathewsii</i>                         | 6938 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. maximiliani</i>                       | 8257 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. sp.1</i>                              | 7080 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. sp.1</i>                              | 7082 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. mediana</i> subsp.                    | 9030 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGAATATG |
| <i>L. mediana</i> subsp.                    | 9031 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGAATATG |
| <i>L. sp.1</i>                              | 9032 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. mediana</i> subsp.                    | 9033 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGAATATG |
| <i>L. mediana</i> subsp. <i>rogersii</i>    | 7079 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGAATATG |
| <i>L. membranacea</i>                       | 9106 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. moniliformis</i>                      | 7884 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. multifolia</i>                        | 8263 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. mutabilis</i>                         | 8264 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. namaquensis</i>                       | 7332 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. namibiensis</i>                       | 8266 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. namibiensis</i>                       | 9112 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. nardousbergensis</i>                  | 8267 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. neilii</i>                            | 7795 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. nervosa</i>                           | 9091 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. obscura</i>                           | 9035 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. obscura</i>                           | 9093 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. obscura</i>                           | 9094 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. orchoides</i> subsp. <i>orchoides</i> | 7805 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. orthopetala</i>                       | 7335 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. orthopetala</i>                       | 7358 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. pallida</i>                           | 8284 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. pallida</i>                           | 8312 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. patula</i>                            | 7363 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. paucifolia</i>                        | 8277 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. peersii</i>                           | 7355 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. peersii</i>                           | 8278 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. perryae</i>                           | 6961 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. polypodantha</i>                      | 8280 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. punctata</i>                          | 7093 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. punctata</i>                          | 7344 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. punctata</i>                          | 8909 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. punctata</i>                          | 8911 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. punctata</i>                          | 8914 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. purpureo-caerulea</i>                 | 6939 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. purpureo-caerulea</i>                 | 7074 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. purpureo-caerulea</i>                 | 7354 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. purpureo-caerulea</i>                 | 7365 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. pusilla</i>                           | 9092 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. pusilla</i>                           | 9098 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. quadricolor</i>                       | 7359 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. rosea</i>                             | 7343 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. rosea</i>                             | 8286 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. salteri</i>                           | 8289 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. schelpei</i>                          | 9089 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. sessiliflora</i>                      | 8314 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. sp.2</i>                              | 8131 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. sp.</i>                               | 9090 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. stayneri</i>                          | 8316 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. suaveolens</i>                        | 7884 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. thomasiae</i>                         | 8292 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. trichophylla</i>                      | 8293 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. trichophylla</i>                      | 9097 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. undulata</i>                          | 8317 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. unifolia</i>                          | 6862 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. unifolia</i>                          | 6863 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. schlechteri</i>                       | 6865 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. schlechteri</i>                       | 6866 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |
| <i>L. unifolia</i>                          | 6867 | GGGCCGATTT | ATCAGATTCT | GATATTCTTG | ATCGATTCCG | TCGGATATG |



```

L._unifolia_6869 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._unifolia_6870 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._unifolia_6871 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._unifolia_6873 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._unifolia_6874 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._unifolia_6878 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._schlechteri_6880 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._valeriae_9104 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._variegata_7097 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._verticillata_7857 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._violacea_7101 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._violacea_7102 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._viridiflora_7098 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._wrightii_8318 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._youngii_7865 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._zebrina_7867 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._zebrina_8305 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
L._zeyheri_7099 GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCGG TCGGATATG
Massonia GGGCCGATTT ATCAGATTCT GATATTCTTG ATCGATTCAG TCGGATATG

```

Appendix F4 – *Lachenalia psbA-trnH*

|                            |            |            |            |            |            |     |
|----------------------------|------------|------------|------------|------------|------------|-----|
| <i>L._bifolia_7064</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA | 50  |
| <i>L._bifolia_7322</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8898</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8899</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8900</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8902</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8903</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8904</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8905</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8906</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_8907</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_9116</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._framesi_8241</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTTTTTA |     |
| <i>L._mediana_9027</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTTTTTA |     |
| <i>L._sp._9028</i>         | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._sp._9029</i>         | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._mediana_9030</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCCC | CCACCAATAG | CTTGTT--TA |     |
| <i>L._mediana_9031</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCCC | CCACCAATAG | CTTGTT--TA |     |
| <i>L._sp._9032</i>         | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._mediana_9033</i>     | GAATCCTTGG | AATTCAAGGA | GCTATCCCCC | CCACCAATAG | CT-GTT--TA |     |
| <i>L._mediana_9034</i>     | NGATCCTTG- | -ATTCAAGGA | GCTATCCCCC | CCACCAATAG | CTTGTT--TA |     |
| <i>L._namaquensis_6970</i> | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----A---- | -TTGTTTTTA |     |
| <i>L._orthopetala_8273</i> | GAATCCTT-G | AATTCAAGGA | GCTGTCCCC- | -----AATAG | TTTGTT--TA |     |
| <i>L._pallida_8284</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTTTTTA |     |
| <i>L._pallida_8294</i>     | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTTTTTA |     |
| <i>L._patula_8276</i>      | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._perryae_9040</i>     | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAG | CTTGTT--TT |     |
| <i>L._perryae_9036</i>     | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._perryae_9039</i>     | GAATCTTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAG | CTTGTT--TT |     |
| <i>L._punctata_8909</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._punctata_8910</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._punctata_8911</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._punctata_8914</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._punctata_9042</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._schlechteri_6880</i> | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._splendida_8290</i>   | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----A---- | -TTGTTTTTA |     |
| <i>L._unifolia_6861</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6862</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6863</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._schlechteri_6865</i> | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._schlechteri_6866</i> | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6867</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6869</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6870</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAG | CTTGTT--TA |     |
| <i>L._unifolia_6871</i>    | GAATCC---- | --TTGAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6873</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6874</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6879</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6881</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_6902</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._unifolia_9038</i>    | GAATCCTT-G | AATTCAAGGA | GCTATCCCC- | -----AATAT | TTTGTT--TA |     |
| <i>L._bifolia_7064</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA | 100 |
| <i>L._bifolia_7322</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8898</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8899</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8900</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8902</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8903</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8904</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8905</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8906</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_8907</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._bifolia_9116</i>     | CGAAAACAAG | CTATTCTATT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |
| <i>L._framesi_8241</i>     | CGAAAACAAG | CTA-----TT | G--GGG---- | -ACAGCTC-C | TGCCAATGAA |     |

```

L._mediana_9027 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._sp._9028 CAAAAACAAG CTA-----TT G--GGG---- -ACAGCTCCT T---AATTAA
L._sp._9029 CAAAAACAAG CTA-----TT G--GGG---- -ACAGCTCCT T---AATTAA
L._mediana_9030 CGAAAACAAG CTATTG-GTG GGTGGGGATA GATAGCCC-C TGCCAATGAA
L._mediana_9031 CGAAAACAAG CTA-----TT GGTGGGGATA GATAGCCC-C TGCCAATGAA
L._sp._9032 CAAAAACAAG CTA-----TT G--GGG---- -ACAGCTCCT T---AAGTAA
L._mediana_9033 CGAAAACAAG CTATTG--GT G--GGGGATA GATAGCCC-C TGCCAATGAA
L._mediana_9034 CGAAAACAAG CTA-----TT G--GTGGATA GATAGCCC-C TGC-AATGAA
L._namaquensis_6970 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._orthopetala_8273 CGAAAACAAG CTA-----TT G--GGG---- -ACAG-TC-C TGCCAATGAA
L._pallida_8284 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._pallida_8294 CGAAAACAAG CTA-----TT G--GGG---- -ATAGCTC-C TGCCAATGAA
L._patula_8276 CGAAAACAAG CTA-----TT G--GGG---- -A-----C TGCCAATGAA
L._perryae_9040 CGTAAACAAA ATA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._perryae_9036 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCC-----AA
L._perryae_9039 CGTAAACAAA ATA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._punctata_8909 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._punctata_8910 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._punctata_8911 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._punctata_8914 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._punctata_9042 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._schlechteri_6880 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCCCC TGCCAATGAA
L._splendida_8290 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCTC-C TGCCAATGAA
L._unifolia_6861 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6862 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6863 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._schlechteri_6865 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._schlechteri_6866 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCCCC TGCCAATGAA
L._unifolia_6867 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6869 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6870 CGAAAACAAG CTA-----TT G--GGG---- -ATAGCCC-C TGCCAATGAA
L._unifolia_6871 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6873 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6874 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_6879 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCCCC TGCCAATGAA
L._unifolia_6881 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCCCC TGCCAATGAA
L._unifolia_6902 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA
L._unifolia_9038 CGAAAACAAG CTA-----TT G--GGG---- -ACAGCCC-C TGCCAATGAA

L._bifolia_7064 TGATTAA--- --ATTA ACTA TTTA----- ---AAATTAA CGACGAGATT 150
L._bifolia_7322 TGATTAAATT AAATTAACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._bifolia_8898 TGATTAAATT AAATTAACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._bifolia_8899 TGATTAAATT AAATTAACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._bifolia_8900 TGATTAAATT AAATTAACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._bifolia_8902 TGATTAA--- --ATTA ACTA TTTA----- ---AAATTAA CGACGAGATT
L._bifolia_8903 TGATTAA--- --ATTA ACTA TTTA----- ---AAATTAA CGACGAGATT
L._bifolia_8904 TGATTAA--- --ATTA ACTA TTT----- --CAAATTAA CGACGAGATT
L._bifolia_8905 TGATTAA--- --ATTA ACTA TTT----- --CAAATTAA CGACGAGATT
L._bifolia_8906 TGATTAAATT AAATTAACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._bifolia_8907 TGATTAA--- --ATTA ACTA TTT----- --CAAATTAA CGACGAGATT
L._bifolia_9116 TGATTAA--- --ATTA ACTA TTT----- --CAAATTAA CGACGAGATT
L._framesi_8241 TGATTAA--- --AGTATCTT ----- -CCAAATTAA CGACGAGATT
L._mediana_9027 TGATTAA--- --AGTATCTT ----- -CCAAATTAA CGACGAGATT
L._sp._9028 TTATT----- ----- -CCAAATTAA CGACGAGATT
L._sp._9029 TTATT----- ----- -CCAAATTAA CGACGAGATT
L._mediana_9030 TGATTAA--- --AGTATCTA TTTATTCCAA ACCAAATTAA CGACGAGATT
L._mediana_9031 TGATTAA--- --AGTATCTA TTTATTCCAA ACCAAATTAA CGACGAGATT
L._sp._9032 TTATT----- ----- -CCAAATTAA CGACGAGATT
L._mediana_9033 TGATTAA--- --AGTATCTA TTTATTCCAA ACCAAATTAA CGACGAGATT
L._mediana_9034 TGATTAA--- --AGTATCTA TTTATTCCAA -CCAAATTAA CGACGAGA-T
L._namaquensis_6970 TGATTAA--- --AGTATCTT CCAAAT---- --CAAATTAA CGACGAGATT
L._orthopetala_8273 TGATTAAATT -AAGTATCTA TTTATTC--- --CAAATTAA CGACGAGATT
L._pallida_8284 TGATTAA--- --AGTATCTT C----- -CAAATTAA CGACGAGATT
L._pallida_8294 TGATTAA--- --AGTATCTT C----- -CAAATTAA CGACGAGATT
L._patula_8276 TGATT----- -----AACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._perryae_9040 TGATTAA--- --ATTAAGTA TTTATTC--- --CAAATTAA CGACGAGATT
L._perryae_9036 TGATT----- -----AAGTA TTTATTC--- --CAAATTAA CGACGAGATT
L._perryae_9039 TGATTAA--- --ATTAAGTA TTTATTC--- --CAAATTAA CGACGAGATT

```

```

L._punctata_8909 TGATTAA--- --ATTA ACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._punctata_8910 TGATTAA--- --ATTA ACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._punctata_8911 TGATTAA--- --ATTA ACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._punctata_8914 TGATTAA--- --ATTA ACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._punctata_9042 TGATTAA--- --ATTA ACTA TTTATTC--- --CAAATTAA CGACGAGATT
L._schlechteri_6880 TGATT----- ----AACTA TTTATTT--- --CAAATTAA CGACGAGATT
L._splendida_8290 TGATT----- --AGTATCTT C----- --CAAATTAA CGACGAGATT
L._unifolia_6861 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6862 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6863 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._schlechteri_6865 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._schlechteri_6866 TGATT----- ----AACTA TTTAATTC-- --CAAATTAA CGACGAGATT
L._unifolia_6867 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6869 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6870 TGATT----- --AGTATCTA TTTATCCAA ACCAAATTAA CGACGAGATT
L._unifolia_6871 TGATT----- ----AACTA TTTTAT--- ----AATTAA CGACGAGATT
L._unifolia_6873 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6874 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_6879 TGATT----- ----AACTA TTTAATTC-- --CAAATTAA CGACGAGATT
L._unifolia_6881 TGATT----- ----AACTA TTTAATTC-- --CAAATTAA CGACGAGATT
L._unifolia_6902 TGATT----- ----AACTA TTTAATTTAT ----AATTAA CGACGAGATT
L._unifolia_9038 TGATT----- ----AACTA TTTAATTTAG ----AATTAA CGACGAGATT

L._bifolia_7064 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT 200
L._bifolia_7322 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8898 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8899 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8900 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8902 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8903 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8904 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8905 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8906 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_8907 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._bifolia_9116 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._framesi_8241 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._mediana_9027 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._sp._9028 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._sp._9029 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._mediana_9030 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._mediana_9031 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._sp._9032 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._mediana_9033 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._mediana_9034 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._namaquensis_6970 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._orthopetala_8273 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._pallida_8284 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._pallida_8294 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._patula_8276 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._perryae_9040 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._perryae_9036 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._perryae_9039 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._punctata_8909 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._punctata_8910 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._punctata_8911 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._punctata_8914 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._punctata_9042 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._schlechteri_6880 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._splendida_8290 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6861 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6862 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6863 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._schlechteri_6865 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._schlechteri_6866 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6867 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6869 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6870 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT
L._unifolia_6871 TATTATCGTT TCTCGCATGT CTCGCGAAAG TCAGAGTAGG CGCGAATTCT

