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HvdWesthuizen.

Hester Maria van der Westhuizen

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Genetic variation in the most primitive *Clivia* species

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Dissertation presented in order to qualify for the degree *Magister Scientiae* in the Faculty of Natural and Agricultural Sciences, Department of Genetics, at the University of the Free State

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CONTENTS

Chapter 2: Potential barcoding regions for species identification in *Clivia nobilis and Clivia mirabilis*

APPENDICES

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ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ATP	Adenosine triphosphate
atpH	Adenosine triphoshate synthase III subunit
atpI	Adenosine triphoshate synthase IV subunit
$CaCl_2$	Calcium cloride
CTAB	Cetyltrimethylammonium bromide
CBOL	Consortium for the Barcode of Life
DNA	Deoxyribonucleic acid
dH_2O	Distilled water
DMSO	Dimethyl sulfoxide
dNTPs	Deoxyribonucleotides
EDTA	Ethylenediaminetetraacetic acid
HCl	Hydrochloric acid
INDEL	Insertion or deletion
matK	*maturase K*
$MgCl_2$	Magnesium chloride
NaCl	Sodium chloride
ng	Nanograms
$ng.\mu l^{-1}$	Nanograms per microlitre
NH_4OAc	Ammonium acetate
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
RAPDs	Random amplified polymorphic DNAs
rbcL	*Ribulose-bisphosphate carboxylase*
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
rpl16	Ribosomal protein L16

rpoB	RNA polymerase B
rpoC1	RNA polymerase C1
TAE	Tris-acetate-EDTA buffer
T_a	Annealing temperature
trnF	transfer RNA gene for phenylalanine
trnK	transfer RNA gene for lysine
trnL	transfer RNA gene for leucine
$\mu g.ml^{-1}$	Micrograms per millilitre
µl	Microlitre
UV	Ultraviolet
$MgCl_2$	Magnesium chloride

Chapter 1:

Literature Review

H. M. van der Westhuizen

1.1. Abstract

The beauty of *Clivia* made these plants a favourite amongst collectors. There are seven different species within the genus *Clivia*. They are *C. nobilis*, *C. caulescens*, *C. miniata*, *C. gardenii*, *C. mirabilis*, *C. robusta* and *Clivia xnimbicola*. *Clivia nobilis* and *C. mirabilis* are believed to be the two most primitive within this genus. Although geographically distinct these two species share phenotypic characteristics. Both these species has a limited distribution range and are regarded as vulnerable.

Different techniques were considered for use during this study. They were restriction fragment length polymorphism, random amplified polymorphic DNA, amplified fragment length polymorphisms, microsatellite markers, single nucleotide polymorphisms and sequencing. Sequencing and microsatellites will be used during this study.

1.2. Introduction

The captivating beauty of *Clivia* Lindl. (1828) has been known to man for decades. The first *Clivia* described*, Clivia nobilis* Lindl. (1828), was discovered in the early 1800's. In honour of Charlotte Florentia Clive, duchess of Northumberland, this genus was named *Clivia* (Duncan, 1999; Koopowitz, 2002). The type specimen was brought from the Cape of Good Hope and was planted in the princely Garden of his Grace, the Duke of Northumberland, at Syon House (Lindley, 1828; Duncan, 1999).

Since this first discovery another six species of *Clivia* were identified namely, *C. miniata* (Lindl.) Regel (1864), *C. gardenii* Hook. (1856), *C. caulescens* R.A.Dyer (1943), *C. mirabilis* Rourke (2002), *C. robusta* Murray, Ran, De Lange, Hammett, Truter & Swanevelder (2004) and most recently *Clivia xnimbicola* Swanevelder, Truter & Van Wyk (2006). The genus *Clivia* belongs to the order Asparagales Link (1829) and the family Amaryllidaceae J. St-Hil. (1805). *Clivia* is also commonly known in Afrikaans as 'Boslelie' (Koopowitz, 2002).

Clivia nobilis was first collected in the Easten Cape Province of South Africa by William J. Burchell in 1815 (Duncan, 1999). James Bowie took this plant to England in 1823 where it was taxonomically described (Lindley, 1828). Dr. R. A. Dyer described *C. caulescens* in 1943. *Clivia caulescens* was the first *Clivia* species which was described in its country of origin, South Africa (Dyer, 1943).

Initially there was confusion amongst taxonomists regarding the exact genus to which *C. miniata* belonged. Lindley described it as *Vallota miniata* in 1854 (Lindley, 1854). In the same year Hooker described this species as *Imantophyllum miniatum* (Hooker, 1854_as cited in Koopowitz, 2002). The name known to us today was only validated in 1864 by Regel.

Major Robert J. Garden of the 45th Regiment collected a *C. gardenii* plant while stationed in the KwaZulu-Natal Province of South Africa. This specimen was sent to the Royal Botanical Gardens at Kew. Here it was given the name

C. gardenii by Sir W. Hooker in 1856 when the plant flowered (Hooker, 1856). *Clivia mirabilis* was found by W. Pretorius, nature conservation officer at the Oorlogskloof Nature Reserve near Nieuwoudtville. This was the second species to be described in its country of origin, South Africa (Rourke, 2002).

Murray, Ran, De Lange, Hammett, Truter and Swanevelder described a plant, previously known as the 'robust form' of *C. gardenii* or Swamp Forest *Clivia*, as a separate species, *C. robusta* (Murray *et al*., 2004). Swanevelder, Truter and Van Wyk teamed up again in 2006 to describe the most recent addition to the genus, namely *C. xnimbicola. Clivia xnimbicola* originated due to a natural hybridization between *C. caulescens* and *C. miniata* (Swanevelder *et al*., 2006).

With the discovery of *C. mirabilis* scientists concluded that this species represents the oldest form of *Clivia* (Fig. 1.1) and that all the other species subsequently evolved from *C. mirabilis* (Conrad & Reeves, 2002). If the distribution of the different species is taken into account (Fig. 1.2) and evolution only occurred in one direction, *C. mirabilis* would represent the oldest species.

Figure 1.1: Hypothetical phylogenetic relationships of different *Clivia* species proposed by Swanevelder (2003).

Most of the *Clivia* species have a limited geographic distribution and are mostly restricted to forests or spots with dense vegetation because they grow best in shady areas (Anonymous, 2008a). The natural habitats of plants are destroyed by humans removing material from the forest, these materials are usually used for fuel production or agricultural use (Duncan, 1999; Swanevelder, 2003). In addition plants found in natural populations get stolen by enthusiasts who sell them for large amounts of money and traditional healers use *Clivia* for medicinal purposes. A survey by Williams *et al.* (2001) showed that *Clivia* species were found in a number of 'muti' shops in the Witwatersrand area. According to Duncan (1999) these plants could be used to treat snakebite, fever and could even accelerate childbirth.

Figure 1.2: Map of South Africa indicating the distribution of the seven *Clivia* species.

- *C. mirabilis*
- *C. nobilis*
- *C. miniata*
- *C. robusta*
- *C. gardenii*
- *C. xnimbicola*
- *C. caulescens*

It is generally believed that veldt fires destroyed the *Clivia* populations once found in the southern part of the Western Cape Province, between the distribution areas of *C. mirabilis* and *C. nobilis.* Unlike their bulbous relatives, clivias has no adaptations to help them survive fires and consequently these populations would be lost forever (Snijman, 2003). Conrad *et al.* (2003) suggested that the increase in aridity and the withdrawal of the subtropical forests during Miocene and Pliocene times respectively could be the reason for the distance between *C. mirabilis* and the other *Clivia* species.

According to the National Red List of South African plants (2010a), *C. mirabilis* and *C. nobilis* are regarded as vulnerable. *Clivia mirabilis* and *C. nobilis* already have very limited distribution ranges and when a species is regarded as vulnerable they have a high risk of becoming extinct in nature (Anonymous, 2010b). Therefore information regarding the genetic variation within and between these two species would be instrumental in the conservation of these species. Extinction can only be fought with knowledge and therefore this study focus on the variation within and between these two primitive *Clivia* species.

1.3. *Clivia nobilis*

Clivia nobilis was discovered in the early 1800's and is the type species for this particular genus (Koopowitz, 2002). Thus, the initial description of the genus *Clivia* was identical to the description of *C. nobilis* (Anonymous, 2008a). The distribution range of *C. nobilis* overlaps with that of *C. miniata* and possibly with *C. robusta*, but not with any of the other *Clivia* species.