```

|                            |            |            |            |            |            |     |
|----------------------------|------------|------------|------------|------------|------------|-----|
| <i>L._unifolia_6873</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
| <i>L._unifolia_6874</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
| <i>L._unifolia_6879</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
| <i>L._unifolia_6881</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
| <i>L._unifolia_6902</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
| <i>L._unifolia_9038</i>    | TATTATCGTT | TCTCGCATGT | CTCGCGAAAG | TCAGAGTAGG | CGCGAATTCT |     |
|                            |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC | 250 |
| <i>L._bifolia_7322</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8898</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8899</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8900</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8902</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8903</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8904</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8905</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8906</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_8907</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._bifolia_9116</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._framesi_8241</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._mediana_9027</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._sp._9028</i>         | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._sp._9029</i>         | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._mediana_9030</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._mediana_9031</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._sp._9032</i>         | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._mediana_9033</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._mediana_9034</i>     | CCCA-TTGT  | GA-CCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._namaquensis_6970</i> | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._orthopetala_8273</i> | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._pallida_8284</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._pallida_8294</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._patula_8276</i>      | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._perryae_9040</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._perryae_9036</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._perryae_9039</i>     | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._punctata_8909</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._punctata_8910</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._punctata_8911</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._punctata_8914</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._punctata_9042</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._schlechteri_6880</i> | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._splendida_8290</i>   | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6861</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6862</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6863</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._schlechteri_6865</i> | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._schlechteri_6866</i> | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6867</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6869</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6870</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6871</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6873</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6874</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6879</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6881</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_6902</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
| <i>L._unifolia_9038</i>    | CCCAATTGT  | GACCCACCAT | ACGATCTGTT | ATATAAATAG | GAAAATGTTC |     |
|                            |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG | 300 |
| <i>L._bifolia_7322</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8898</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8899</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8900</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8902</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8903</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8904</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |
| <i>L._bifolia_8905</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG | GGTATAATGG |     |

|                            |            |            |            |             |            |     |
|----------------------------|------------|------------|------------|-------------|------------|-----|
| <i>L._bifolia_8906</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._bifolia_8907</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._bifolia_9116</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._framesi_8241</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._mediana_9027</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._sp._9028</i>         | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._sp._9029</i>         | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._mediana_9030</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._mediana_9031</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._sp._9032</i>         | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._mediana_9033</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._mediana_9034</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._namaquensis_6970</i> | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._orthopetala_8273</i> | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._pallida_8284</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._pallida_8294</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._patula_8276</i>      | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._perryae_9040</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._perryae_9036</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._perryae_9039</i>     | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._punctata_8909</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._punctata_8910</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._punctata_8911</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._punctata_8914</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._punctata_9042</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._schlechteri_6880</i> | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._splendida_8290</i>   | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6861</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6862</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6863</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._schlechteri_6865</i> | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._schlechteri_6866</i> | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6867</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6869</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6870</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6871</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6873</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6874</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6879</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6881</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_6902</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
| <i>L._unifolia_9038</i>    | CTTTCCATTA | TGAATAGCGA | TTGTATGGCC | AATCATTTTG  | GGTATAATGG |     |
|                            |            |            |            |             |            | 350 |
| <i>L._bifolia_7064</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_7322</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8898</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8899</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8900</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8902</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8903</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8904</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8905</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8906</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_8907</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._bifolia_9116</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._framesi_8241</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._mediana_9027</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._sp._9028</i>         | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._sp._9029</i>         | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._mediana_9030</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._mediana_9031</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._sp._9032</i>         | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._mediana_9033</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._mediana_9034</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._namaquensis_6970</i> | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._orthopetala_8273</i> | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._pallida_8284</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |
| <i>L._pallida_8294</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTTCCTCCTC | CCTCATGTTG |     |

|                            |            |            |            |            |            |     |
|----------------------------|------------|------------|------------|------------|------------|-----|
| <i>L._patula_8276</i>      | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._perryae_9040</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._perryae_9036</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._perryae_9039</i>     | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._punctata_8909</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._punctata_8910</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._punctata_8911</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._punctata_8914</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._punctata_9042</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._schlechteri_6880</i> | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._splendida_8290</i>   | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6861</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6862</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6863</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._schlechteri_6865</i> | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._schlechteri_6866</i> | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6867</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6869</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6870</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6871</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6873</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6874</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6879</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6881</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_6902</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
| <i>L._unifolia_9038</i>    | TAGATGCCCG | AGACCAAGTT | ACTATTATTT | CTTCTCCTC  | CCTCATGTTG |     |
|                            |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT | 400 |
| <i>L._bifolia_7322</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8898</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8899</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8900</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8902</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8903</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8904</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8905</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8906</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_8907</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._bifolia_9116</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._framesi_8241</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._mediana_9027</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._sp._9028</i>         | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._sp._9029</i>         | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._mediana_9030</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._mediana_9031</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._sp._9032</i>         | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._mediana_9033</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._mediana_9034</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._namaquensis_6970</i> | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._orthopetala_8273</i> | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._pallida_8284</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._pallida_8294</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._patula_8276</i>      | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._perryae_9040</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._perryae_9036</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._perryae_9039</i>     | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._punctata_8909</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._punctata_8910</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._punctata_8911</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._punctata_8914</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._punctata_9042</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._schlechteri_6880</i> | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._splendida_8290</i>   | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._unifolia_6861</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._unifolia_6862</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._unifolia_6863</i>    | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._schlechteri_6865</i> | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |
| <i>L._schlechteri_6866</i> | AGTTTTTCAA | TTTTTCTAGA | TAAATGATTA | GCTACAAAAG | GATTTTTTTT |     |

```

L._unifolia_6867 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6869 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6870 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6871 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6873 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6874 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6879 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6881 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_6902 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT
L._unifolia_9038 AGTTTTTCAA TTTTCTAGA TAAATGATTA GCTACAAAAG GATTTTTTTT

L._bifolia_7064 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT 450
L._bifolia_7322 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8898 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8899 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8900 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8902 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8903 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8904 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8905 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8906 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_8907 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._bifolia_9116 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._framesi_8241 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._mediana_9027 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TT-TAAAGAT
L._sp._9028 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._sp._9029 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._mediana_9030 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTCCA TTTTAAAGAT
L._mediana_9031 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTCCA TTTTAAAGAT
L._sp._9032 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._mediana_9033 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTCCA TTTTAAAGAT
L._mediana_9034 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTCCA TTTTAAAGAT
L._namaquensis_6970 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._orthopetala_8273 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._pallida_8284 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._pallida_8294 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._patula_8276 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._perryae_9040 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._perryae_9036 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._perryae_9039 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._punctata_8909 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._punctata_8910 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._punctata_8911 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._punctata_8914 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._punctata_9042 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._schlechteri_6880 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._splendida_8290 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6861 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6862 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6863 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._schlechteri_6865 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._schlechteri_6866 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6867 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6869 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6870 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTCCA TTTTAAAGAT
L._unifolia_6871 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6873 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6874 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6879 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6881 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_6902 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT
L._unifolia_9038 TAGTGAACGT GTCACAGCCG ATTACTCC-T TTTTTTTTACA TTTTAAAGAT

L._bifolia_7064 TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG 500
L._bifolia_7322 TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG
L._bifolia_8898 TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG
L._bifolia_8899 TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG
L._bifolia_8900 TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG

```



*L.\_bifolia\_8902* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_8903* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_8904* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_8905* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_8906* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_8907* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_bifolia\_9116* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_framesi\_8241* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_mediana\_9027* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_sp.\_9028* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_sp.\_9029* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_mediana\_9030* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_mediana\_9031* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_sp.\_9032* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_mediana\_9033* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_mediana\_9034* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_namaquensis\_6970* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_orthopetala\_8273* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_pallida\_8284* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_pallida\_8294* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_patula\_8276* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_perryae\_9040* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_perryae\_9036* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_perryae\_9039* TGGCATTCTA TGTCCAATAG AATATAATA- ATATCCCGAT CTAAGCTATG  
*L.\_punctata\_8909* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_punctata\_8910* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_punctata\_8911* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_punctata\_8914* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_punctata\_9042* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_schlechteri\_6880* NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
*L.\_splendida\_8290* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6861* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6862* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6863* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_schlechteri\_6865* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_schlechteri\_6866* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6867* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6869* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6870* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6871* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6873* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6874* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6879* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6881* TGGCATTCTA TGTCCAATAG AATATAATAA ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_6902* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG  
*L.\_unifolia\_9038* TGGCATTCTA TGTCCAATAG AATATAATAG ATATCCCGAT CTAAGCTATG

*L.\_bifolia\_7064* AAGGTAAGAA TAAATACAT 519  
*L.\_bifolia\_7322* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8898* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8899* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8900* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8902* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8903* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8904* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8905* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8906* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_8907* AAGGTAAGAA TAAATACAT  
*L.\_bifolia\_9116* AAGGTAAGAA TAAATACAT  
*L.\_framesi\_8241* AAGGTAAGAA TAAATACAT  
*L.\_mediana\_9027* AAGGTAAGAA TAAATACAT  
*L.\_sp.\_9028* AAGGTAAGAA TAAATACAT  
*L.\_sp.\_9029* AAGGTAAGAA TAAATACAT  
*L.\_mediana\_9030* AAGGTAAGAA TAAATACAT  
*L.\_mediana\_9031* AAGGTAAGAA TAAATACAT  
*L.\_sp.\_9032* AAGGTAAGAA TAAATACAT  
*L.\_mediana\_9033* AAGGTAAGAA TAAATACAT  
*L.\_mediana\_9034* AAGGTAAGAA TAAATACAT

```

L._namaquensis_6970 AAGGTAAGAA TAAATACAT
L._orthopetala_8273 AAGGTAAGAA TAAATACAT
  L._pallida_8284 AAGGTAAGAA TAAATACAT
  L._pallida_8294 AAGGTAAGAA TAAATACAT
  L._patula_8276 AAGGTAAGAA TAAATACAT
  L._perryae_9040 AAGGTAAGAA TAAATACAT
  L._perryae_9036 AAGGTAAGAA TAAATACAT
  L._perryae_9039 AAGGT-AGAA TAAATACAT
L._punctata_8909 AAGGTAAGAA TAAATACAT
L._punctata_8910 AAGGTAAGAA TAAATACAT
L._punctata_8911 AAGGTAAGAA TAAATACAT
L._punctata_8914 AAGGTAAGAA TAAATACAT
L._punctata_9042 AAGGTAAGAA TAAATACAT
L._schlechteri_6880 NNNNNNNNNN NNNNNNNNNN
  L._splendida_8290 AAGGTAAGAA TAAATACAT
  L._unifolia_6861 AAGGTAAGAA TAAATACAT
  L._unifolia_6862 AAGGTAAGAA TAAATACAT
  L._unifolia_6863 AAGGTAAGAA TAAATACAT
L._schlechteri_6865 AAGGTAAGAA TAAATACAT
L._schlechteri_6866 AAGGTAAGAA TAAATACAT
  L._unifolia_6867 AAGGTAAGAA TAAATACAT
  L._unifolia_6869 AAGGTAAGAA TAAATACAT
  L._unifolia_6870 AAGGTAAGAA TAAATACAT
  L._unifolia_6871 AAGGTAAGAA TAAATACAT
  L._unifolia_6873 AAGGTAAGAA TAAATACAT
  L._unifolia_6874 AAGGTAAGAA TAAATACAT
  L._unifolia_6879 AAGGTAAGAA TAAATACAT
  L._unifolia_6881 AAGGTAAGAA TAAATACAT
  L._unifolia_6902 AAGGT-AGAA TAAATACAT
  L._unifolia_9038 AAGGTAAGAA TAAATACAT

```

## Appendix F5 – *Lachenalia rbcLa*

[illegible]

[illegible]

|                            |            |            |            |            |            |     |
|----------------------------|------------|------------|------------|------------|------------|-----|
| <i>L._sp.1_7081</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._sp.1_7082</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._sp.1_9028</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._sp.1_9029</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._sp.1_9032</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._sp.2_8131</i>        | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6861</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6862</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6863</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6867</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6869</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6870</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6871</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6873</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6874</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
| <i>L._unifolia_6878</i>    | CAGGGGCTGC | GGTAGCTGCC | GAATCTTCTA | CTGGTACATG | GACAACTGTG |     |
|                            |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA | 200 |
| <i>L._bifolia_8893</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8895</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8896</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8898</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8899</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8900</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8901</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8902</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8903</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8904</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8905</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8906</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._bifolia_8907</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._mediana_7078</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._mediana_9030</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._mediana_9031</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._mediana_9033</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._pallida_7089</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._pallida_8284</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._pallida_9089</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._pallida_9113</i>     | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._patula_7363</i>      | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_7093</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_7344</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_8909</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_8910</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_8911</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._punctata_8914</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._schlechteri_6865</i> | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._schlechteri_6866</i> | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._schlechteri_6880</i> | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_7080</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_7081</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_7082</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_9028</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_9029</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.1_9032</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._sp.2_8131</i>        | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6861</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6862</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6863</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6867</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6869</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6870</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6871</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6873</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6874</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
| <i>L._unifolia_6878</i>    | TGGACCGATG | GACTTACCAG | TCTTGATCGT | TACAAAGGAC | GATGCTACCA |     |
|                            |            |            |            |            |            |     |
| <i>L._bifolia_7064</i>     | CATTGAGGCC | GTTGTTGGAG | AAGAAAATCA | ATATATTGCT | TATGTAGCTT | 250 |

[illegible]



```

L._sp.1_9032 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._sp.2_8131 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6861 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6862 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6863 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6867 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6869 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6870 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6871 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6873 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6874 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA
L._unifolia_6878 ATTGTGGGTA ATGTATTTGG TTTCAAAGCC CTACGAGCTC TACGTCTGGA

L._bifolia_7064 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC 400
L._bifolia_8893 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8895 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8896 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8898 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8899 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8900 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8901 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8902 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8903 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8904 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8905 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8906 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._bifolia_8907 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._mediana_7078 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._mediana_9030 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._mediana_9031 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._mediana_9033 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._pallida_7089 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._pallida_8284 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._pallida_9089 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._pallida_9113 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._patula_7363 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_7093 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_7344 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_8909 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_8910 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_8911 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._punctata_8914 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._schlechteri_6865 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._schlechteri_6866 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._schlechteri_6880 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_7080 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_7081 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_7082 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_9028 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_9029 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.1_9032 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._sp.2_8131 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6861 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6862 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6863 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6867 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6869 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6870 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6871 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6873 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6874 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC
L._unifolia_6878 GGATCTGCGA ATTCCCCCTT CTTATTCCAA AACTTTCCAA GGCCCGCCCC

L._bifolia_7064 ATGGCATCCA AGTTGAAAGA GA 422
L._bifolia_8893 ATGGCATCCA AGTTGAAAGA GA
L._bifolia_8895 ATGGCATCCA AGTTGAAAGA GA
L._bifolia_8896 ATGGCATCCA AGTTGAAAGA GA
L._bifolia_8898 ATGGCATCCA AGTTGAAAGA GA

```



*L.\_bifolia\_8899* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8900* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8901* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8902* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8903* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8904* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8905* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8906* ATGGCATCCA AGTTGAAAGA GA  
*L.\_bifolia\_8907* ATGGCATCCA AGTTGAAAGA GA  
*L.\_mediana\_7078* ATGGCATCCA AGTTGAAAGA GA  
*L.\_mediana\_9030* ATGGCATCCA AGTTGAAAGA GA  
*L.\_mediana\_9031* ATGGCATCCA AGTTGAAAGA GA  
*L.\_mediana\_9033* ATGGCATCCA AGTTGAAAGA GA  
*L.\_pallida\_7089* ATGGCATCCA AGTTGAAAGA GA  
*L.\_pallida\_8284* ATGGCATCCA AGTTGAAAGA GA  
*L.\_pallida\_9089* ATGGCATCCA AGTTGAAAGA GA  
*L.\_pallida\_9113* ATGGCATCCA AGTTGAAAGA GA  
*L.\_patula\_7363* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_7093* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_7344* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_8909* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_8910* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_8911* ATGGCATCCA AGTTGAAAGA GA  
*L.\_punctata\_8914* ATGGCATCCA AGTTGAAAGA GA  
*L.\_schlechteri\_6865* ATGGCATCCA AGTTGAAAGA GA  
*L.\_schlechteri\_6866* ATGGCATCCA AGTTGAAAGA GA  
*L.\_schlechteri\_6880* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_7080* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_7081* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_7082* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_9028* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_9029* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.1\_9032* ATGGCATCCA AGTTGAAAGA GA  
*L.\_sp.2\_8131* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6861* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6862* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6863* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6867* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6869* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6870* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6871* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6873* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6874* ATGGCATCCA AGTTGAAAGA GA  
*L.\_unifolia\_6878* ATGGCATCCA AGTTGAAAGA GA

Appendix F6 – *Lachenalia trnL-F*

|                                                  |            |            |            |            |            |     |
|--------------------------------------------------|------------|------------|------------|------------|------------|-----|
| <i>L. aloides</i> _7698                          | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT | 50  |
| <i>L. bifolia</i> _6932                          | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. bifolia</i> _7322                          | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. bifolia</i> _7720                          | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. bifolia</i> _8903                          | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. carnososa</i> _7723                        | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. flava</i> _7060                            | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. flava</i> _7699                            | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. mediana</i> _subsp._ <i>mediana</i> _7780  | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079 | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7782 | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. orthopetala</i> _7807                      | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. pallida</i> _7809                          | AAAACTAGAC | TAGAATAAAA | AAAA---GGG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. pallida</i> _7823                          | AAAACTAGAC | TAGAATAAAA | AAAA---GGG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. pallida</i> _7844                          | AAAACTAGAC | TAGAATAAAA | AAAA---GGG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. patula</i> _7363                           | AAAACTAGAC | TAGAAAAAAA | A-----GG   | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. patula</i> _7811                           | AAAACTAGAC | TAGAAAAAAA | A-----GG   | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. perryae</i> _7814                          | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. perryae</i> _9036                          | NNNNNNNNNN | NNNNNNNNNN | NNNNNNNNNG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. punctata</i> _7344                         | AAA-----AC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. punctata</i> _7829                         | AAA-----AC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. sp.</i> _9028                              | NNAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _6869                         | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _6870                         | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _6871                         | AAAACTAGAC | TAGAATAAAA | AAA---GGG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _6873                         | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _6874                         | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. schlechteri</i> _6880                      | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. unifolia</i> _7849                         | AAAACTAGAC | TAGAATAAAA | AAAA---GG  | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. vanzylliae</i> _7707                       | AAAACTAGAC | TAGAATAAAA | AAAAA---GG | GATAGGTGCA | GAGACTCAAT |     |
| <i>M. depressa</i> _7120                         | AAAACTAGAC | TAGAATAAAA | A-----GG   | GATAGGTGCA | GAGACTCAAT |     |
| <i>M. echinata</i> _7871                         | AAAACTAGAC | TAGAATAAAA | A-----GG   | GATAGGTGCA | GAGACTCAAT |     |
| <i>M. jasminiflora</i> _7115                     | AAAACTAGAC | TAGAATAAAA | A-----GG   | GATAGGTGCA | GAGACTCAAT |     |
| <i>L. aloides</i> _7698                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT | 100 |
| <i>L. bifolia</i> _6932                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. bifolia</i> _7322                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. bifolia</i> _7720                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. bifolia</i> _8903                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. carnososa</i> _7723                        | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CG-----    | ---TTATAAT |     |
| <i>L. flava</i> _7060                            | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. flava</i> _7699                            | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. mediana</i> _subsp._ <i>mediana</i> _7780  | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079 | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7782 | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. orthopetala</i> _7807                      | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CGTTGACGTT | GTGTTATAAT |     |
| <i>L. pallida</i> _7809                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CG-----    | ---TTATAAT |     |
| <i>L. pallida</i> _7823                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CG-----    | ---TTATAAT |     |
| <i>L. pallida</i> _7844                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CG-----    | ---TTATAAT |     |
| <i>L. patula</i> _7363                           | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. patula</i> _7811                           | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>L. perryae</i> _7814                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. perryae</i> _9036                          | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. punctata</i> _7344                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CGTT-----  | --ATAATAAA |     |
| <i>L. punctata</i> _7829                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACTA | CGTT-----  | --ATAATAAA |     |
| <i>L. sp.</i> _9028                              | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _6869                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _6870                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _6871                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _6873                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _6874                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. schlechteri</i> _6880                      | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. unifolia</i> _7849                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTATAAT |     |
| <i>L. vanzylliae</i> _7707                       | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GTGTTATAAT |     |
| <i>M. depressa</i> _7120                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTA-ATA |     |
| <i>M. echinata</i> _7871                         | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTA-ATA |     |
| <i>M. jasminiflora</i> _7115                     | GGAAGCTGTT | CTAACGAATG | GAGTTGACT- | -----ACGTT | GCGTTA-ATA |     |
| <i>L. aloides</i> _7698                          | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG | 150 |
| <i>L. bifolia</i> _6932                          | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. bifolia</i> _7322                          | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. bifolia</i> _7720                          | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. bifolia</i> _8903                          | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. carnososa</i> _7723                        | -AACAGGAAT | AGAATA---  | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |

|                                                  |            |            |            |            |            |     |
|--------------------------------------------------|------------|------------|------------|------------|------------|-----|
| <i>L. flava</i> _7060                            | -----      | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. flava</i> _7699                            | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. mediana</i> _subsp._ <i>mediana</i> _7780  | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGCG |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079 | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGCG |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7782 | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGCG |     |
| <i>L. orthopetala</i> _7807                      | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. pallida</i> _7809                          | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. pallida</i> _7823                          | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. pallida</i> _7844                          | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. patula</i> _7363                           | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. perryae</i> _7811                          | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. perryae</i> _7814                          | AACAGGAAT  | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. perryae</i> _9036                          | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. punctata</i> _7344                         | TAACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. punctata</i> _7829                         | TAACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. sp.</i> _9028                              | -AACAGGAAT | AGAATA---- | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>L. unifolia</i> _6869                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGG  |     |
| <i>L. unifolia</i> _6870                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGCG |     |
| <i>L. unifolia</i> _6871                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGG  |     |
| <i>L. unifolia</i> _6873                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGG  |     |
| <i>L. unifolia</i> _6874                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGNG |     |
| <i>L. schlechteri</i> _6880                      | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGGG |     |
| <i>L. unifolia</i> _7849                         | -AACAGGAAT | A-----     | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGG  |     |
| <i>L. vanzylliae</i> _7707                       | -AACAGGAAT | AGAATAATCA | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>M. depressa</i> _7120                         | -AACAGGA-- | -----      | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>M. echinata</i> _7871                         | -AACAGGA-- | -----      | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
| <i>M. jasminiflora</i> _7115                     | -AACAGGA-- | -----      | ATCCTTCTAT | CGAAATTAAA | GA-AAGGGTG |     |
|                                                  |            |            |            |            |            |     |
| <i>L. aloides</i> _7698                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC | 200 |
| <i>L. bifolia</i> _6932                          | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. bifolia</i> _7322                          | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. bifolia</i> _7720                          | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. bifolia</i> _8903                          | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. carnosa</i> _7723                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. flava</i> _7060                            | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. flava</i> _7699                            | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. mediana</i> _subsp._ <i>mediana</i> _7780  | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079 | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7782 | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. orthopetala</i> _7807                      | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. pallida</i> _7809                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. pallida</i> _7823                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. pallida</i> _7844                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. patula</i> _7363                           | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. patula</i> _7811                           | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. perryae</i> _7814                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. perryae</i> _9036                          | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. punctata</i> _7344                         | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. punctata</i> _7829                         | ACCTA----- | ATATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. sp.</i> _9028                              | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _6869                         | ACCTA----- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _6870                         | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _6871                         | ACCTA----- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _6873                         | ACCTA----- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _6874                         | ACCTATATA- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. schlechteri</i> _6880                      | ACCTA----- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. unifolia</i> _7849                         | ACCTA----- | -TATATCTAA | TACGTACGT- | ---ATACTGA | CATATCAAAC |     |
| <i>L. vanzylliae</i> _7707                       | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>M. depressa</i> _7120                         | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>M. echinata</i> _7871                         | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
| <i>M. jasminiflora</i> _7115                     | ACCTA----- | -TATATCTAA | TACGTACGTA | TACATACTGA | CATATCAAAC |     |
|                                                  |            |            |            |            |            |     |
| <i>L. aloides</i> _7698                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT | 250 |
| <i>L. bifolia</i> _6932                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. bifolia</i> _7322                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. bifolia</i> _7720                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. bifolia</i> _8903                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. carnosa</i> _7723                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. flava</i> _7060                            | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. flava</i> _7699                            | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. mediana</i> _subsp._ <i>mediana</i> _7780  | AATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7079 | AATTAATCAT | GACCCGAATC | CATATATTAT | ATATATATAC | AC----GTAT |     |
| <i>L. mediana</i> _subsp._ <i>rogersii</i> _7782 | AATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. orthopetala</i> _7807                      | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. pallida</i> _7809                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. pallida</i> _7823                          | GATTAATCAT | GACCCGAATC | CATGTAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. pallida</i> _7844                          | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |

|                                                |            |            |            |            |            |     |
|------------------------------------------------|------------|------------|------------|------------|------------|-----|
| <i>L. patula</i> _7363                         | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. patula</i> _7811                         | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. perryae</i> _7814                        | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. perryae</i> _9036                        | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. punctata</i> _7344                       | GATTAATCAT | GACCCAAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. punctata</i> _7829                       | GATTAATCAT | GACCCAAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. sp.</i> _9028                            | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. unifolia</i> _6869                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. unifolia</i> _6870                       | AATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>L. unifolia</i> _6871                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. unifolia</i> _6873                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. unifolia</i> _6874                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. schlechteri</i> _6880                    | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. unifolia</i> _7849                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ACGTATGTAT |     |
| <i>L. vanzyliae</i> _7707                      | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | AC----GTAT |     |
| <i>M. depressa</i> _7120                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ATG-----   |     |
| <i>M. echinata</i> _7871                       | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ATG-----   |     |
| <i>M. jasminiflora</i> _7115                   | GATTAATCAT | GACCCGAATC | CATATAT--- | -TATATATAC | ATG-----   |     |
|                                                |            |            |            |            |            |     |
| <i>L. aloides</i> _7698                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG | 300 |
| <i>L. bifolia</i> _6932                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. bifolia</i> _7322                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. bifolia</i> _7720                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. bifolia</i> _8903                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. carnosa</i> _7723                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. flava</i> _7060                          | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. flava</i> _7699                          | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. mediana</i> subsp. <i>mediana</i> _7780  | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> _7079 | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> _7782 | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. orthopetala</i> _7807                    | ATGCAATATA | TGCAAAATTC | AG--TCAGAG | TGGATCTATT | CCAATCGAAG |     |
| <i>L. pallida</i> _7809                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. pallida</i> _7823                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. pallida</i> _7844                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. patula</i> _7363                         | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. patula</i> _7811                         | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. perryae</i> _7814                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. perryae</i> _9036                        | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. punctata</i> _7344                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. punctata</i> _7829                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. sp.</i> _9028                            | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. unifolia</i> _6869                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. unifolia</i> _6870                       | ATGCAATATA | TGCAAAATTC | ATAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>L. unifolia</i> _6871                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. unifolia</i> _6873                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. unifolia</i> _6874                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. schlechteri</i> _6880                    | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. unifolia</i> _7849                       | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCTATCGAAG |     |
| <i>L. vanzyliae</i> _7707                      | ATGCAATATA | TGCAAAATTC | AGAGG----- | TGGATCTATT | CCAATCGAAG |     |
| <i>M. depressa</i> _7120                       | -----TATA  | TGCAAAATTC | AGAATTATTG | TGGATCTATT | CCAATCGAAG |     |
| <i>M. echinata</i> _7871                       | -----TATA  | TGCAAAATTC | AGAATTATTG | TGGATCTATT | CCAATCGAAG |     |
| <i>M. jasminiflora</i> _7115                   | -----TATA  | TGCAAAATTC | AGAATTATTG | TGGATCTATT | CCAATCGAAG |     |
|                                                |            |            |            |            |            |     |
| <i>L. aloides</i> _7698                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- | 350 |
| <i>L. bifolia</i> _6932                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. bifolia</i> _7322                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. bifolia</i> _7720                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. bifolia</i> _8903                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. carnosa</i> _7723                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. flava</i> _7060                          | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. flava</i> _7699                          | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. mediana</i> subsp. <i>mediana</i> _7780  | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> _7079 | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> _7782 | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. orthopetala</i> _7807                    | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | T-CAGA---- |     |
| <i>L. pallida</i> _7809                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. pallida</i> _7823                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. pallida</i> _7844                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. patula</i> _7363                         | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AGTCATTCAT | TCCAGA---- |     |
| <i>L. patula</i> _7811                         | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AGTCATTCAT | TCCAGA---- |     |
| <i>L. perryae</i> _7814                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. perryae</i> _9036                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. punctata</i> _7344                       | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. punctata</i> _7829                       | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. sp.</i> _9028                            | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |
| <i>L. unifolia</i> _6869                       | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATTATTAT  | TTCAGA---- |     |
| <i>L. unifolia</i> _6870                       | TTGACGGAAG | AGTCGAATAT | TCAGTGATCA | AATCATTCAT | TCCAGA---- |     |

|                                                  |            |            |            |             |             |     |
|--------------------------------------------------|------------|------------|------------|-------------|-------------|-----|
| <i>L._unifolia</i> _6871                         | TTGACGGAAG | AATC-AATAT | TCAGTGATCA | AATTATTTCAT | TTCAGA----  |     |
| <i>L._unifolia</i> _6873                         | TTGACGGAAG | AATC-AATAT | TCAGTGATCA | AATTATTTCAT | TTCAGA----  |     |
| <i>L._unifolia</i> _6874                         | TTGACGGAAG | AATC-AATAT | TCAGTGATCA | AATTATTTCAT | TTCAGA----  |     |
| <i>L._schlechteri</i> _6880                      | TTGACGGAAG | AATC-AATAT | TCAGTGATCA | AATTATTTCAT | TTCAGA----  |     |
| <i>L._unifolia</i> _7849                         | TTGACGGAAG | AATC-AATAT | TCAGTGATCA | AATTATTTCAT | TTCAGA----  |     |
| <i>L._vanzyliae</i> _7707                        | TTGACGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT  | TCCAGA----  |     |
| <i>M._depressa</i> _7120                         | TTGATGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT  | TCCAGAGTTT  |     |
| <i>M._echinata</i> _7871                         | TTGATGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT  | TCCAGAGTTT  |     |
| <i>M._jasminiflora</i> _7115                     | TTGATGGAAG | AATCGAATAT | TCAGTGATCA | AATCATTCAT  | TCCAGAGTTT  |     |
|                                                  |            |            |            |             |             |     |
| <i>L._aloides</i> _7698                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  | 400 |
| <i>L._bifolia</i> _6932                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._bifolia</i> _7322                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._bifolia</i> _7720                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._bifolia</i> _8903                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._carnosa</i> _7723                          | -----TAG   | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._flava</i> _7060                            | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._flava</i> _7699                            | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._mediana</i> _subsp._ <i>mediana</i> _7780  | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7079 | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7782 | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._orthopetala</i> _7807                      | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._pallida</i> _7809                          | -GTTTAATAG | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._pallida</i> _7823                          | -GTTTAATAG | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._pallida</i> _7844                          | -GTTTAATAG | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._patula</i> _7363                           | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._patula</i> _7811                           | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._perryae</i> _7814                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._perryae</i> _9036                          | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._punctata</i> _7344                         | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._punctata</i> _7829                         | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._sp.</i> _9028                              | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _6869                         | -ATTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _6870                         | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _6871                         | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _6873                         | -ATTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _6874                         | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._schlechteri</i> _6880                      | -ATTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._unifolia</i> _7849                         | -ATTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>L._vanzyliae</i> _7707                        | -GTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>M._depressa</i> _7120                         | AGTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>M._echinata</i> _7871                         | AGTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
| <i>M._jasminiflora</i> _7115                     | AGTTTA---- | ATAGACCCC- | --TTTTTTGA | AAAACGTATT  | AATCGGACGA  |     |
|                                                  |            |            |            |             |             |     |
| <i>L._aloides</i> _7698                          | GAATAAAGAG | AGAGTCCGT  | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  | 450 |
| <i>L._bifolia</i> _6932                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._bifolia</i> _7322                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._bifolia</i> _7720                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._bifolia</i> _8903                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._carnosa</i> _7723                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._flava</i> _7060                            | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._flava</i> _7699                            | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._mediana</i> _subsp._ <i>mediana</i> _7780  | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7079 | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7782 | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._orthopetala</i> _7807                      | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._pallida</i> _7809                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._pallida</i> _7823                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._pallida</i> _7844                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._patula</i> _7363                           | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA? |     |
| <i>L._patula</i> _7811                           | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._perryae</i> _7814                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._perryae</i> _9036                          | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._punctata</i> _7344                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._punctata</i> _7829                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._sp.</i> _9028                              | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _6869                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _6870                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _6871                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _6873                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _6874                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._schlechteri</i> _6880                      | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._unifolia</i> _7849                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>L._vanzyliae</i> _7707                        | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>M._depressa</i> _7120                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>M._echinata</i> _7871                         | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |
| <i>M._jasminiflora</i> _7115                     | GAATAAAGAG | AGAGTCCCGT | TCTACATGTC | AATAC-CGAC  | AACAATGAAA  |     |