1.3.1. Structure of the plant and leaves

The leaves are strap-shaped, stiff and of a dark green colour. Some of the plants have a pale green stripe down the middle. Two characteristics of *C. nobilis* are that the leaf tip is notched or bluntly rounded (Fig. 1.3a) and the sides of the leaves are rough to the touch. The density of the shade would influence the length of the leaves. In light shade the leaves are about 300 mm long and when the shade is more intense the leaves could grow to a length of 800 mm. The dark green leaf is 25–50 mm wide. *Clivia nobilis* has a very slow growth rate and can take up to 12 years to reach maturity. Seedlings have their leaves parallel to the ground for the first few seasons but as they grow older the leaves became almost vertical. These plants reached heights of 500 cm to 1.1 m (Duncan, 1999; Koopowitz, 2002; Anonymous, 2008a).

1.3.2. Flowers and berries

Between July to December 20–60 pendulous tubular flowers (Fig. 1.3b) appear on a peduncle which is about 300 mm long. Sporadically this plant can flower at any other time of the year pending on environmental factors. The tubes are 11 mm wide and 25–40 mm in length. The flower colour range from orange to red, with contrasting green tepal tips. A pure yellow form is known but is very rarely observed. With close observation the stamens (Fig. 1.3c) can be seen at the opening of the tubes. After the flowering period berries the size of marbles appears. These berries can take nine months to a year to ripen and each contain a variable number of seeds (Duncan, 1999; Koopowitz, 2002; Anonymous, 2008a). The berries found on a particular plant ripen at different time intervals (Fig. 1.3d-f).

1.3.3. Habitat and distribution

Like most clivias, *C. nobilis* grows best in shady areas. They can be found in evergreen forests and amongst dune vegetation (Fig. 1.3g & h). The leaf length of each plant will be greatly affected by the intensity of the light shining through. The humus (decomposing leaves) necessary for optimal growth is provided by the canopy above. There are some plants located on top of dunes which grow in full sun. The habitat of these plants range from the beach, only a few meters away from the sea side, to almost 48 km inland (Duncan, 1999; Koopowitz, 2002; Anonymous, 2008a; Swanevelder & Fisher, 2009).

The distribution range from close to Port Elizabeth in the Eastern Cape to the former Transkei. The temperatures differ depending on how far or close to the coast the population is found. The coastal area has a climate range from 9℃ to 25℃ with a summer rainfall of between 600 mm and 900 mm. The inland areas have an annual rainfall of approximately 250 mm and temperatures which range from below zero in the winter to 45℃ during the summer months (Duncan, 1999; Koopowitz, 2002; Anonymous, 2008a).

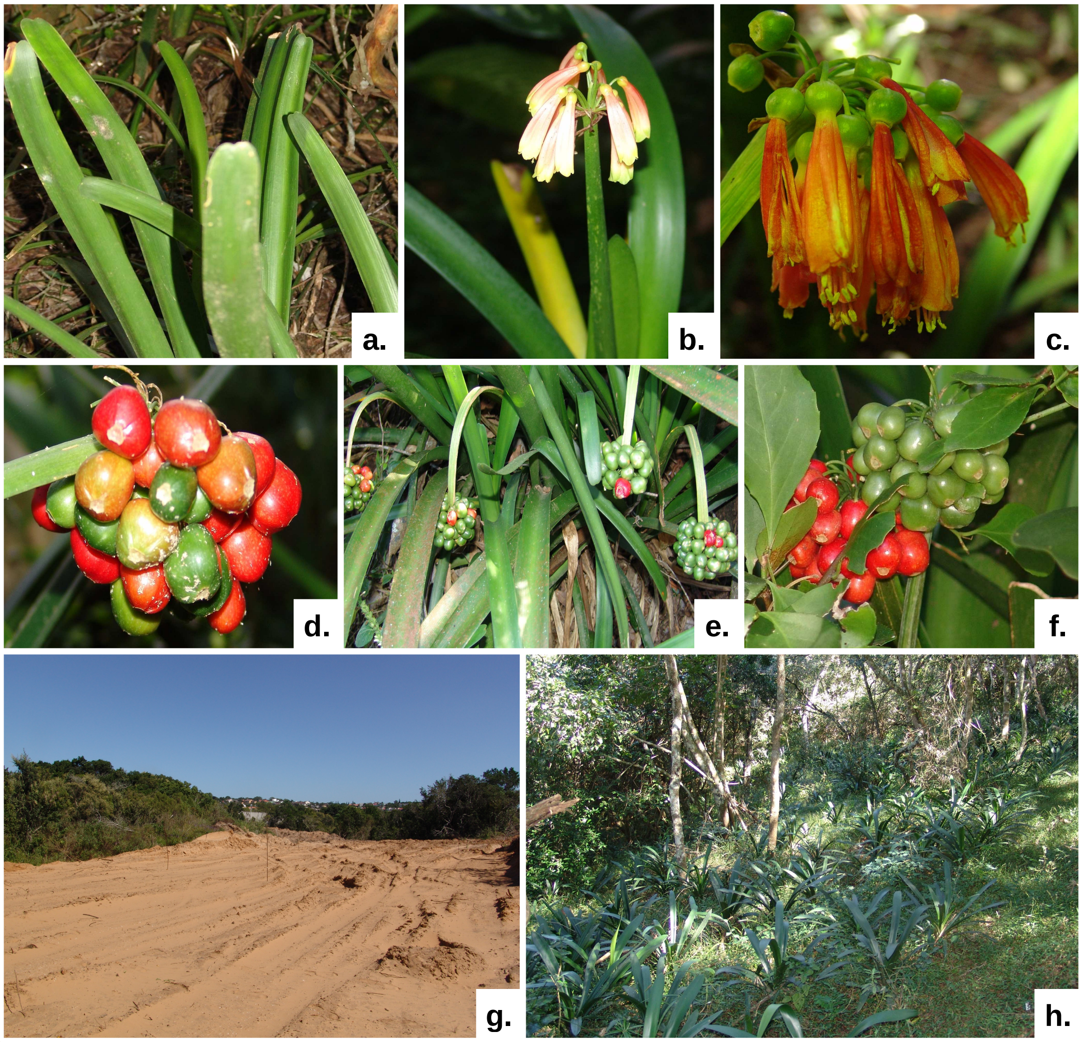

Figure 1.3 Photo's of *C. nobilis* plants in habitat. **(a).** The leaf tips are notched or bluntly rounded. **(b).** *C. nobilis* flowering. **(c).** The stamens of the *C. nobilis* flowers can be seen at the opening of the tubes. **(d-f).** The number of berries indicates that fertilization is very successful in nature. Ripening of berries at different times, increase the probability of survival. **(g).** Population growing in sandy soil and **(h).** evergreen forests.

1.4. *Clivia mirabilis*

Surprisingly this species is found in the Northern Cape Province and the adjacent area of the Western Cape Province. This means that the distribution range of *C. mirabilis* doesn't overlap with any of the other known species (Rourke, 2002; Duncan, 2008). *Clivia nobilis* is the closes spatially to *C. mirabilis* but these two species are still separated by a distance of 700 km (Google maps, 2010). *Clivia mirabilis* has an extremely slow growth rate and might take up to 16 years to reach maturity. Due to the unlikely distribution of these species and the time interval between the previous discoveries and this one, it is understandable that this species name is derived from the word miracle (Rourke, 2002; Duncan, 2008).

1.4.1. Structure of the plant and leaves

The leaves of this species are firm, with smooth margins. The dark green coloured leaves has a pale green stripe down the middle of the leaf (Fig 1.4a), but there are plants however, which has no apparent line. Some *C. mirabilis* plants have a notched leaf tip and no line on the surface of the leaf (Fig. 1.4b). The notched tip is supposed to be a unique characteristic of *C. nobilis*. The leaves has an average length of 1000 mm and are generally between 25 and 45 mm wide (Fig. 1.4c) (Rourke, 2002; Anonymous, 2008b).

1.4.2. Flowers and berries

During October and November pendulous flowers appear (Fig. 1.4d). As these flowers open they will be visibly bi-coloured. These flowers are connected to drooping pedicels which are found on a peduncle (Fig 1.4e). This peduncle has a purple to carmine colour. The developing berries turn from green to yellow and then to a sort of pink colour. Bright red coloured berries will be an indication that they are ripe. This usually occurs during March, thus, before the first winter rains. Normally each berry contains one to three seeds each, but sporadically up to seven has been recorded. The berries of *C. mirabilis* ripen within about three months after pollination, thus faster than all the other species of *Clivia* (Rourke, 2002; Anonymous, 2008b).

1.4.3. Habitat and distribution

Clivia mirabilis is found in the Oorlogskloof Nature reserve near Nieuwoudtville in the Northern Cape Province and neighbouring areas. Cracks in the sandstone of the Oorlogskloof Canyon provide an ideal rooting place for this species (Fig. 1.4f & g). These cracks are usually rich in humus. They grow at about 850 m to 900 m above sea level and, therefore, plants occasionally experience light frost in winter. This species is usually found in a gorge (Fig. 1.4h) because there are trees which could provide shade for the population. However there are clusters which grow in full sun. The leaves and flowers of these plants have visible signs of water stress. The area where *C. mirabilis* are found, commonly has a semi-arid Mediterranean climate thus experiencing hot dry summers and winter rainfall. The mean annual rainfall for this area is 400 mm (Rourke, 2002; Anonymous, 2008b; Swanevelder & Fisher, 2009).

1.4.4. Adaptations to habitat

The *C. mirabilis* plants have much thicker roots (Fig. 1.4i) in comparison to the other *Clivia* species. This is due to the semi-arid Mediterranean climate which these species has to endure. The roots acts as water storage organs and will therefore be instrumental in the survival of the long dry summers (Anonymous, 2008b). The fact that the berries only take three months to ripen will also be a direct result of this type of weather. Furthermore when these plants experience a draught they will allow the older leaves to die in order to keep more resources for the survival of the plant (Fig. 1.4j & k).

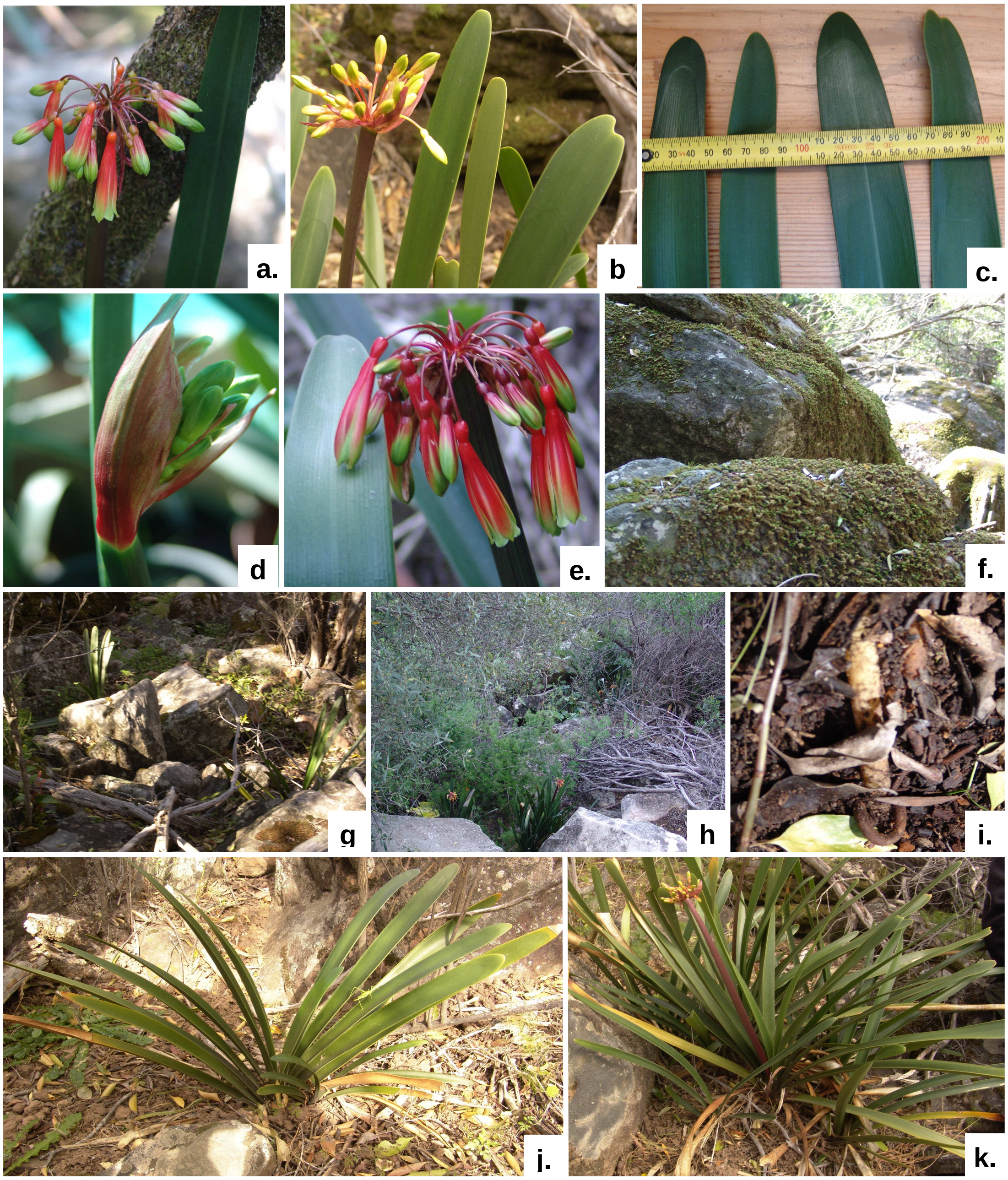

Figure 1.4 Pictures of *C. mirabilis* specimens in habitat. **(a).** *C. mirabilis* with a median stripe. **(b).** Leaf tips of some *C. mirabilis* plants are notched. **(c).** Leaves of different *C. mirabilis* plants ranging from 25 to 45 mm. **(d).** Inflorescence starting to open. **(e).** Peduncle are reduce and long. **(f&g).** Cracks in the sandstone provide an ideal rooting place for this species. **(h).** A typical gorge where trees provide shade for the *C. mirabilis* populations. **(i).** Thick root of a *C. mirabilis* plant which may act as water storage organs. **(j&k).** Older leaves in *C. mirabilis* may die when experiencing drought.

1.5. Genetic variation

Genetic variation is the genetic diversity found within a species (Solomon, 2002). Mutations and gene flow are two of the major sources of genetic variation. Mutations are changes found in the DNA sequence of an organism (Fairbanks & Andersen, 1999; Solomon, 2002). Gene flow is an indication of any movement between populations which result in genetic exchange (Hedrick, 2000). Consequently allele movement will be observed between local populations. Mutations and gene flow can have a considerable influence on the evolutionary development of a specific species (Solomon, 2002).

Changes in the environment force organisms to adapt in order to survive. A population with a high level of genetic variation has more alleles to “choose” from and therefore has a better chance to survive. A small population size can be an indication of a low level of genetic diversity found in this particular population (Grassi *et al*., 2004). The genetic richness decrease when alleles become lost from the gene pool in a specific population. This population has an increased change of becoming inbred and experiencing inbreeding depression (Hedrick, 2000).

A small population has an increased chance of becoming extinct in future (Grassi *et al*., 2004). It’s clear that a healthy level of genetic variation is essential for species survival. Therefore, an estimation of the genetic variation of these two *Clivia* species under discussion would be instrumental in the conservation of these species.

Gene exchange between different populations can be beneficial because it will lead to an improved allele pool which will increase the effective population size (Grassi *et al*., 2004). The genetic diversity of a small population can be improved by the addition of new individuals of the same species.

If there is more variation within a specific species than between two different species, the two species probably belong to the same species. A study regarding the genetic variation within and between *C. nobilis* and *C. mirabilis* will provide answers to all the questions surrounding the evolutionary development of the genus *Clivia*. This study will indicate which of the two species should be regarded as the oldest and specify how closely related the two species are.

The population size of *C. mirabilis* is very small and therefore no time can be wasted. An estimation of the genetic variation found within each species and between the respective species can help to make an informed decision regarding the future of these populations. A decision based on anything other than genetic evidence will only be a shot in the dark which might be a detriment rather than a benefit.

1.6. Different techniques

To date several different techniques had been introduced through which one could determine genetic variation within and between species. These techniques had been tried and tested by different scientists working on different genera. Some of the relevant techniques were,

Restriction fragment length polymorphism (RFLP): This technique relies on different DNA fragment lengths, created by means of restriction endonuclease cleavage, to reveal sequence variation. The differences in fragment lengths are brought about by mutations (Fairbanks & Andersen, 1999). Alterations to the restriction sites are caused by base substitutions, indels and rearrangements (Liu & Cordes, 2004).

By means of this technique maps had been constructed for maize (Gardiner *et al*., 1993; Lin *et al*., 1997) and soybeans (Cregan *et al*., 1999). This technique provided more information on the *Leymus* (Anamthawat-Jónsson & Bödvarsdóttir, 2001) and *Ceramium* (Wattier *et al*., 2001) species.

There are no probes available which has been especially designed for *Clivia* and there are a lack of sequence information regarding this genus. With these facts in consideration it is best not to use RFLPs during this study.