|                                          |      |             |             |            |            |            |     |
|------------------------------------------|------|-------------|-------------|------------|------------|------------|-----|
| <i>L. aloides</i>                        | 7698 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      | 500 |
| <i>L. bifolia</i>                        | 6932 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. bifolia</i>                        | 7322 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. bifolia</i>                        | 7720 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. bifolia</i>                        | 8903 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. carnosa</i>                        | 7723 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. flava</i>                          | 7060 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. flava</i>                          | 7699 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. mediana</i> subsp. <i>mediana</i>  | 7780 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> | 7079 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> | 7782 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. orthopetala</i>                    | 7807 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. pallida</i>                        | 7809 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. pallida</i>                        | 7823 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. pallida</i>                        | 7844 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. patula</i>                         | 7363 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. patula</i>                         | 7811 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. perryae</i>                        | 7814 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. perryae</i>                        | 9036 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. punctata</i>                       | 7344 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. punctata</i>                       | 7829 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. sp.</i>                            | 9028 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 6869 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 6870 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 6871 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 6873 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 6874 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. schlechteri</i>                    | 6880 | TTTATAGTAA  | G-AGGAAAAAT | CCGTCGACTT | TAGAAATCGT | GAGGGTTCAA |     |
| <i>L. unifolia</i>                       | 7849 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>L. vanzyliae</i>                      | 7707 | TTTATAGTAA  | G-AGGAA---  | -----      | -----      | -----      |     |
| <i>M. depressa</i>                       | 7120 | TTTATAGTAA  | G-AGGA----  | -----      | -----      | -----      |     |
| <i>M. echinata</i>                       | 7871 | TTTATAGTAA  | G-AGGA----  | -----      | -----      | -----      |     |
| <i>M. jasminiflora</i>                   | 7115 | TTTATAGTAA  | G-AGGA----  | -----      | -----      | -----      |     |
| <i>L. aloides</i>                        | 7698 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC | 550 |
| <i>L. bifolia</i>                        | 6932 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. bifolia</i>                        | 7322 | GTCCCTCTAT  | CCCCAATAAA  | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. bifolia</i>                        | 7720 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. bifolia</i>                        | 8903 | GTCCCTCTAT  | CCCCAATAAA  | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. carnosa</i>                        | 7723 | -----       | -----       | AAGATTATTT | GACTTCTTAA | CTATCTATCC |     |
| <i>L. flava</i>                          | 7060 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. flava</i>                          | 7699 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. mediana</i> subsp. <i>mediana</i>  | 7780 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> | 7079 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. mediana</i> subsp. <i>rogersii</i> | 7782 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. orthopetala</i>                    | 7807 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATCTATCC |     |
| <i>L. pallida</i>                        | 7809 | -----       | -----       | AAGATTATTT | GACTTCTTAA | CTATCTATCC |     |
| <i>L. pallida</i>                        | 7823 | -----       | -----       | AAGATTATTT | GACTTCTTAA | CTATCTATCC |     |
| <i>L. pallida</i>                        | 7844 | -----       | -----       | AAGATTATTT | GACTTCTTAA | CTATCTATCC |     |
| <i>L. patula</i>                         | 7363 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. patula</i>                         | 7811 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. perryae</i>                        | 7814 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. perryae</i>                        | 9036 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. punctata</i>                       | 7344 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. punctata</i>                       | 7829 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. sp.</i>                            | 9028 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 6869 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 6870 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 6871 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 6873 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 6874 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. schlechteri</i>                    | 6880 | GTCCCTCTAT  | CCCCAATAAA  | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. unifolia</i>                       | 7849 | -----       | -----       | AAGACCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>L. vanzyliae</i>                      | 7707 | -----       | -----       | AAGATCATTT | GACTTCTTAA | CTATTTATCC |     |
| <i>M. depressa</i>                       | 7120 | -----       | -----TAAA   | AAGACCATTT | GACTTCTTAA | CTATTTATCT |     |
| <i>M. echinata</i>                       | 7871 | -----       | -----TAAA   | AAGACCATTT | GACTTCTTAA | CTATTTATCT |     |
| <i>M. jasminiflora</i>                   | 7115 | -----       | -----TAAA   | AAGACCATTT | GACTTCTTAA | CTATTTATCT |     |
| <i>L. aloides</i>                        | 7698 | TCCC-----T  | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC | 600 |
| <i>L. bifolia</i>                        | 6932 | TCCC-----TT | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. bifolia</i>                        | 7322 | TCCC-----TT | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. bifolia</i>                        | 7720 | TCCC-----TT | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. bifolia</i>                        | 8903 | TCCC-----TT | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. carnosa</i>                        | 7723 | TCCCC-----T | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. flava</i>                          | 7060 | TCCC-----TT | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |
| <i>L. flava</i>                          | 7699 | TCCC-----T  | TTTTTTCGTA  | AGCGGTTCAA | A-TAAATTC- | AATATCTTTC |     |

|                                        |             |            |             |            |            |     |
|----------------------------------------|-------------|------------|-------------|------------|------------|-----|
| <i>L._mediana_subsp._mediana_7780</i>  | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AACCGCTTTC |     |
| <i>L._mediana_subsp._rogersii_7079</i> | TCCCC---TTT | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AACCGCTTTC |     |
| <i>L._mediana_subsp._rogersii_7782</i> | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AACCGCTTTC |     |
| <i>L._orthopetala_7807</i>             | TCCC---TT   | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._pallida_7809</i>                 | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._pallida_7823</i>                 | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._pallida_7844</i>                 | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._patula_7363</i>                  | TCCC---TT   | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._patula_7811</i>                  | TCCC---TT   | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._perryae_7814</i>                 | TCCC---TTT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._perryae_9036</i>                 | TCCC---TTT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._punctata_7344</i>                | TCCC---TTT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._punctata_7829</i>                | TCCC---TTT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._sp._9028</i>                     | TCCC---TT   | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._unifolia_6869</i>                | TCCCCCTTT   | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._unifolia_6870</i>                | TCCCC---TT  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AACCGCTTTC |     |
| <i>L._unifolia_6871</i>                | TCCCCCTTT   | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._unifolia_6873</i>                | TCCCCCTTT   | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._unifolia_6874</i>                | TCCCCCTT    | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._schlechteri_6880</i>             | TCCCCCTTT   | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._unifolia_7849</i>                | TCCCCCTTT   | TTTTTTCGTA | AGCGGTTCAAG | TTCAAAT--- | ---ATCTTTC |     |
| <i>L._vanzyliae_7707</i>               | TCCC-----T  | TTTTTTCGTA | AGCGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>M._depressa_7120</i>                | TCC-----TT  | TTTTTTCGTA | AGTGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>M._echinata_7871</i>                | TCC-----TT  | TTTTTTCGTA | AGTGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>M._jasminiflora_7115</i>            | TCC-----TT  | TTTTTTCGTA | AGTGGTTCAA  | A-TAAATTC- | AATATCTTTC |     |
| <i>L._aloides_7698</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC | 650 |
| <i>L._bifolia_6932</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._bifolia_7322</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._bifolia_7720</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._bifolia_8903</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._carnosa_7723</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._flava_7060</i>                   | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._flava_7699</i>                   | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._mediana_subsp._mediana_7780</i>  | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._mediana_subsp._rogersii_7079</i> | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._mediana_subsp._rogersii_7782</i> | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._orthopetala_7807</i>             | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._pallida_7809</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._pallida_7823</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._pallida_7844</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._patula_7363</i>                  | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._patula_7811</i>                  | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._perryae_7814</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._perryae_9036</i>                 | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._punctata_7344</i>                | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._punctata_7829</i>                | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._sp._9028</i>                     | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._unifolia_6869</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._unifolia_6870</i>                | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._unifolia_6871</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._unifolia_6873</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._unifolia_6874</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._schlechteri_6880</i>             | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._unifolia_7849</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTG---- |     |
| <i>L._vanzyliae_7707</i>               | ----TCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>M._depressa_7120</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>M._echinata_7871</i>                | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>M._jasminiflora_7115</i>            | TCATTCATTA  | TACTCTTTCG | CAAATAGATC  | CGGGCAGAAA | TCTTTGGATC |     |
| <i>L._aloides_7698</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT | 700 |
| <i>L._bifolia_6932</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._bifolia_7322</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._bifolia_7720</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._bifolia_8903</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._carnosa_7723</i>                 | TTAT----CC  | CTAT----TT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._flava_7060</i>                   | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._flava_7699</i>                   | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._mediana_subsp._mediana_7780</i>  | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._mediana_subsp._rogersii_7079</i> | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._mediana_subsp._rogersii_7782</i> | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._orthopetala_7807</i>             | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._pallida_7809</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._pallida_7823</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._pallida_7844</i>                 | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._patula_7363</i>                  | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |
| <i>L._patula_7811</i>                  | TTAT----CC  | CTATTAGGTT | T---GAATAG  | ATACCTGTAC | AAACGAACAT |     |

|                                                  |            |            |            |            |            |            |  |
|--------------------------------------------------|------------|------------|------------|------------|------------|------------|--|
| <i>L._perryae</i> _7814                          | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._perryae</i> _9036                          | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._punctata</i> _7344                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._punctata</i> _7829                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._sp.</i> _9028                              | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _6869                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _6870                         | TTAT-TATCC |            | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _6871                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _6873                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _6874                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._schlechteri</i> _6880                      | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._unifolia</i> _7849                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>L._vanzyliae</i> _7707                        | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAACGAACAT |  |
| <i>M._depressa</i> _7120                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAATGAACAT |  |
| <i>M._echinata</i> _7871                         | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAATGAACAT |  |
| <i>M._jasminiflora</i> _7115                     | TTAT----   | CC         | CTATTAGGTT | T---GAATAG | ATACCTGTAC | AAATGAACAT |  |
|                                                  |            |            |            |            |            |            |  |
| <i>L._aloides</i> _7698                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- | 750        |  |
| <i>L._bifolia</i> _6932                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._bifolia</i> _7322                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._bifolia</i> _7720                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._bifolia</i> _8903                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._carnosa</i> _7723                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._flava</i> _7060                            | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._flava</i> _7699                            | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._mediana</i> _subsp._ <i>mediana</i> _7780  | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTTACAGTC | CATATCATT  |            |  |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7079 | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTTACAGTC | CATATCATT  |            |  |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7782 | ATATGGTCAA | GGAATTCCCA | TTATTGA--- | ATTTACAGTC | CATATCATT  |            |  |
| <i>L._orthopetala</i> _7807                      | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._pallida</i> _7809                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._pallida</i> _7823                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._pallida</i> _7844                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._patula</i> _7363                           | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._patula</i> _7811                           | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._perryae</i> _7814                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._perryae</i> _9036                          | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._punctata</i> _7344                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._punctata</i> _7829                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._sp.</i> _9028                              | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._unifolia</i> _6869                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._unifolia</i> _6870                         | ATATGGTCAA | GGAATTCCCA | TTATTGA--- | ATTTACAGTC | CATATCATT  |            |  |
| <i>L._unifolia</i> _6871                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._unifolia</i> _6873                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._unifolia</i> _6874                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._schlechteri</i> _6880                      | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._unifolia</i> _7849                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>L._vanzyliae</i> _7707                        | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>M._depressa</i> _7120                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>M._echinata</i> _7871                         | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
| <i>M._jasminiflora</i> _7115                     | ATATGGTCAA | GGAATTCCCA | TTATTGAATT | ATTCACAGCC | CATATCA--- |            |  |
|                                                  |            |            |            |            |            |            |  |
| <i>L._aloides</i> _7698                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA | 800        |  |
| <i>L._bifolia</i> _6932                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._bifolia</i> _7322                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAG--- |            |  |
| <i>L._bifolia</i> _7720                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._bifolia</i> _8903                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._carnosa</i> _7723                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._flava</i> _7060                            | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._flava</i> _7699                            | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._mediana</i> _subsp._ <i>mediana</i> _7780  | CATTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7079 | CATTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._mediana</i> _subsp._ <i>rogersii</i> _7782 | CATTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._orthopetala</i> _7807                      | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._pallida</i> _7809                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._pallida</i> _7823                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._pallida</i> _7844                          | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._patula</i> _7363                           | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAG--- |            |  |
| <i>L._patula</i> _7811                           | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAG--- |            |  |
| <i>L._perryae</i> _7814                          | --TTATCCTT | ACATTCAC-A | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._perryae</i> _9036                          | --TTATCCTT | ACATTCAC-A | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._punctata</i> _7344                         | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._punctata</i> _7829                         | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._sp.</i> _9028                              | --TTATCCTT | ACATTCAC-A | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._unifolia</i> _6869                         | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._unifolia</i> _6870                         | CATTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._unifolia</i> _6871                         | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |
| <i>L._unifolia</i> _6873                         | --TTATCCTT | ACATTCACAA | AAAAAGTCTT | CTTTTGAAG  | ATCTAAGAAA |            |  |



```

    L._unifolia_6874 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAAAAA
L._schlechteri_6880 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAAAAA
    L._unifolia_7849 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAAAAA
    L._vanzyliae_7707 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAGAAA
    M._depressa_7120 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAGAAA
    M._echinata_7871 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAGAAA
M._jasminiflora_7115 --TTATCCTT ACATTCACAA AAAAAGTCTT CTTTTTGAAG ATCTAAGAAA

    L._aloides_7698 TTCGGGGACT GGG   813
    L._bifolia_6932 TTCGGGGACT GGG
    L._bifolia_7322 --CGGGGACT GGG
    L._bifolia_7720 TTCGGGGACT GGG
    L._bifolia_8903 TTCGGGGACT GGG
    L._carnosa_7723 TTCGGGGACT AGG
    L._flava_7060 TTCGGGGACT GGG
    L._flava_7699 TTCGGGGACT GGG
    L._mediana_subsp._mediana_7780 TTCGGGGACT AGG
L._mediana_subsp._rogersii_7079 TTCGGGGACT AGG
L._mediana_subsp._rogersii_7782 TTCGGGGACT AGG
    L._orthopetala_7807 TTCGGGGACT AGG
    L._pallida_7809 TTCGGGGACT AGG
    L._pallida_7823 TTCGGGGACT AGG
    L._pallida_7844 TTCGGGGACT AGG
    L._patula_7363 --CGGGGACT AGG
    L._patula_7811 --CGGGGACT AGG
    L._perryae_7814 TTCGGGGACT AGG
    L._perryae_9036 TTCGGGGACT AGG
    L._punctata_7344 TTCGGGGACT GGG
    L._punctata_7829 TTCGGGGACT GGG
    L._sp._9028 TTCGGGGACT AGG
    L._unifolia_6869 TTCGGGGACT AGG
    L._unifolia_6870 TTCGGGGACT AGG
    L._unifolia_6871 TTCGGGGACT AGG
    L._unifolia_6873 TTCGGGGACT AGG
    L._unifolia_6874 TTCGGGGACT AGG
    L._schlechteri_6880 TTCGGGGACT AGG
    L._unifolia_7849 TTCGGGGACT AGG
    L._vanzyliae_7707 TTCGGGGACT GGG
    M._depressa_7120 TTCAGGGACT AGG
    M._echinata_7871 TTCAGGGACT AGG
M._jasminiflora_7115 TTCAGGGACT AGG

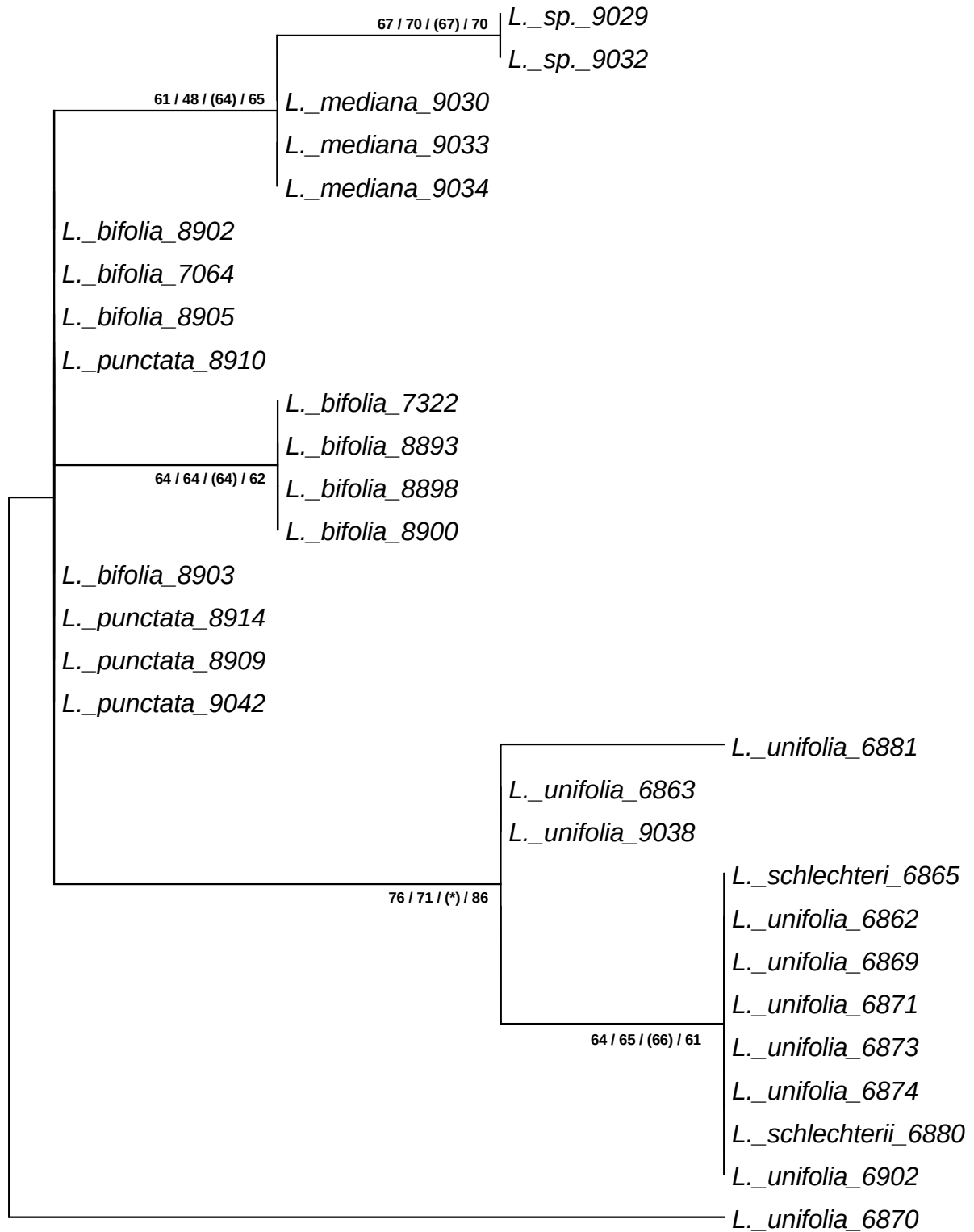
```