Random amplified polymorphic DNA (RAPD): This technique literally enables the random amplification of DNA. During PCR short primers (about

10 nucleotides long) bind to the template DNA and amplify the DNA sequence between the two primers (Fairbanks & Anderson, 1999). Primers would bind to several complementary sites to give rise to amplification products (Fairbanks & Anderson, 1999; Liu & Cordes, 2004).

These markers had been used to determine variation within plant species, *Gentianella germanica* (Fisher & Matthies, 1998) and *Ranunculus reptans* (Fisher *et al*., 2000). RAPD's had also been used for analysis done on trees, for example *Eucalyptus urophylla* (Gaiotto *et al*., 1997).

This technique would enable one to detect genetic variation among closely related genotypes (Ran *et al*., 2001). We don't know exactly how closely related *C. mirabilis* and *C. nobilis* are but we suspect that they are closer related to each other than to any of the four other species of *Clivia*. The low level of reproducibility of this technique made it unsuitable for use during this study. Plants could still be closely related despite of differences in their outwards appearance.

Amplified fragment length polymorphism (AFLP): Vos *et al.* (1995) introduced this multi-locus fingerprinting technique. As the name implies this technique focus on the amplification of fragments which are created by means of restriction enzymes. The entire genome could be digested, but only selected fragments would be amplified (Vos *et al.*, 1995). The use of adaptors enabled the amplification of restriction fragments even without any known molecular information (Vos *et al*, 1995; Liu & Cordes, 2004; Primrose & Twyman, 2006).

In the past AFLP's had been used in fungi: *Arbuscular mycorrhizal* (Rosendahl & Taylor, 1997); bacteria: *Bacillus anthracis* (Keim *et al*., 1997) and animals: *Artemia* (Triantaphyllidis *et al*., 1997). These markers had also successfully been used on plants: *Eucalyptus urophylla* (Gaiotto *et al*., 1997), *Pedicularis palustris* (Schmidt & Jensen, 2000), *Miscanthus* (Poaceae) (Hodkinson, 2002) and more recently on the genus *Clivia* (Gagiano, 2006).

A technique with a high level of reproducibility would be vital during this study. AFLP results vary significantly between different experiments. Paternal testing would not be possible when AFLP's is used.

Microsatellites: A region of DNA that contains a high frequency of mono-, di-, tri- or tetra-nucleotide repeats (Fairbanks & Andersen, 1999). They are also often called variable-number of tandem repeats (VNTR), short tandem repeats (STR) or simple sequence repeats (SSR) (Selkoe & Toonen, 2006).

The DNA sequence before and after a specific microsatellite region is called the flanking region. These flanking regions are found to have a low mutation rate. The nucleotide sequence would, therefore, stay the same between successive generations and even between different species. Complementary primers would bind to the flanking region and amplify the microsatellite region during PCR (Selkoe & Toonen, 2006).

Microsatellites were previously used in different plants in the Amaryllidaceae family, for example in the wild daffodil *Narcissus triandrus* (Hodgins *et al*., 2007), in *Phaedranassa tunguraguae* (Oleas *et al*., 2005) and in *Hymenocallis coronaria* (Markwith & Scanlon, 2005).

The high mutation rate observed within the microsatellite regions would make this technique ideal for revealing allelic diversity. This study deals with relatively small population sizes and, therefore, microsatellite information would be useful during this particular study.

Single nucleotide polymorphisms (SNPs): Single nucleotide polymorphisms occur due to variation at a single nucleotide position (Liu & Cordes, 2004; Strachan & Read, 2004). This variation is usually caused by a point mutation (Liu & Cordes, 2004), for example insertion, deletion or a single nucleotide being substituted by a different nucleotide (Fairbanks & Andersen, 1999).

SNPs were used in humans to give more insight into genetic variation (Collins *et al*., 1998; Sachidanandam *et al.,* 2001). These markers also enabled scientists to estimate diversity within the Bovine family (Konfortov *et al*., 1999; Heaton *et al.,* 2001).

SNP analysis could be a relevant technique to use during this study on *C. nobilis* and *C. mirabilis*.

Sequencing: In 1977 Frederick Sanger developed the Sanger sequencing method. Today the basis of sequencing is still based on this method Sanger explained thirty years ago but modern machines makes it easier to analyse the results. Nowadays the four ddNTP's added to the reaction mixture would respectively be labelled with a red, blue, green or yellow fluorescent dye (Fairbanks & Andersen, 1999; Primrose & Twyman, 2006).

Sequencing data of the *rbcL* and *trnL-F* regions provided systematic insight into the family Amaryllidaceae (Meerow *et al*., 1999). Different primer pairs had been tested on a variety of land plants in order to develop universal primers for amplification (Taberlet *et al.*, 1991; Demesure *et al.*, 1995; Dumolin-Lapegue *et al*., 1997). Over the last few years the complete genome sequence of a wide variety of organisms had been published. Amongst these organisms were *Escherichia coli* K-12 (Blattner *et al.,* 1997), *Caenorhabditis elegans* (Ainscough *et al.*, 1998), *Arabidopsis thaliana* (*Arabidopsis* genome initiative, 2000), *Drosophila melanogaster* (Adams *et al*., 2000), *Streptococcus pneumonia* (Tettelin *et al*., 2001), *Phanerochaete chrysosporium* (Martinez *et al*., 2004) and *Cryptococcus neoformans* (Loftus *et al*., 2005).

The chloroplast region is maternally inherited in the majority of plant species. A comparison can be drawn between plants within a specific population if the nucleotide sequences for the different plants are obtained. It would also be possible to compare different species and thus get insight into their phylogenetic relationship.

All the advantages and the disadvantages of the different techniques were measured up against each other. The RFLP technique needed highly informative probes (Liu & Cordes, 2004; Primrose & Twyman, 2006). The two species under discussion are closely related (Conrad *et al.,* 2003), therefore, RFLP's would not be able to show variation between these two species.

RAPD's can result in a low level of reproducibility between individual experiments (Pérez *et al*., 1998; Liu & Cordes, 2004). It was important to choose a technique with a high level of reproducibility and, therefore, AFLPs were also excluded. Microsatellites proofed to have a high level of allelic diversity. Sequencing information would reveal genetic variation between species and allow the reconstruction of the phylogenetic relationship within the genus *Clivia*. Microsatellites and sequencing would definitely be the best methods for genetic analyses during this study.

1.7. Chloroplast genes

1.7.1. Chloroplast region and haplotypes

The Eve theory use mitochondrial DNA, which is only inherited maternally, to trace back the family tree of all human beings to one woman called the mitochondrial Eve. Seven different haplotypes were identified in a study conducted by Sykes (2001). According to this study all humans could trace back their ancestors to one of these seven women, now known as the seven daughters of Eve.

The chloroplast is a membranous organelle found in plants and is the site where photosynthesis occur (Solomon *et al.,* 2002). Similar to the mitochondrial genes found in humans, the genes found in the chloroplast region of the majority of plants are inherited maternally. By analysing genes found in the chloroplast region, it would be possible to predict phylogentic relatedness between different plant species (Wallace & Cota, 1996; Conrad *et al.,* 2003). Structural rearrangements (insertions, deletions or the occurrence of inversions) within the chloroplast genome would also help to identify related organisms (Wallace & Cota, 1996). The reconstruction of a phylogenetic tree for *C. nobilis* and *C. mirabilis* would reveal genetic variation between and within these species.

Strachan and Read (2004) define a haplotype as, 'a series of alleles found at linked loci on a single chromosome'. These linked genes would be inherited together, thus maternally or paternally. The reconstruction of a haplotype network would establish historical relationships among species and populations. A haplotype network measures relationships among different haplotypes and not among different individuals and would indicate the amount of mutations separating different haplotypes (Beerli, 2005).

1.7.2. Barcoding

The development of a unique barcode for plant species would help scientist and taxonomists to distinguish between different plant species (Taberlet *et al*., 2007). With a barcoding system in place new species would be indentified faster (Rubinoff *et al*., 2006). In order to develop such a barcode, genes must be identified which is variable enough between different species (Rubinoff *et al*., 2006; Taberlet *et al*., 2007), but still conserved enough within species. The same genes should be used across different taxonomic groups and the target region should provide sufficient phylogenetic information in order to place these plants within the correct families and genera (Taberlet *et al*., 2007). The results obtained from sequencing has to be reliable and, therefore, the primer binding sites has to be conserved (Rubinoff *et al*., 2006; Taberlet *et al*., 2007). A short target region would allow amplification even when the DNA has been degraded (Taberlet *et al*., 2007).

Barcoding has been done on animals with great success, but in plants it is still in the developmental stage. In animals the mitochondrial gene *cox1* is used. The mitochondrial genes of land plants showed low levels of variation and could not be used for barcoding (Chase *et al.,* 2007). Developing a barcode which match all the above mentioned characteristics led to different opinions amongst scientists (Rubinoff *et al*., 2006). Some of the proposed gene combinations are *rpoC1*, *rpoB* and *matK* or *rpoC1*, *matK* and *trnH-pshA* (Chase *et al*., 2007). Taberlet *et al*. (2007) argues that the *trnL* intron alone would provide enough information to use as a plant barcode. The Consortium for the Barcode of Life (CBOL) decided on the *matK* and *rbcL* regions for land plant barcoding (CBOL plant working group, 2009).

1.7.3. Genes sequenced during this study

The genes located in the chloroplast region of the majority of plants are maternally inherited and, therefore, these genes would not contain any recombination. Although recombination is vital for species survival, it is very difficult to obtain ancestral information from recombined genes. The genes located within the chloroplast region, however, show a low evolutionary rate and would, therefore, indicate interspecific (between species) variation but not intraspecific (within species) variation (Taberlet *et al.,* 1991). Non-coding regions (introns and intergenic spacers) are more prone to mutations because selection against mutations is not as strong as in the regions essential for gene function (Taberlet *et al.,* 1991; Hamilton, 1999). Non-coding regions would show more variation when sequenced than coding regions would. By sequencing introns and intergenic spacers, it would be possible to study intraspecific variation if the genes sequenced is informative enough (Hamilton, 1999). The more conserved exon regions would provide ideal binding sites for the primer pairs and would ensure that the same primers could be used within a variety of different families (Taberlet *et al.,* 1991).

Based on these facts, gene regions which were sequenced regularly in our laboratory were chosen for analysis. The six gene regions were *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16* and the *trnL-F* region. In earlier studies each of these regions showed an adequate amount of variation (Taberlet *et al.,* 1991; Liston, 1992; Johnson & Soltis, 1995; Campagna & Downie, 1998; Drancourt & Raoult, 2002). These regions would each be discussed in further detail.

1.7.3.1. *atpH-I* region

The *atp* genes is responsible for the expression of ATP synthase, better known as transporter proteins (Hicks *et al*., 2003). The *atpH* and *atpI* regions are responsible for the production of the ATP synthase III subunit and ATP synthase IV subunit respectively (Woessner *et al*., 1987; Wakasugi *et al*., 1998). An autonomously replicating sequence, *ars2*, is located between *atpH* and *atpI* (Wakasugi *et al*., 1998; Heinze, 2007). The *atpH-I* region is approximately 740 bp in length (Fig 1.5) (Wakasugi *et al*., 1998; Heinze, 2007). The *ars2* region would expectedly have a high level of variation.

Figure 1.5: Diagram representing the *atpH-I* region (Heinze, 2007). The *ars2* sequence is located between *atpH* and *atpI*. (Not according to scale).

1.7.3.2. *matK* region

The *maturase K* region is located within the intron region of the *trnK* gene (Fig. 1.6) (Neuhaus & Link, 1987; Wakasugi *et al*., 1998). The *trnK* gene is a transfer RNA gene which codes for lysine (Neuhaus & Link, 1987; Hilu *et al.,* 1999). *matK* plays an important role in the splicing of group II introns (Neuhaus & Link, 1987). This region is about 1500 bp long (Wakasugi *et al*., 1998). In earlier studies, *matK* was one of the chloroplast regions in which a high relative rate of substitutions were observed (Olmstead & Palmer, 1994; Johnson & Soltis, 1995).

Figure 1.6: Diagram of the *matK* area located in the intron region of the *trnK* gene (Hilu *et al*., 1999). (Not according to scale).

1.7.3.3. *rpoB* region

The *rpoB* region (Fig. 1.7) encodes the β subunit part of RNA polymerase which is responsible for RNA synthesis (Yepiz-Plascencia *et al*., 1990; Zeltz *et al*., 1993; Wakasugi *et al*., 1998). In tobacco the *rpoB* region are found to be 3212 bp long (Wakasugi *et al*., 1998) and in the *Staphylococcus* species this region is between 3452 bp to 3845 bp in length (Drancourt & Raoult, 2002). The *Staphylococcus* sequence results show between 36.8% and 39.2% GC content (Drancourt & Raoult, 2002).

Figure 1.7: Diagram of the *rpoB* and *rpoC1* regions located next to each other. The exon region of *rpoC1* is interrupted and therefore contains two exon and one intron region (Heinze, 2007). (Not according to scale).

1.7.3.4. *rpoC1* region

RNA polymerase C1 is positioned in the chloroplast genome, at the single copy region of the genome (Liston, 1992). The *rpoC1* region is functionally involved in the expression of the β' subunit, a part of the enzyme (RNA polymerase) involved in RNA synthesis (Wakasugi *et al*., 1998). The *rpoC1* region contains a 738 bp intron (Fig. 1.7) which would expectantly show more variation than the coding regions (Liston, 1992; Wakasugi *et al*., 1998).

1.7.3.5. *rpl16* region

The *rpl16* gene is found in the chloroplast and codes for the ribosomal protein L16 (Jordan *et al*., 1996; Campagna & Downie, 1998). An intron region interrupts the *rpl16* gene in most land plants (Fig. 1.8). This intron is reported to be 536 bp long in *Marchantia* (Campagna & Downie, 1998), 1020 bp long in tobacco (Wakasugi *et al*., 1998) and up to 1400 bp in length in Duckweed (Campagna & Downie, 1998). A longer intron would expectedly result in more variation.

Figure 1.8: Diagram of the *rpl16* gene which is interrupted by an intron region (Hosokawa *et al*., 2005). The *rpl16* gene is located between *rps3* and *rpl14*. (Not according to scale).

1.7.3.6. *trnL-F* region

This region consists of the *trnL* intron, the *trnL* (UAA) exon and the *trnL-F* intergenic spacer (Fig. 1.9). These no-coding regions would expectantly show more variation due to a higher mutation rate observed within no-coding regions. The primer binding sites are located in highly conserved regions which would increase their effectiveness amongst a wide variety of taxa. The *trnL-F* region is approximately 1000 bp in length but length would vary between different taxanomic groups (Taberlet *et al.,* 1991). Universal primers c-f divides this region into smaller parts which would make amplification of the entire *trnL-F* region easier (Taberlet *et al.,* 1991).

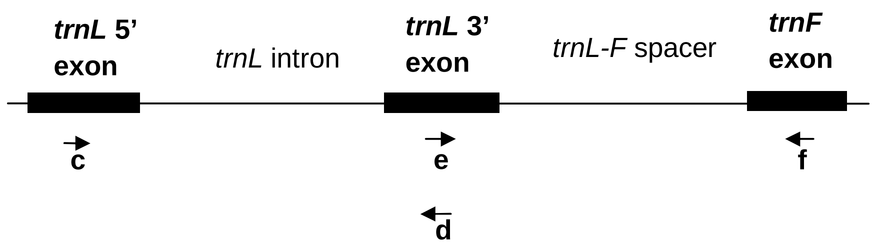

Figure 1.9: The diagram represents the *trnL-F* region. The *trnL-F* region contains an intron and intergenic spacer. The arrows indicate the direction of the four universal primers, c to f (Taberlet *et al.,* 1991). (Not according to scale).

1.8. Nuclear gene

1.8.1. *ITS* region

One nuclear region, *ITS1*, is also used for sequencing. This region is known for its high evolutionary rate and would, therefore, be ideal for species discrimination and even detection of intra specific variation (White *et al*., 1990; Schnabel & Wendel, 1998).

There are two internal transcribed spacer regions in the nuclear ribosomal DNA. The two *ITS* regions is separated by the 5.8S rDNA (Fig. 1.10). There are universal primers designed for the amplification of the *ITS1* region namely ITSL and ITS2 (White *et al*., 1990; Hsiao *et al*., 1995). The two *ITS* regions range between 290 bp and 330 bp in length.

Figure 1.10: Diagram of the internal transcribed spacer regions. The 5.8S rDNA separates the two *ITS* regions. The five universal primers are indicated on the diagram (Modified from White *et al*., 1990). (Not according to scale).

1.9. Microsatellites

1.9.1. Primers for microsatellite analysis

Swanevelder (2003) designed microsatellite primers for the genus *Clivia* while studying *C. miniata*. The four primers designed by Swanevelder were used and to ensure thorough analysis additional primers were also included. Other plants in the Amaryllidaceae family had been subjected to microsatellite analysis before. After analyzing different primer pairs designed for some of these plants, the *Phaedranassa tunguraguae* (Oleas *et al*., 2005) and *Hymenocallis coronia* (Markwith & Scanlon, 2006) primers were tested on the genus *Clivia*.