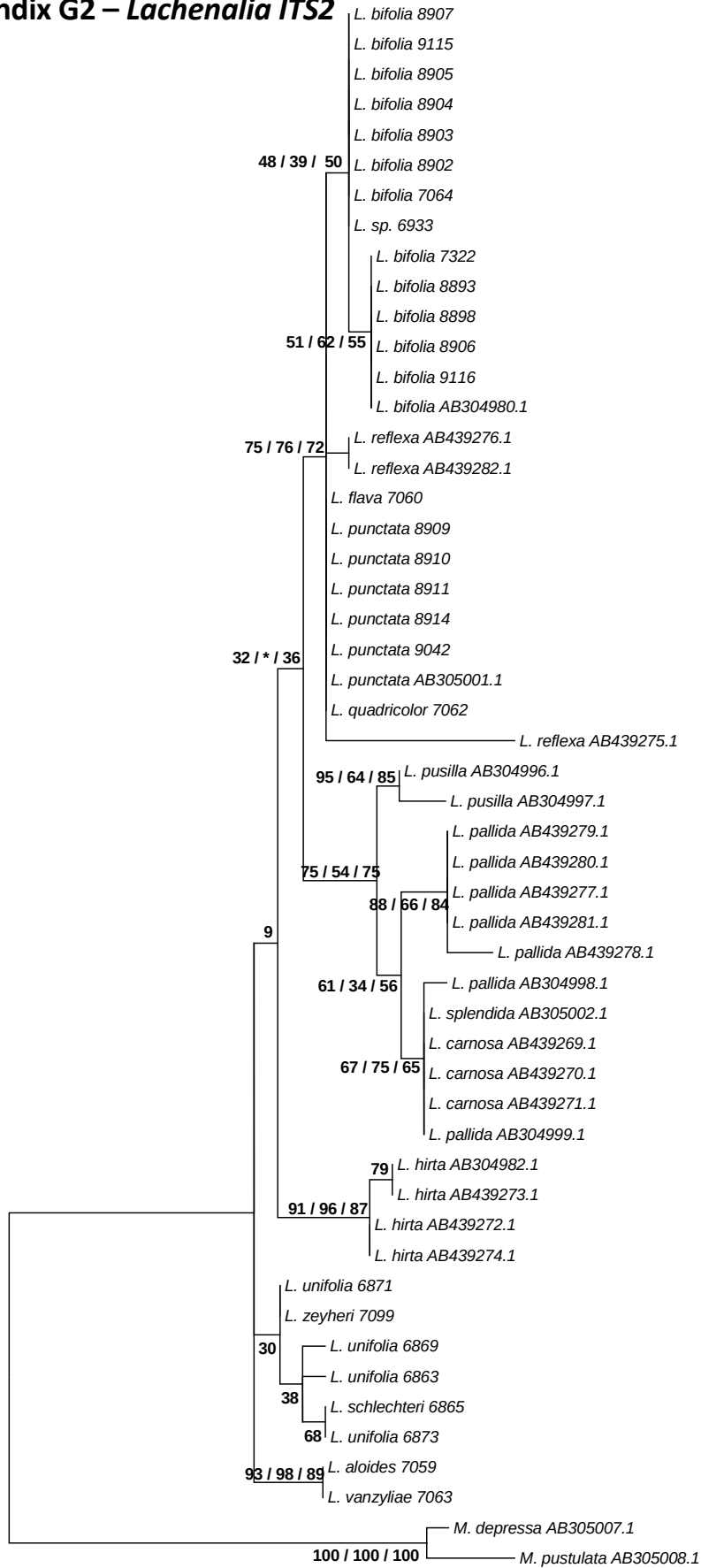
# Appendix G

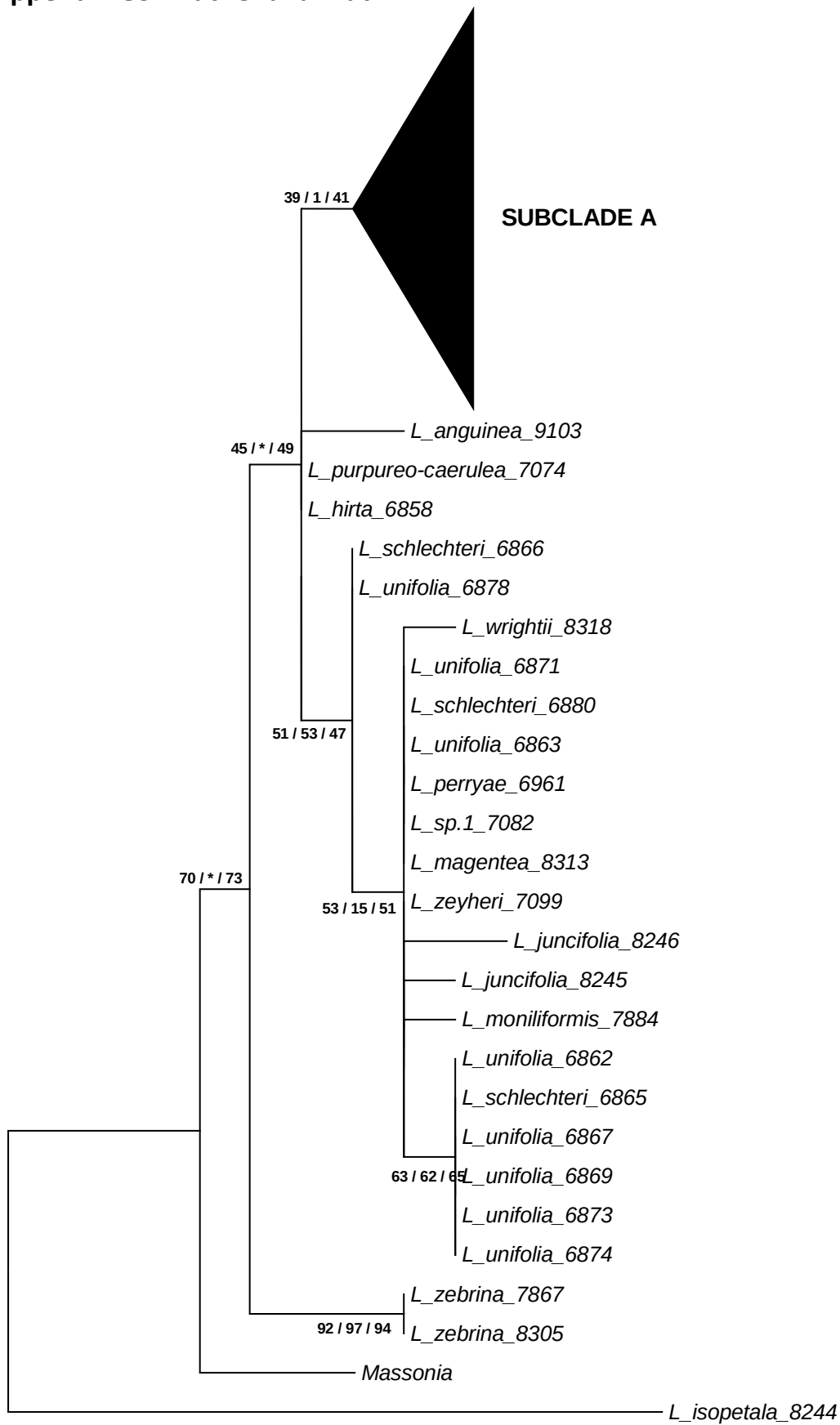
## **Lachenalia *cladograms***

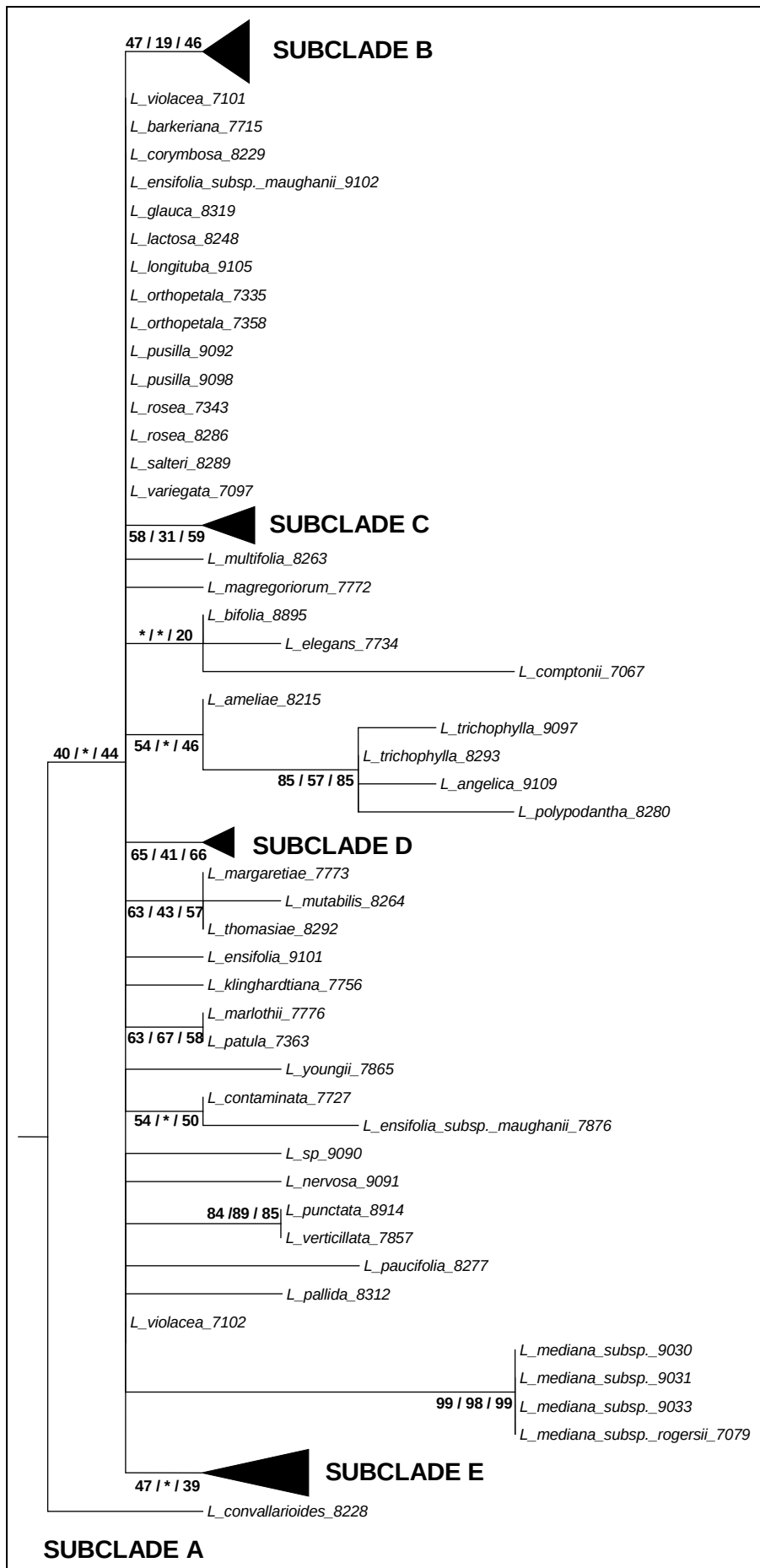
---

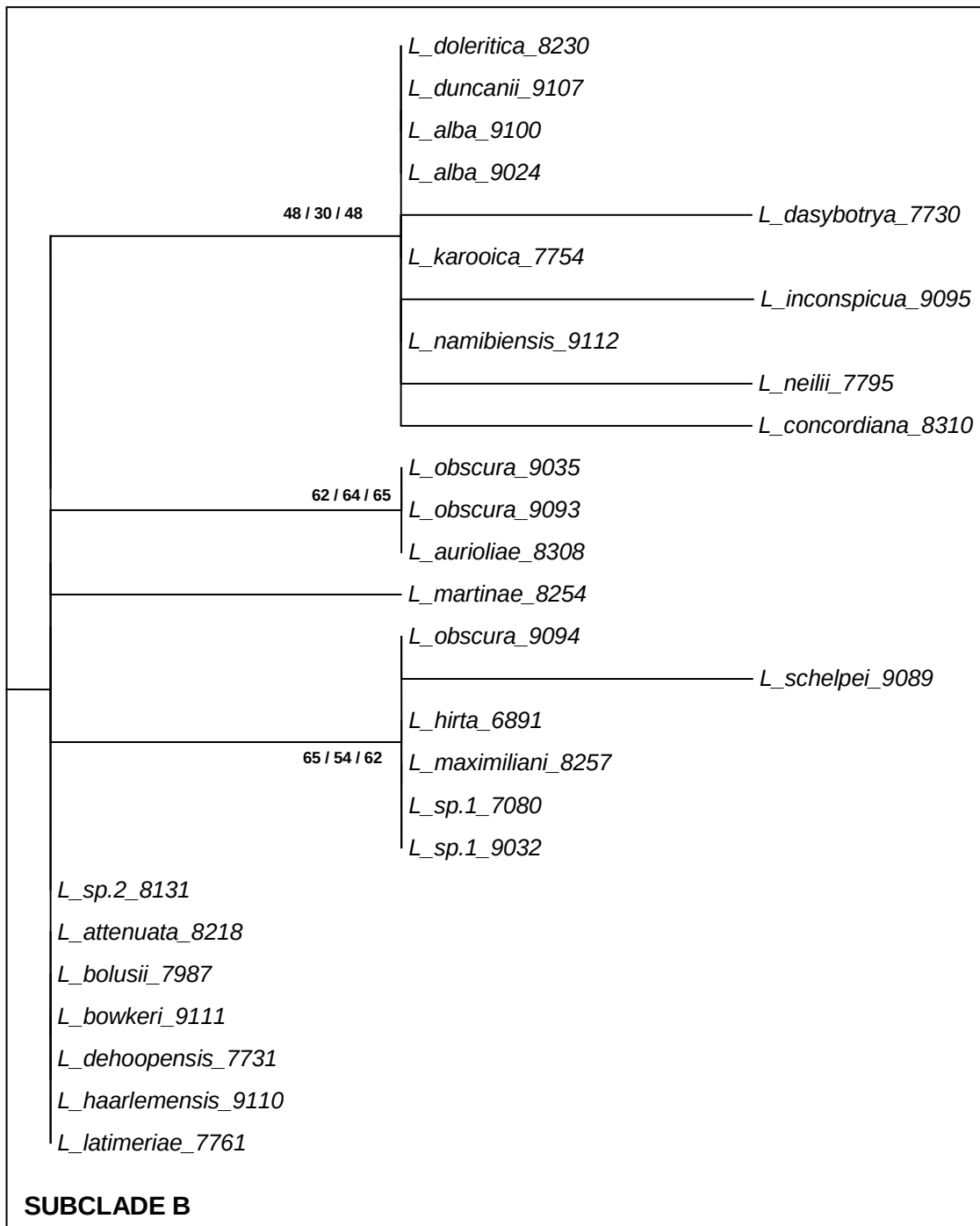
Phylogenetic trees for each gene region were constructed using the Neighbor-Joining (NJ), UPGMA, Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Since the tree topologies of the different methods were highly similar, a ML cladogram from one of the tree-drawing methods was chosen to represent the phylogenies. The bootstrap and Bayesian Inference values are next to or on the branches in the following order: NJ / UPGMA / (MP) / ML. An unsupported branch is indicated with an asterisk (\*).

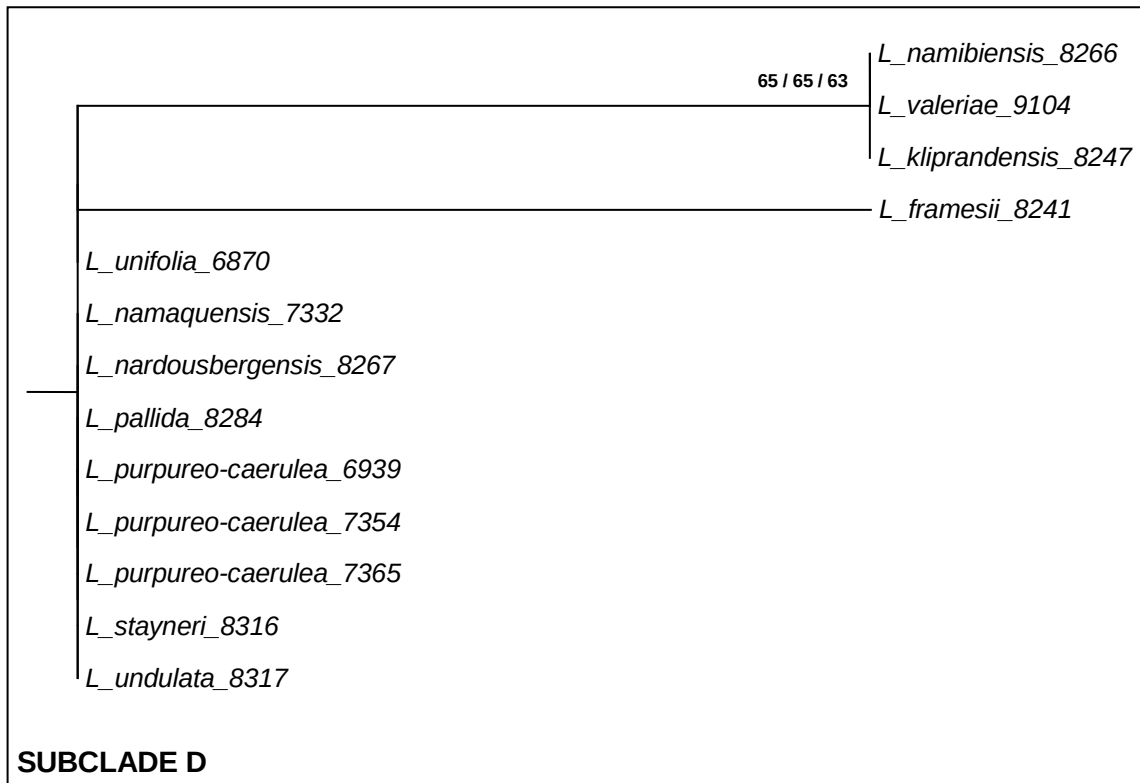
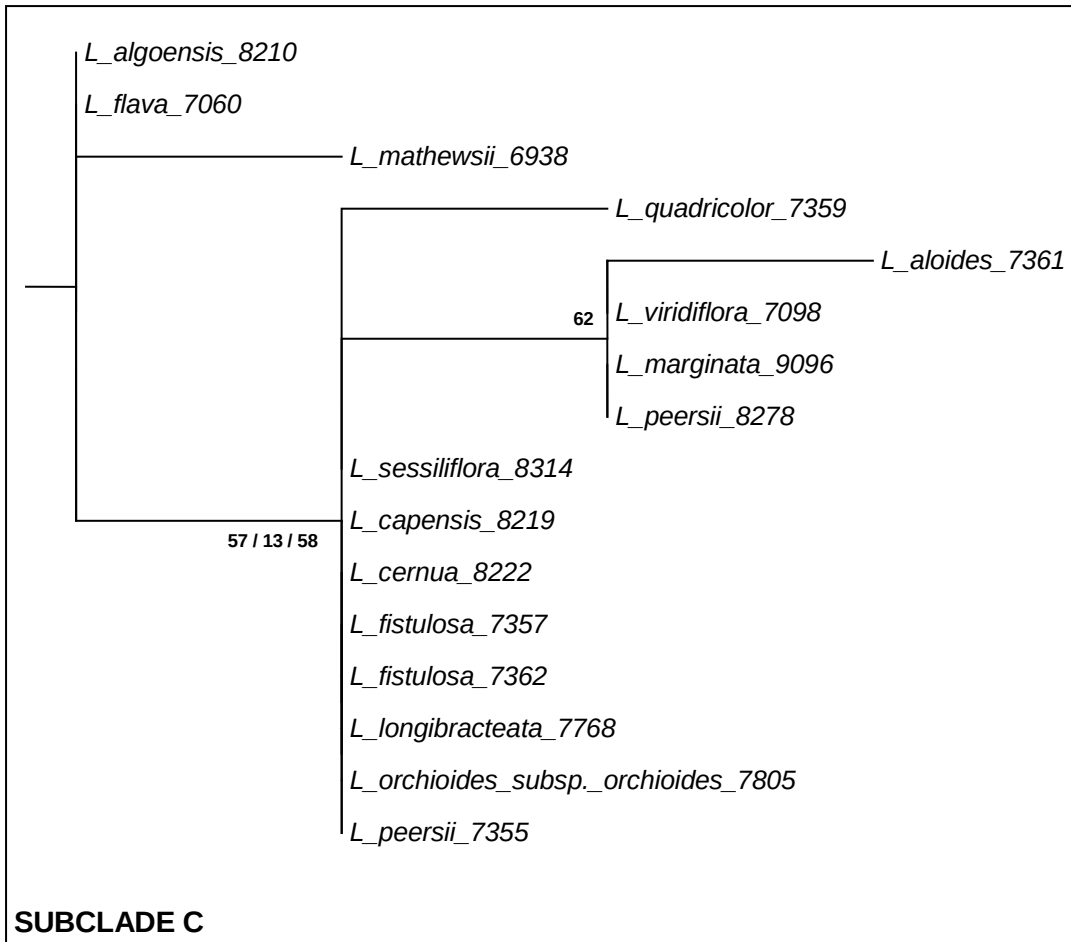
Appendix G1 – *Lachenalia* atpH-atpI