1.9.2. Potential problems

1.9.2.1. Species specificity

The species specificity of microsatellite makers is a major advantage which would prevent cross-contamination with other DNA present in the reaction (Selkoe & Toonen, 2006). However, this same advantage could prevent microsatellite primers developed for a specific species to bind and amplify in other species. The low mutation rate observed among the nucleotides at the primer binding sites could possibly solve this problem. Due to the low mutation rate, different species in the same genus or family might have the same nucleotide sequence at the flanking regions (Selkoe & Toonen, 2006).

Cross-species amplification had been done successfully before (Rossetto *et al*., 2001; Gupta *et al*., 2003; Eujayl *et al*., 2004; Saha *et al*., 2004; Markwith & Scanlon, 2006; Cotrim *et al*., 2009). White & Powell (1997), Peakall *et al*. (1998) and Roa *et al*. (2000) reported that the results obtained for cross-species makers were inadequate.

1.9.2.2. Polyploidy in *Clivia*

A polyploid organism has multiple chromosome sets (Strachan & Read, 2004). *Clivia* has 22 chromosomes (Gibbs Russell *et al*., 1987) and a secondary basic chromosome number of 11 (Spies & van der Westhuizen, 2009). All basic chromosome numbers above nine are secondarily derived (Goldblatt, 1982). This indicates that *Clivia* was a polyploid plant with more than two sets of chromosomes present in each cell. However we are dealing with an ancient polyploid plant and consequently some of the individuals or certain genes might no longer act as polyploids. This would greatly influence the number of alleles observed and might complicate the analysis.

1.9.2.3. Amplification of non-microsatellite regions

With the use of cross-species makers the primer pairs might bind to a complimentary region other than the target region. This could lead to the amplification of a non-microsatellite region. The results obtained would subsequently not be reliable. To eliminate this problem each microsatellite region has to be sequenced individually in order to prove that it is microsatellite regions used for analysis.

1.10. Data analysis

The availability of specialized computer programs simplified data analysis. The programs used during this study were GeneMarker (Anonymous, 2010c)., DnaSP v 5.0 (Rozas *et al*., 2003), Geneious Pro 4.7.5 (Rozen & Skaletsky, 2000), Network 4.5.1.0 (Anonymous, 2010d) and MEGA 4.1 (Kumar *et al*., 2008). All five programs had unique features and when used together they bridge each other’s short comings.

GeneMarker is a research friendly genotyping analysis tool. Slab gels or capillary electrophoresis can be analyzed with GeneMarker because off its compatibility with most systems. Up to a thousand lanes of four coloured data sets can be analyzed, with a lane by lane overview of each sample. After manipulation according to report requirements, the data can be stored in a variety of different formats (Anonymous, 2010c).

As the name indicated, DnaSP v 5.0 (DNA sequence polymorphism), examines DNA polymorphism when nucleotide sequence data is available. This particular program can use noncoding, synonymous and nonsynonymous sites to measure the sequence variation found within and between populations (Rozas *et al*., 2003). Gene flow, gene conversion (Betrán *et al*., 1997), recombination and linkage disequilibrium are all parameters which can be measured with DnaSP. This program can also perform a variety of neutrality tests and enables one to analyse a large number of sequences simultaneously. Another advantage of DnaSP is its ability to exchange information with other programs which can perform functions which DnaSP cannot perform itself (Rozas *et al*., 2003).

The software developed for Geneious Pro enables scientists to manipulate and share DNA sequences. Geneious Pro allows the assembly of the forward and reverse sequencing strands and makes sequence alignment relatively easy. This program allows phylogenetic analysis and primers can be designed for a specific target region (Rozen and Skaletsky, 2000).

The computer program Network 4.5.1.0 (Anonymous, 2010d) enables the reconstruction of phylogenetic trees and networks (Bandelt *et al*., 1995; Bandelt *et al*., 1999). The trees with the maximum parsimony (Polzin *et al.,* 2003) are reconstructed. Furthermore ancestral types and potential types can be inferred and evolutionary branching can be predicted. With the help of Network it is possible to estimate the time frame in which each event occurred (Forster *et al*., 1996).

With the help of Molecular Evolutionary Genetics Analysis (MEGA), sequence data can be assembled and based on these results evolutionary relationships can be predicted. This program also enables visualization of the data as a phylogenetic tree. Another advantage of MEGA is its ability to calculate evolutionary distance matrices and it provides tools for statistical analysis (Kumar *et al.,* 2008).

1.11. Dissertation outline

This dissertation will be presented as individual articles with a general introduction at the beginning and a discussion at the end in order to bind all the chapters as a unit. In **Chapter 1** general information regarding the species and the different techniques used were presented. **Chapter 2** focused mainly on the barcoding potential revealed by the gene regions used for sequencing and the tremendous impact which these barcodes would have for implementation of conservation strategies. The phylogenetic relationships within and between *C. nobilis* and *C. mirabilis* were discussed in **Chapter 3**. In **Chapter 4** intraspecific variation were tested using sequencing and cross-species microsatellite markers. A summary of the entire dissertation would be found in **Chapter 5**.

The exact locality of plant samples used during this study will not be given in order to protect these populations from *Clivia* enthusiasts who steal and sell plants for huge amounts of money. In the past natural populations have been wiped out by enthusiasts and should therefore be protected. In order to distinguish between the gene regions and the primers used, gene regions will be written in italics in this study. The DNA sequences obtained during this study is currently being submitted to GenBank.

1.12. Aim of this study

The aim of this study was

1.) to determine if microsatellite maker designed for *Phaedranassa tunguraguae* (Oleas *et al*., 2005) and *Hymenocallis coronia* (Markwith & Scanlon, 2006) would work on the genus *Clivia*, 2.) to evaluate the six chloroplast regions (*atpH-I, matK, rpoB, rpoC1, rpl16,* and the *trnL-F*) for future barcoding potential in *C. nobilis* and *C. mirabilis*,

3.) to determine if the data obtained from sequencing and the microsatellite analysis provide enough informative sites to determine the phylogenetic relationship within *Clivia*,

4.) to determine the genetic variation between *C. nobilis* and *C. mirabilis*,

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Chapter 2:

Potential barcoding regions for species identification in *Clivia nobilis and Clivia mirabilis*

modified from South African Journal of Botany

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2.1. Abstract

The two most primitive species in the genus *Clivia*, *C. nobilis* and *C. mirabilis*, shares phenotypic characteristics. For conservation purposes it is essential to discriminate between these two species. Since phenotypic identification is difficult, genotypic information had to be obtained for identification purposes. Sequencing information was obtained for seven different gene regions. Six chloroplast regions and one nuclear region were analysed, *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F* and *ITS1* regions. All the regions showed variation between *C. nobilis* and *C. mirabilis* accept the *rpoB* gene region. The *atpH-I*, *matK*, *rpoC1, rpl16*, *trnL-F* and *ITS1* regions showed potential as future barcoding regions in *C. nobilis* and *C. mirabilis*. We propose the combined use of the *matK, rpl16*, and *trnL-F* regions for these *Clivia* species.

2.2. Introduction

Clivia nobilis and *C. mirabilis* are geographically distinct but still share phenotypic characteristics. Difficulty in distinguishing between *C. nobilis* and *C. mirabilis* is brought about by these overlapping characteristics (Duncan, 1999; Koopowitz, 2002). The original *C. mirabilis* population, found at the Oorlogskloof Nature Reserve near Nieuwoudtville (Rouke, 2002), had almost been wiped out entirely by unscrupulous collectors. Both *C. nobilis* and *C. mirabilis* have a limited distribution rage and are regarded as vulnerable by the National Red List of South African plants (2010).

The shared characteristics observed between these two species led to *C. mirabilis* being mistaken for *C. nobilis* and *vice versa*. *Clivia nobilis* currently has a bigger distribution range than *C. mirabilis*. Although both species are regarded as vulnerable, at the moment the conservation strategy for *C. mirabilis* is enforced more strictly. As a result *C. mirabilis* is extremely rare in cultivation and is, therefore, one of the most expensive plants in the genus. Based on the phenotype of these plants, *C. nobilis* can be sold for a higher price when posed as *C. mirabilis*.