Appendix G2 – *Lachenalia* ITS2

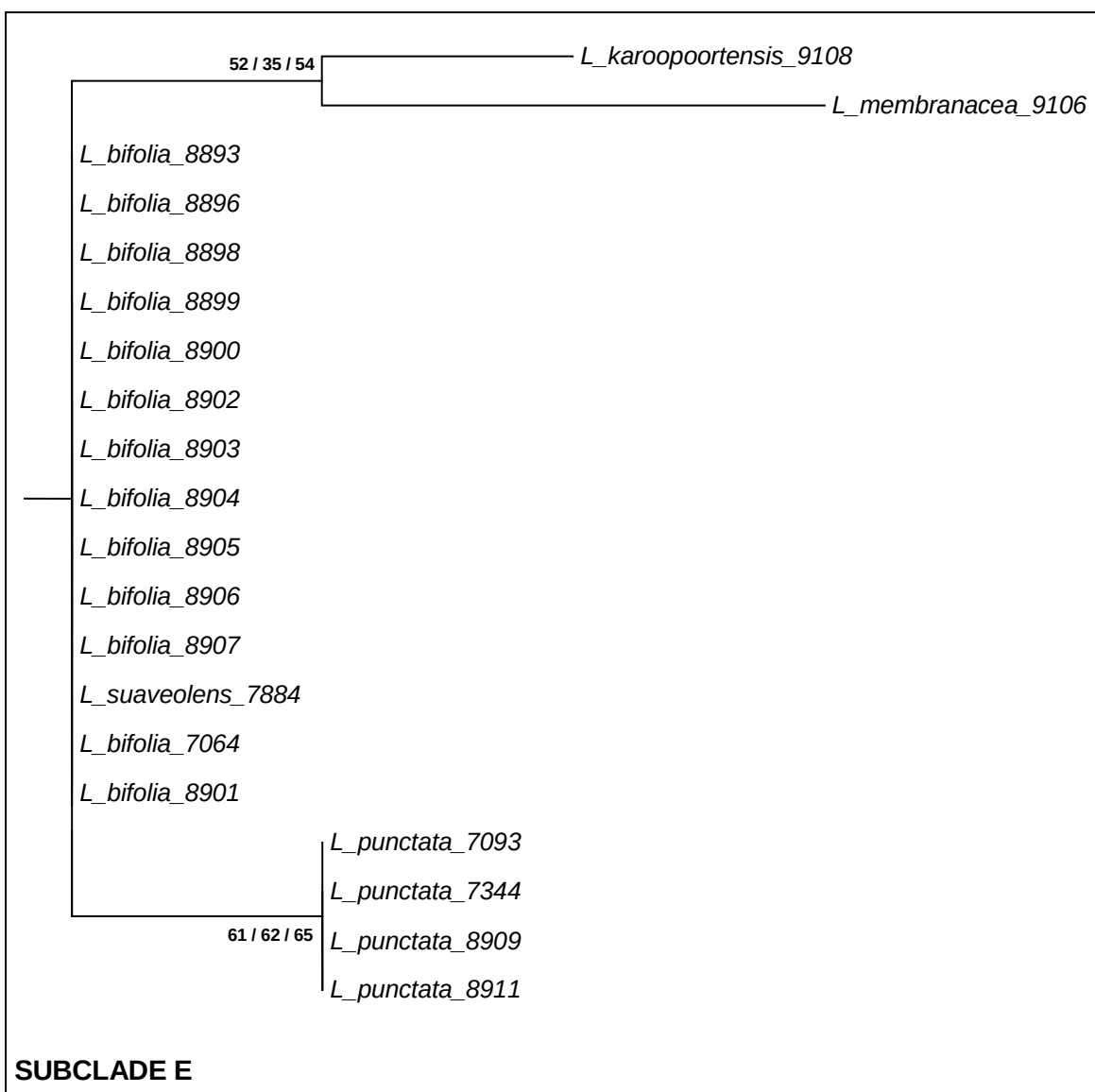
Appendix G3 – *Lachenalia* matK

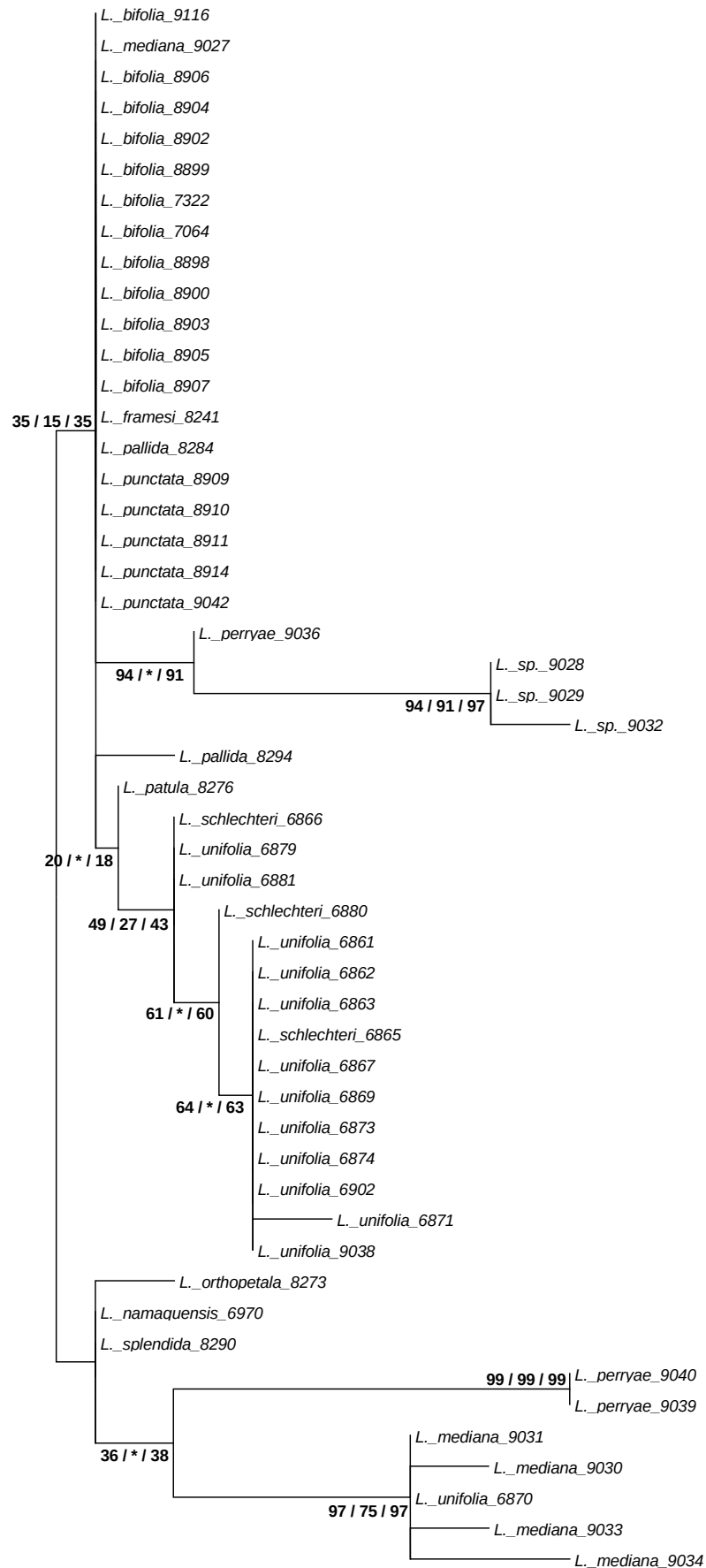


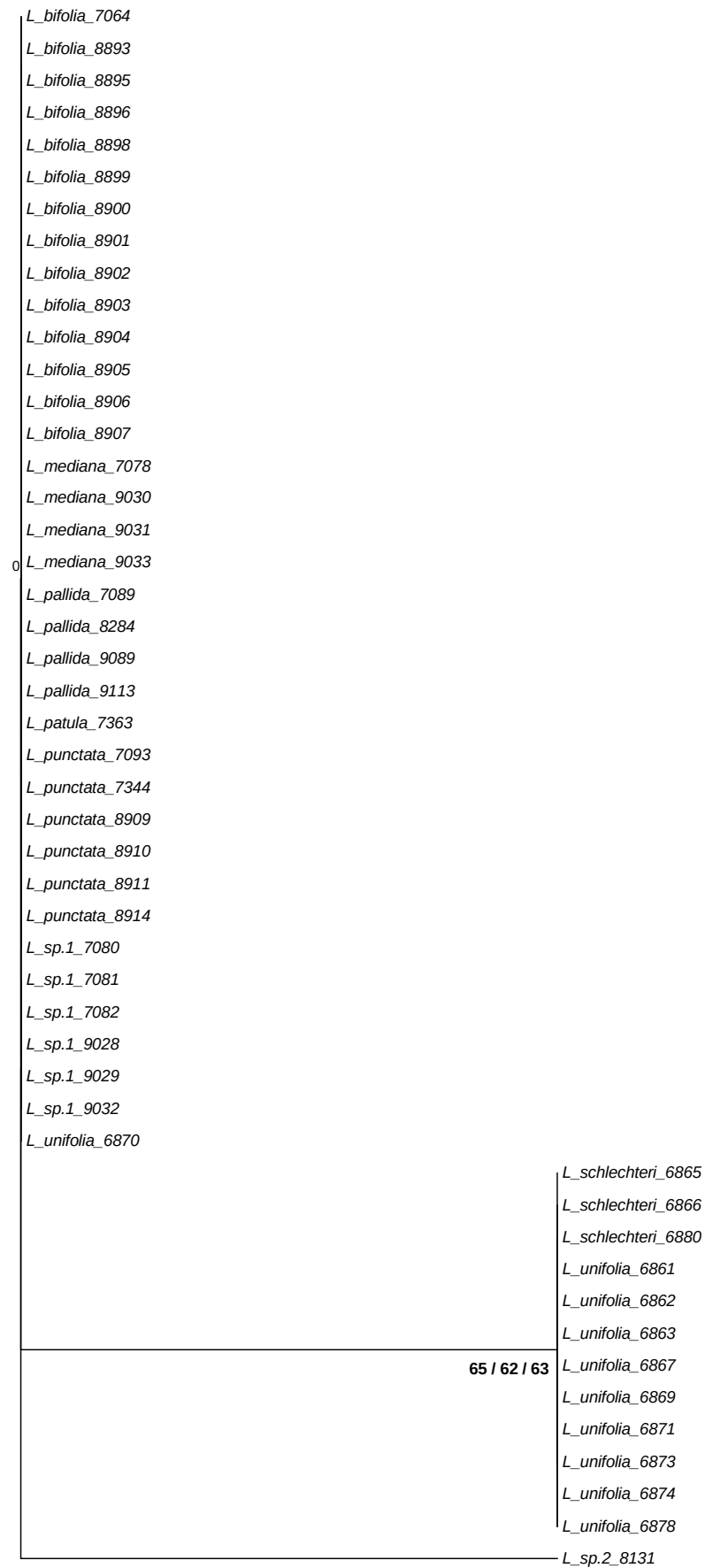


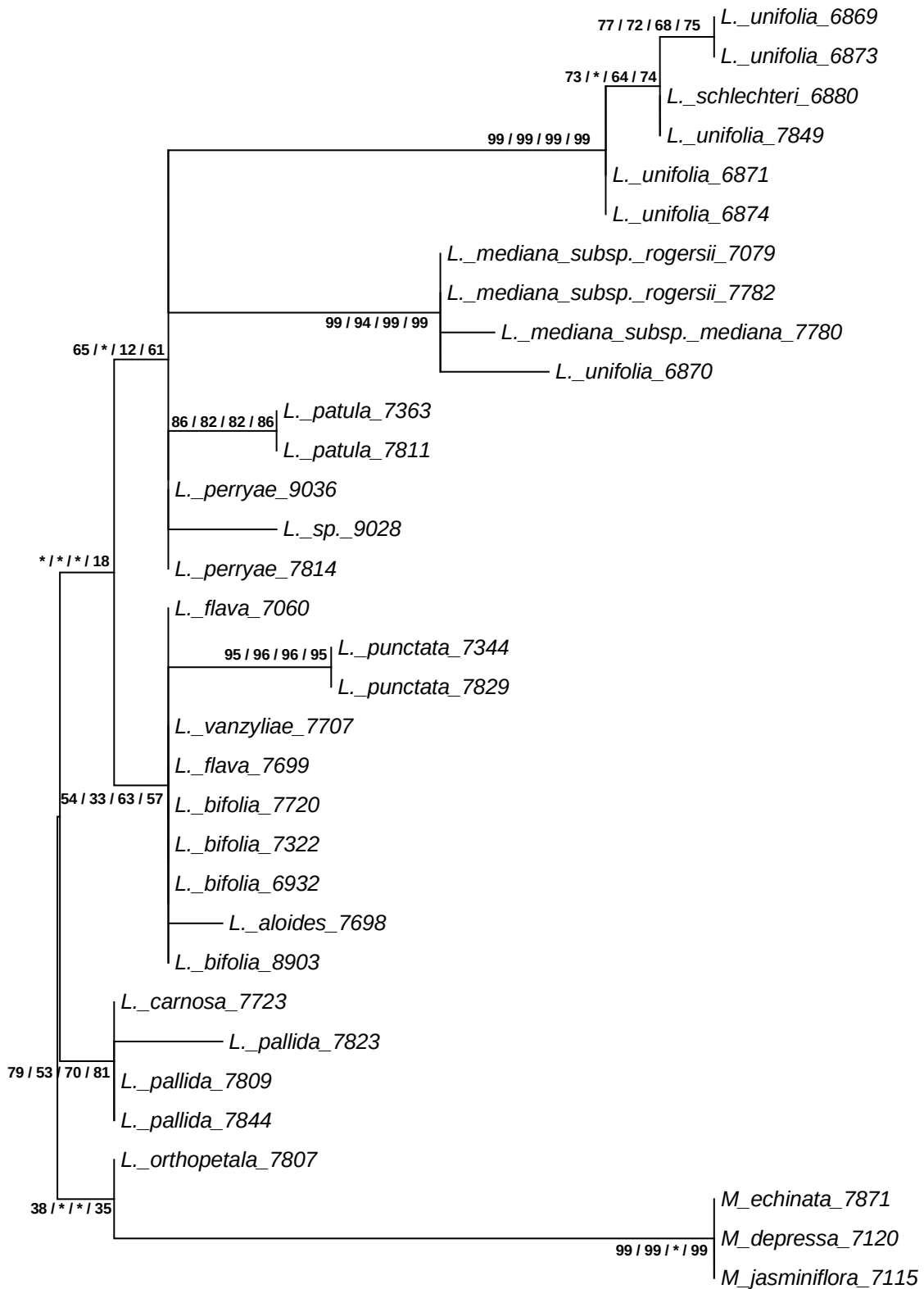






Appendix G4 – *Lachenalia psbA-trnH*

Appendix G5 – *Lachenalia* rbcLa

Appendix G6 – *Lachenalia* *trnL-F*

# Appendix H

## **Lachenalia *MOTUs***

---

The software jMOTU calculated the number of Molecular Operational Taxonomic Units (MOTUs) that are created at different cut-off values (measured in bp) from zero to 20. A separate excel spreadsheet was created for all twenty cut-off values for each gene region. These spreadsheets were manually screened. Only the selected data are represented in this appendix in the original output format from jMOTU.

The data is set out as follows:

Column 1: Genus\_region\_cut-off value (bp)\_MOTU cluster number

Column 2: The list of taxa corresponding to a MOTU (or cluster) in column 1

**Appendix H1 – *Lachenalia atpH-atpI***

35 sequences have been clustered into 10 clusters

|                   |                                     |
|-------------------|-------------------------------------|
| atpH_6bp_MOTU0001 | <i>L._bifolia_7064</i>              |
| atpH_6bp_MOTU0001 | <i>L._bifolia_7322_2n_=_42+0-1B</i> |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8893_2n_=_42</i>      |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8898_2n_=_42</i>      |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8900_2n_=_56</i>      |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8902_2n_=_28</i>      |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8903_2n_=_28</i>      |
| atpH_6bp_MOTU0001 | <i>L._bifolia_8905_2n_=_42+0-1B</i> |
| atpH_6bp_MOTU0002 | <i>L._schlechteri_6865</i>          |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6862</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6867</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6869</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6871</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6873</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6874</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6880</i>             |
| atpH_6bp_MOTU0002 | <i>L._unifolia_6902</i>             |
| atpH_6bp_MOTU0003 | <i>L._unifolia_6870</i>             |
| atpH_6bp_MOTU0004 | <i>L._perryae_9036</i>              |
| atpH_6bp_MOTU0004 | <i>L._perryae_9039</i>              |
| atpH_6bp_MOTU0005 | <i>L._mediana_9030</i>              |
| atpH_6bp_MOTU0005 | <i>L._mediana_9033</i>              |
| atpH_6bp_MOTU0005 | <i>L._mediana_9034</i>              |
| atpH_6bp_MOTU0006 | <i>L._schlechteri_6866</i>          |
| atpH_6bp_MOTU0006 | <i>L._unifolia_6881</i>             |
| atpH_6bp_MOTU0007 | <i>L._mediana_9029</i>              |
| atpH_6bp_MOTU0007 | <i>L._mediana_9032</i>              |
| atpH_6bp_MOTU0008 | <i>L._bifolia_8907_2n_=_42</i>      |
| atpH_6bp_MOTU0008 | <i>L._punctata_8909_2n_=_14**</i>   |
| atpH_6bp_MOTU0008 | <i>L._punctata_8910_2n_=_14</i>     |