Over the last few years the development of a unique barcode for land plants had been the focus of various research studies (Chase *et al*., 2005; Kress *et al*., 2005; Rubinoff *et al*., 2006; Chase *et al.,* 2007; Taberlet *et al*., 2007). Due to the low evolutionary rate of the chloroplast genome (Taberlet *et al.,* 1991), non-coding regions (which are prone to mutations) would be more suitable for barcoding purposes. The use of primers with conserved binding sites would increase their effectiveness amongst a wide variety of taxa (Taberlet *et al.,* 1991). Good barcoding loci have conserved flanking regions and will be able to discriminate between species (Hollingsworth *et al*., 2009).

Seven different gene regions will be sequenced. Six chloroplast regions, namely the *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F* and one nuclear region, *ITS1*. Our aim was to determine if the molecular information obtained could differentiate between *C. nobilis* and *C. mirabilis* and to evaluate these chloroplast regions as potential barcoding regions for future use.

2.3. Materials and methods

2.3.1. Sample locality and extraction

Three different *C. mirabilis* populations and twenty *C. nobilis* populations were sampled. A preliminary study was conducted using eight *C. nobilis* samples and nine samples of *C. mirabilis* (Table 2.1). These seventeen samples represented the different populations and phenotypic characters observed. A sample from each of the other *Clivia* species, i.e. *C. caulescens*, *C. miniata*, *C. gardenii*, *C. robusta* and *C. xnimbicola*, were also included in the study (Table 2.1).

Table 2.1: List representing the plant samples used for analysis.

Collection Number	Species	Locality
142	*C. nobilis*	Qora
144	*C. nobilis*	Unknown
173	*C. nobilis*	Qora (Cobb Inn)
174	*C. nobilis*	Chulumna (location 1)
175	*C. nobilis*	Keiskamma (location 2)
177	*C. nobilis*	Pirates Creek, Bonza Bay
178	*C. nobilis*	Pirates Creek, Bonza Bay
193	*C. nobilis*	Fish River
241	*C. mirabilis*	Donkerhoek (Population 1)
242	*C. mirabilis*	Donkerhoek (Population 1)
253	*C. mirabilis*	Donkerhoek (Population 2)
256	*C. mirabilis*	Donkerhoek (Population 2)
257	*C. mirabilis*	Donkerhoek (Population 2)
264	*C. mirabilis*	Klein Koebee
265	*C. mirabilis*	Klein Koebee
269	*C. mirabilis*	Klein Koebee
533	*C. mirabilis*	Oorlogskloof Nature Reserve

139	*C. gardenii*	Ingubevu (Greytown)
164	*C. robusta*	Port Shepstone
210	*C. caulescens*	God's Window
325	*C. miniata*	Cultivated by Kirstenbosch
329	*C. xnimbicola*	Bearded Man

Leaves of the *C. mirabilis* samples were stored in a saturated Sodium chloride-Cetyltrimethylammonium bromide (NaCl-CTAB) solution for five months prior to DNA extraction whereas fresh leaf material was used for DNA extraction of the six remaining species. A modified CTAB extraction method proposed by Rogstad (1992) was followed. (Results are shown in Appendices A and B).

2.3.2. Amplification and sequencing

The primer pairs used to amplify the *atpH-I* (Grivet *et al*., 2001)*, trnL-F* (Taberlet *et al*., 1991; Pfosser & Speta, 1999)*, rpl16* (Jordan *et al*., 1996) *and ITS1* (White *et al*., 1990; Hsiao *et al*., 1995) regions have been developed previously. These publications were used as an initial starting point, but alterations were made to the concentrations used and the PCR programs, in order to increase the quality of the amplification product. Standard protocols for the amplification of the *matK*, *rpoB* and *rpoC1* regions were retrieved from the barcoding webpage of the Royal Botanical Gardens, Kew (http://www.kew.org/barcoding/iupdate.html). For *trnL-F* eight primers were used for amplification and sequencing. These primers divide the region into small fractions which would be easier to amplify. The primers c, d, e and f (Taberlet *et al*., 1991) were used for amplification and nested primers (Pfosser & Speta, 1999) for sequencing. In this text the four primers designed by Pfosser and Speta were named PS1, PS2, PS3 and PS4.

The 20 µl amplification reaction consisted of: **1.) *atpH-I*** and ***trnL-F* region:** 4 µl 5x Buffer (10 µl 10 mM dNTP mix, 500 µl 10x Buffer, 0.001 g Gelatine, 455 µl dH_2O, 5 µl 100x Triton), 0.2 µl 5 units/µl Super-Therm Taq Polymerase, 1 µl 25 mM $MgCl_2$, 0.2 µl 50 µM of each Primer, 11.4 µl dH_2O, 3 µl 20 ng/µl Template DNA. **2.) *matK*, *rpoB*** and ***rpoC1* regions:** 2 µl 10x Buffer, 0.16 µl

10 mM dNTP's mix, 0.2 µl 5 units/µl Super-Therm Taq Polymerase, 1.2 µl 25mM $MgCl_2$, 2 µl 5 µM of each Primer, 8.64 µl dH_2O, 3 µl 20 ng/µl Template DNA and 0.8 µl DMSO. **3.) *rpl16* region:** 4 µl 10x Buffer, 0.4 µl 10 mM dNTP mix, 0.4 µl 5 units/µl Super-Therm Taq Polymerase, 2 µl 25 mM $MgCl_2$, 2 µl 5 µM of each Primer, 6.2 µl dH_2O and 3 µl 20ng/µl Template DNA. **4.) *ITS* region:** 2 µl 10x Buffer, 0.4 µl 10 mM dNTP mix, 0.4 µl 5 units/µl Super-Therm Taq Polymerase, 1 µl 25 mM $MgCl_2$, 1 µl 10 µM of each Primer, 11.4 µl dH_2O, 3 µl 20 ng/µl Template DNA and 0.8 µl DMSO.

The following programs for DNA amplification were used: **1.)** for the ***atpH-I*** and ***trnL-F* region:** 4 min at 94ºC; 35 cycles of (1 min at 94ºC, 1 min at 58-50ºC, 2 min at 72ºC); 5 min at 72ºC and stored at 4ºC. **2.) *matK*, *rpoB*** and ***rpoC1* regions:** 1 min at 94ºC; 30 s at 94ºC, 40 s at 53ºC, 40 s at 72ºC repeated 35 times; 5 min at 72ºC and stored at 4ºC. **3.) *rpl16* region:** 3 min at 94ºC; 1 min at 94ºC, 1 min at 52ºC, 3 min at 72ºC repeated 28 times; 7 min at 72ºC and stored at 4ºC. **4.) *ITS1* region:** 3 min at 94ºC; a touchdown program was run over a 58ºC to 50ºC temperature range for 16 cycles; 40 s at 94ºC, 40 s at 49ºC, 90 s at 72ºC repeated 25 times; 7 min at 72ºC and stored at 4ºC.

Table 2.2: The twelve primer pairs used during sequencing analysis and their respective nucleotide sequences.

Region	Primer Name	Direction	Primer Sequence (5'-3')	Reference
matK	**2.1**	**f**	CCT ATC CAT CTG GAA ATC TTA G	http://www.kew.org/
	5	**r**	GTT CTA GCA CAA GAA AGT CG	barcoding/iupdate.html
rpoB	**2**	**f**	ATG CAA CGT CAA GCA GTT CC	http://www.kew.org/
	4	**r**	GAT CCC AGC ATC ACA ATT CC	barcoding/iupdate.html
rpoC1	**2**	**f**	GGC AAA GAG GGA AGA TTT CG	http://www.kew.org/
	4	**r**	CCA TAA GCA TAT CTT GAG TTG G	barcoding/iupdate.html
atpH-I	**H-P**	**f**	CCA GCA GCA ATA ACG GAA GC	Grivet *et al*., 2001
	I-M	**r**	ATA GGT GAA TCC ATG GAG GG	
rpl16	**F71**	**f**	GCT ATG CTT AGT GTG TGA CTC GTT G	Jordan *et al*., 1996
	R1661	**r**	CGT ACC CAT ATT TTT CCA CCA CGA C	
trnL-F	**c**	**f**	CGA AAT CGG TAG ACG CTA CG	Taberlet *et al*., 1991
	d	**r**	GGG GAT AGA GGG ACT TGA AC	
	e	**f**	GGT TCA AGT CCC TCT ATC CC	

	f	**r**	ATT TGA ACT GGT GAC ACG AG	
	PS1	**f**	CTA CGG ACT TAA TTG GAT TGA GC	Pfosser & Speta, 1999
	PS2	**r**	GGG GAT AGA GGG ACT TGA AC	
	PS3	**f**	GGT TCA AGT CCC TCT ATC CC	
	PS4	**r**	AGG ATT TTC AGT CCT CTG CTC	
ITS1	**L**	**f**	TCG TAA CAA GGT TTC CGT AGG TG	Hsiao *et al*., 1995
	2	**r**	GCT GCG TTC TTC ATC GAT GC	White *et al*., 1990

The BioFlux Biospin Gel Extraction Kit was used to clean the PCR products after pre-sequencing. For sequencing the ABI Prism BigDye Terminator v 3.1 Cycle Sequencing Kit was used. The sequencing reaction mixture consisted of Premix, 3.2 pmol Primer, 20 ng Template DNA (diluted), 5x Sequencing Buffer, DMSO and dH_2O. The PCR sequencing program was as follows: 3 min at 94ºC; 10 s at 94ºC, 5 s at 50ºC, 4 min at 60ºC repeated 25 times; 5 min at 72ºC and hold at 4ºC. The Ethanol/EDTA post-reaction cleanup was used as described in the ABI Prism BigDye Terminator v 3.1 Cycle Sequencing Kit protocol.