| atpH_6bp_MOTU0008 | <i>L._punctata_8914_2n_=_12</i>     |
| atpH_6bp_MOTU0008 | <i>L._punctata_9042</i>             |
| atpH_6bp_MOTU0008 | <i>L._punctata_9042_2n_=_14</i>     |
| atpH_6bp_MOTU0009 | <i>L._unifolia_6863</i>             |
| atpH_6bp_MOTU0010 | <i>L._unifolia_9038</i>             |

**Appendix H2 – *Lachenalia* ITS2**

52 sequences have been clustered into 21 clusters

|                   |                                     |
|-------------------|-------------------------------------|
| ITS2_0bp_MOTU0001 | <i>L._unifolia_6869</i>             |
| ITS2_0bp_MOTU0002 | <i>L._carnosa_ AB439269.1</i>       |
| ITS2_0bp_MOTU0002 | <i>L._carnosa_ AB439270.1 </i>      |
| ITS2_0bp_MOTU0002 | <i>L._carnosa_ AB439271.1 </i>      |
| ITS2_0bp_MOTU0002 | <i>L._pallida_ AB304999.1 </i>      |
| ITS2_0bp_MOTU0002 | <i>L._splendida_ AB305002.1 </i>    |
| ITS2_0bp_MOTU0003 | <i>L._hirta_ AB304982.1 </i>        |
| ITS2_0bp_MOTU0003 | <i>L._hirta_ AB439273.1 </i>        |
| ITS2_0bp_MOTU0004 | <i>L._schlechteri_6865</i>          |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_7064</i>              |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_8902_2n_=_28</i>      |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_8903</i>              |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_8904_2n_=_42+0-1B</i> |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_8905</i>              |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_8907</i>              |
| ITS2_0bp_MOTU0005 | <i>L._bifolia_91/57/7</i>           |
| ITS2_0bp_MOTU0005 | <i>L._sp._6933AdV</i>               |
| ITS2_0bp_MOTU0006 | <i>L._pallida_ AB304998.1 </i>      |
| ITS2_0bp_MOTU0007 | <i>L._unifolia_6873</i>             |
| ITS2_0bp_MOTU0008 | <i>L._unifolia_6871AdV</i>          |
| ITS2_0bp_MOTU0008 | <i>L._zeyheri_7099AdV</i>           |
| ITS2_0bp_MOTU0009 | <i>L._pallida_ AB439277.1 </i>      |
| ITS2_0bp_MOTU0009 | <i>L._pallida_ AB439279.1 </i>      |
| ITS2_0bp_MOTU0009 | <i>L._pallida_ AB439280.1 </i>      |
| ITS2_0bp_MOTU0009 | <i>L._pallida_ AB439281.1 </i>      |
| ITS2_0bp_MOTU0010 | <i>L._reflexa_ AB439276.1 </i>      |
| ITS2_0bp_MOTU0010 | <i>L._reflexa_ AB439282.1 </i>      |
| ITS2_0bp_MOTU0011 | <i>L._unifolia_6863</i>             |
| ITS2_0bp_MOTU0012 | <i>L._pusilla_ AB304997.1 </i>      |
| ITS2_0bp_MOTU0013 | <i>L._flava_7060</i>                |
| ITS2_0bp_MOTU0013 | <i>L._punctata_8909_2n_=_14**</i>   |
| ITS2_0bp_MOTU0013 | <i>L._punctata_8910_2n_=_14</i>     |
| ITS2_0bp_MOTU0013 | <i>L._punctata_8911_2n_=_14</i>     |
| ITS2_0bp_MOTU0013 | <i>L._punctata_8914</i>             |
| ITS2_0bp_MOTU0013 | <i>L._punctata_9042</i>             |
| ITS2_0bp_MOTU0013 | <i>L._punctata_ AB305001.1 </i>     |
| ITS2_0bp_MOTU0013 | <i>L._quadricolor_7062AdV</i>       |
| ITS2_0bp_MOTU0014 | <i>M._pustulata_ AB305008.1 </i>    |
| ITS2_0bp_MOTU0015 | <i>L._hirta_ AB439272.1 </i>        |
| ITS2_0bp_MOTU0015 | <i>L._hirta_ AB439274.1 </i>        |
| ITS2_0bp_MOTU0016 | <i>L._bifolia_7322_2n_=_42+0-1B</i> |
| ITS2_0bp_MOTU0016 | <i>L._bifolia_8893</i>              |

ITS2\_0bp\_MOTU0016  
ITS2\_0bp\_MOTU0016  
ITS2\_0bp\_MOTU0016  
ITS2\_0bp\_MOTU0016  
ITS2\_0bp\_MOTU0017  
ITS2\_0bp\_MOTU0018  
ITS2\_0bp\_MOTU0019  
ITS2\_0bp\_MOTU0019  
ITS2\_0bp\_MOTU0020  
ITS2\_0bp\_MOTU0021

*L. bifolia*\_8898\_2n\_=\_42  
*L. bifolia*\_8906\_2n\_=\_49+0-3B  
*L. bifolia*\_9116  
*L. bifolia*\_|AB304980.1|  
*L. reflexa*\_|AB439275.1|  
*L. pallida*\_|AB439278.1|)  
*L. aloides*\_7059  
*L. vanzyliae*\_7063  
*L. pusilla*\_|AB304996.1|  
*M. depressa*\_|AB305007.1|



**Appendix H3 – *Lachenalia* matK**

83 sequences have been clustered into 42 clusters

|                        |                                        |
|------------------------|----------------------------------------|
| Lach_matK_1bp_MOTU0001 | <i>L._mediana_9030</i>                 |
| Lach_matK_1bp_MOTU0001 | <i>L._mediana_9031</i>                 |
| Lach_matK_1bp_MOTU0001 | <i>L._unifolia_6870_x=_11</i>          |
| Lach_matK_1bp_MOTU0002 | <i>L._punctata_7093_x=_7</i>           |
| Lach_matK_1bp_MOTU0002 | <i>L._punctata_7344_x=_7</i>           |
| Lach_matK_1bp_MOTU0002 | <i>L._punctata_8911_x=_7</i>           |
| Lach_matK_1bp_MOTU0002 | <i>L._punctata_8914_x=_6</i>           |
| Lach_matK_1bp_MOTU0002 | <i>L._punctata_9042_x=_7</i>           |
| Lach_matK_1bp_MOTU0003 | <i>L._unifolia_6867_x=_11</i>          |
| Lach_matK_1bp_MOTU0004 | <i>L._mediana_9034</i>                 |
| Lach_matK_1bp_MOTU0005 | <i>L._mathewsii_6938_x=_7</i>          |
| Lach_matK_1bp_MOTU0006 | <i>L._bifolia_West_Coast_x=_7</i>      |
| Lach_matK_1bp_MOTU0007 | <i>L._mediana_9032</i>                 |
| Lach_matK_1bp_MOTU0008 | <i>L._zeyheri_7099_x=_11</i>           |
| Lach_matK_1bp_MOTU0009 | <i>L._punctata_8910_x=_7</i>           |
| Lach_matK_1bp_MOTU0010 | <i>L._peersii_7355_x=_7</i>            |
| Lach_matK_1bp_MOTU0010 | <i>L._sessiliflora_8314_x=_7</i>       |
| Lach_matK_1bp_MOTU0011 | <i>L._aloides_JX090373.1_x=_7</i>      |
| Lach_matK_1bp_MOTU0011 | <i>L._quadricolor_7359_x=_7</i>        |
| Lach_matK_1bp_MOTU0012 | <i>L._mediana_subsp._rogersii_7079</i> |
| Lach_matK_1bp_MOTU0013 | <i>Massonia_depressa_AB017321.1</i>    |
| Lach_matK_1bp_MOTU0014 | <i>L._hirta_6858_x=_11</i>             |
| Lach_matK_1bp_MOTU0014 | <i>L._purpureo_caerulea_7074_x=_8</i>  |
| Lach_matK_1bp_MOTU0015 | <i>L._ensifolia_8908</i>               |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_7322_2n=_42+0-1B</i>     |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8893_2n=_42</i>          |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8899_2n=_56</i>          |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8900_2n=_56</i>          |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8902_2n=_28</i>          |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8903_2n=_28</i>          |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8904_2n=_421B</i>        |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8905_2n=_42+0-1B</i>     |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8907_x=_7</i>            |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_8909_x=_7</i>            |
| Lach_matK_1bp_MOTU0016 | <i>L._bifolia_9115</i>                 |
| Lach_matK_1bp_MOTU0016 | <i>L._pusilla_JX090375.1</i>           |
| Lach_matK_1bp_MOTU0017 | <i>L._namaquensis_7332_x=_8</i>        |
| Lach_matK_1bp_MOTU0017 | <i>L._pallida_6849_x=_8</i>            |
| Lach_matK_1bp_MOTU0017 | <i>L._pallida_8284_x=_8</i>            |
| Lach_matK_1bp_MOTU0017 | <i>L._purpureo_caerulea_7354_x=_8</i>  |
| Lach_matK_1bp_MOTU0017 | <i>L._purpureo_caerulea_7365_x=_8</i>  |

|                        |                                          |
|------------------------|------------------------------------------|
| Lach_matK_1bp_MOTU0017 | <i>L. reflexa</i> _7091_x =_7            |
| Lach_matK_1bp_MOTU0017 | <i>L. undulata</i> _8317_x =_10?         |
| Lach_matK_1bp_MOTU0018 | <i>L. unifolia</i> _6880_x =_11          |
| Lach_matK_1bp_MOTU0019 | <i>L. mediana</i> _7080                  |
| Lach_matK_1bp_MOTU0019 | <i>L. mediana</i> _7081                  |
| Lach_matK_1bp_MOTU0019 | <i>L. mediana</i> _7082                  |
| Lach_matK_1bp_MOTU0019 | <i>L. obscura</i> _8900_x =_9            |
| Lach_matK_1bp_MOTU0019 | <i>L. unifolia</i> _6891_x =_11          |
| Lach_matK_1bp_MOTU0020 | <i>L. ensifolia</i> _JX090374.1          |
| Lach_matK_1bp_MOTU0020 | <i>L. lactosa</i> _8248_x =_7            |
| Lach_matK_1bp_MOTU0020 | <i>L. orthopetala</i> _7335_x =_8        |
| Lach_matK_1bp_MOTU0020 | <i>L. orthopetala</i> _7358_x =_8        |
| Lach_matK_1bp_MOTU0020 | <i>L. pusilla</i> _8896_x =_7            |
| Lach_matK_1bp_MOTU0020 | <i>L. pusilla</i> _8911_x =_7            |
| Lach_matK_1bp_MOTU0020 | <i>L. rosea</i> _7343_x =_7              |
| Lach_matK_1bp_MOTU0020 | <i>L. rosea</i> _8286_x =_7              |
| Lach_matK_1bp_MOTU0020 | <i>L. variegata</i> _7097_x =_7?         |
| Lach_matK_1bp_MOTU0020 | <i>L. violacea</i> _7101_x =_7           |
| Lach_matK_1bp_MOTU0020 | <i>L. violacea</i> _7102_x =_7           |
| Lach_matK_1bp_MOTU0021 | <i>L. unifolia</i> _9038_x =_11          |
| Lach_matK_1bp_MOTU0022 | <i>L. mediana</i> _9028                  |
| Lach_matK_1bp_MOTU0023 | <i>L. mediana</i> _9029                  |
| Lach_matK_1bp_MOTU0024 | <i>L. anguinea</i> _8917_x =_15          |
| Lach_matK_1bp_MOTU0025 | <i>L. obscura</i> _8898_x =_9            |
| Lach_matK_1bp_MOTU0026 | <i>L. unifolia</i> _6869_x =_11          |
| Lach_matK_1bp_MOTU0027 | <i>L. unifolia</i> _6879_x =_11          |
| Lach_matK_1bp_MOTU0028 | <i>L. nervosa</i> _8893_x =_8            |
| Lach_matK_1bp_MOTU0029 | <i>L. perryae</i> _9039                  |
| Lach_matK_1bp_MOTU0030 | <i>Massonia</i> _sp._8918                |
| Lach_matK_1bp_MOTU0031 | <i>L. framesii</i> _8241_x =_8           |
| Lach_matK_1bp_MOTU0032 | <i>L. carnosae</i> _HM640625.1_x =_8     |
| Lach_matK_1bp_MOTU0033 | <i>L. unifolia</i> _6873_x =_11          |
| Lach_matK_1bp_MOTU0034 | <i>L. unifolia</i> _6874_x =_11          |
| Lach_matK_1bp_MOTU0035 | <i>L. peersii</i> _8921_x =_7            |
| Lach_matK_1bp_MOTU0035 | <i>L. viridiflora</i> _7098_x =_7        |
| Lach_matK_1bp_MOTU0036 | <i>Massonia angustifolia</i> _HM640627.1 |
| Lach_matK_1bp_MOTU0037 | <i>L. contaminata</i> _7727_x =_8        |
| Lach_matK_1bp_MOTU0038 | <i>L. unifolia</i> _6871_x =_11          |
| Lach_matK_1bp_MOTU0039 | <i>L. attenuata</i> _8218_x =_7          |
| Lach_matK_1bp_MOTU0040 | <i>L. schlechteri</i> _6866_x =_11       |
| Lach_matK_1bp_MOTU0041 | <i>L. unifolia</i> _6902_x =_11          |
| Lach_matK_1bp_MOTU0042 | <i>L. perryae</i> _9036                  |

**Appendix H4 – *Lachenalia* *psbA-trnH***

|                        |                                 |
|------------------------|---------------------------------|
| Lach_trnH_Obp_MOTU0001 | <i>L._mediana_9028</i>          |
| Lach_trnH_Obp_MOTU0001 | <i>L._mediana_9029</i>          |
| Lach_trnH_Obp_MOTU0001 | <i>L._patula_8276</i>           |
| Lach_trnH_Obp_MOTU0001 | <i>L._perryae_9036</i>          |
| Lach_trnH_Obp_MOTU0001 | <i>L._schlechteri_6880</i>      |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_6866</i>         |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_6871</i>         |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_6879</i>         |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_6881</i>         |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_6902</i>         |
| Lach_trnH_Obp_MOTU0001 | <i>L._unifolia_P4</i>           |
| Lach_trnH_Obp_MOTU0002 | <i>L._unifolia_6870</i>         |
| Lach_trnH_Obp_MOTU0003 | <i>L._mediana_9033</i>          |
| Lach_trnH_Obp_MOTU0004 | <i>L._mediana_9034</i>          |
| Lach_trnH_Obp_MOTU0005 | <i>L._mediana_9031</i>          |
| Lach_trnH_Obp_MOTU0006 | <i>L._mediana_9032</i>          |
| Lach_trnH_Obp_MOTU0007 | <i>L._mediana_9030</i>          |
| Lach_trnH_Obp_MOTU0008 | <i>L._punctata_8909</i>         |
| Lach_trnH_Obp_MOTU0008 | <i>L._punctata_8914</i>         |
| Lach_trnH_Obp_MOTU0008 | <i>L._punctata_9042</i>         |
| Lach_trnH_Obp_MOTU0009 | <i>L._framesi_8241</i>          |
| Lach_trnH_Obp_MOTU0009 | <i>L._pallida_8284</i>          |
| Lach_trnH_Obp_MOTU0010 | <i>L._punctata_8910</i>         |
| Lach_trnH_Obp_MOTU0010 | <i>L._punctata_8911</i>         |
| Lach_trnH_Obp_MOTU0011 | <i>L._bifolia_7322</i>          |
| Lach_trnH_Obp_MOTU0011 | <i>L._bifolia_8898</i>          |
| Lach_trnH_Obp_MOTU0011 | <i>L._bifolia_8899</i>          |
| Lach_trnH_Obp_MOTU0011 | <i>L._bifolia_8900</i>          |
| Lach_trnH_Obp_MOTU0011 | <i>L._bifolia_8906</i>          |
| Lach_trnH_Obp_MOTU0012 | <i>L._bifolia_7064</i>          |
| Lach_trnH_Obp_MOTU0012 | <i>L._bifolia_8902</i>          |
| Lach_trnH_Obp_MOTU0012 | <i>L._bifolia_8903</i>          |
| Lach_trnH_Obp_MOTU0013 | <i>L._pallida_8294</i>          |
| Lach_trnH_Obp_MOTU0014 | <i>L._mediana_9027</i>          |
| Lach_trnH_Obp_MOTU0015 | <i>L._bifolia_8904</i>          |
| Lach_trnH_Obp_MOTU0015 | <i>L._bifolia_8907</i>          |
| Lach_trnH_Obp_MOTU0016 | <i>L._bifolia_8905</i>          |
| Lach_trnH_Obp_MOTU0016 | <i>L._bifolia_9116</i>          |
| Lach_trnH_Obp_MOTU0017 | <i>L._perryae/unifolia_9040</i> |
| Lach_trnH_Obp_MOTU0018 | <i>L._perryae_9039</i>          |
| Lach_trnH_Obp_MOTU0019 | <i>L._unifolia_9038</i>         |
| Lach_trnH_Obp_MOTU0020 | <i>L._unifolia_6861</i>         |
| Lach_trnH_Obp_MOTU0020 | <i>L._unifolia_6862</i>         |
| Lach_trnH_Obp_MOTU0020 | <i>L._unifolia_6863</i>         |

Lach\_trnH\_0bp\_MOTU0020  
Lach\_trnH\_0bp\_MOTU0020  
Lach\_trnH\_0bp\_MOTU0020  
Lach\_trnH\_0bp\_MOTU0020  
Lach\_trnH\_0bp\_MOTU0020  
Lach\_trnH\_0bp\_MOTU0021  
Lach\_trnH\_0bp\_MOTU0022  
Lach\_trnH\_0bp\_MOTU0023

*L.\_unifolia\_6865*  
*L.\_unifolia\_6867*  
*L.\_unifolia\_6869*  
*L.\_unifolia\_6873*  
*L.\_unifolia\_6874*  
*L.\_splendida\_8290*  
*L.\_orthopetala\_8273*  
*L.\_namaquensis\_6970*