2.3.3. Data analysis

The forward and reverse strands were assembled using Geneious Pro. The edited sequence results for all the samples were aligned with this program (Rozen & Skaletsky, 2000).

2.4. Results

2.4.1. DNA amplification

After the initial amplification, all the samples showed amplification after separation on a 1% agarose gel (Appendix C). A 1 X TAE buffer (40mM Tris-acetate, 1mM EDTA at a pH of 8.0) was used during electrophoresis and the intercalation of ethidium bromide enabled visualization on an ultraviolet light.

2.4.2. Unique barcodes and SNP’s

Twenty-two unique SNPs were obtained for *C. nobilis* and *C. mirabilis* when the sequencing data of the seven regions were combined. Geneious4.5.1.0

was used during analysis. Five of these SNPs can be seen in Figure 2.1 to 2.4. (Refer to Appendix D for all the barcodes/SNPs).

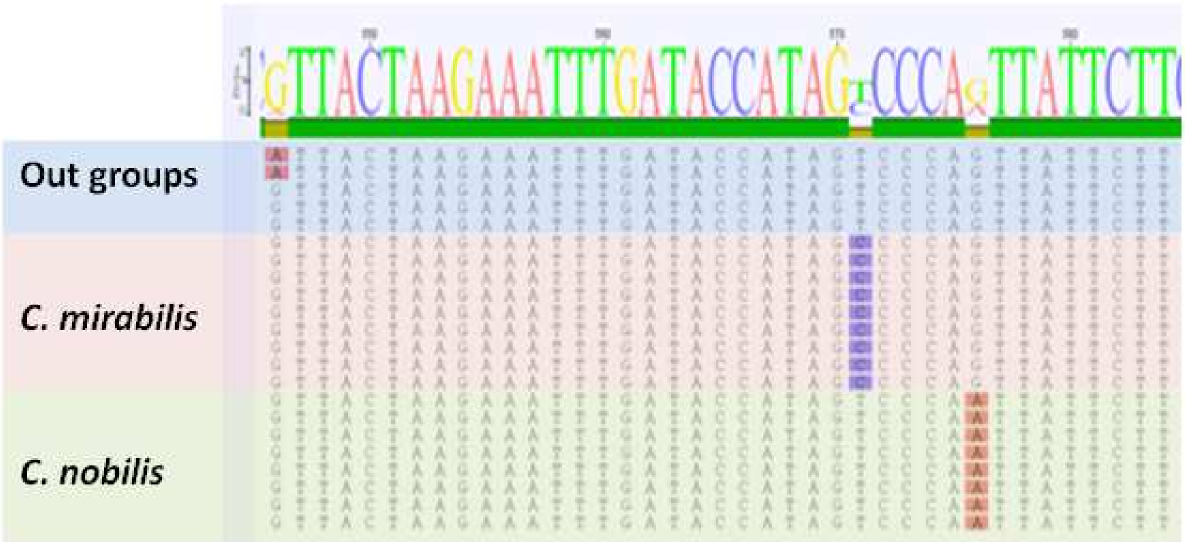

Figure 2.1: Two unique one base pair barcodes obtained respectively for *C. nobilis* and *C. mirabilis* within the *matK* region (Geneious4.5.1.0).

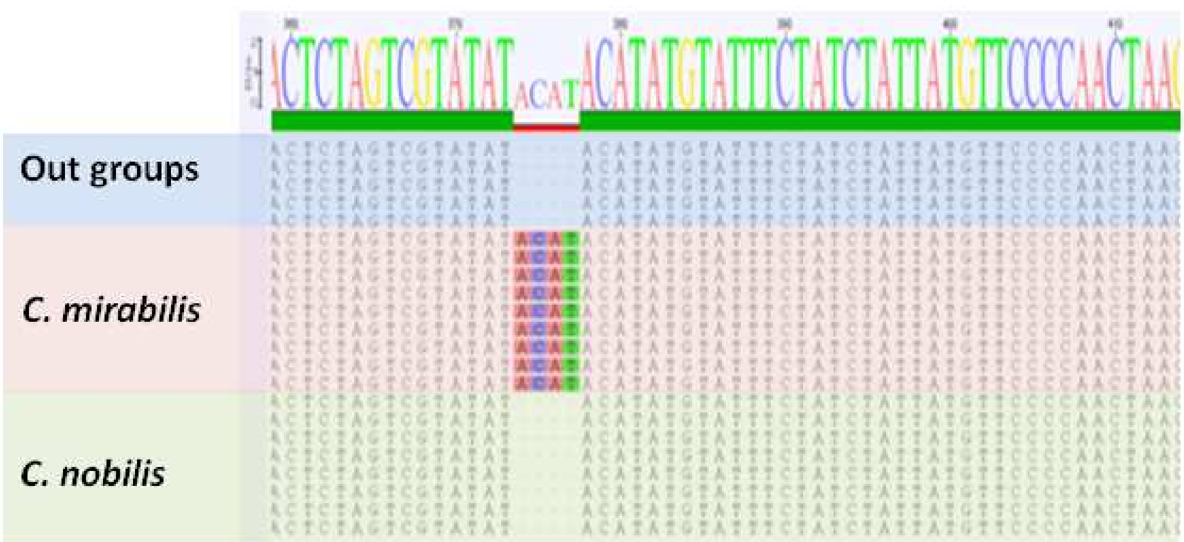

Figure 2.2: A four base pair indel obtained which differentiate between *C. nobilis* and *C. mirabilis* when the *atpH-I* region was analysed (Geneious4.5.1.0).

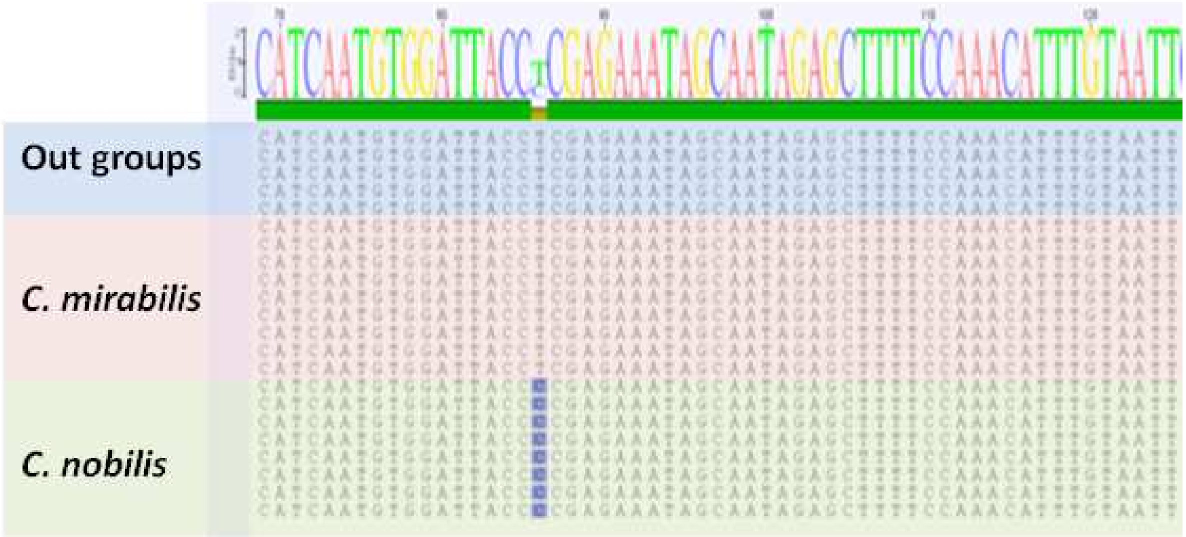

Figure 2.3: A unique one base pair difference within the *rpoC1* region for all *C. nobilis* samples (Geneious4.5.1.0).

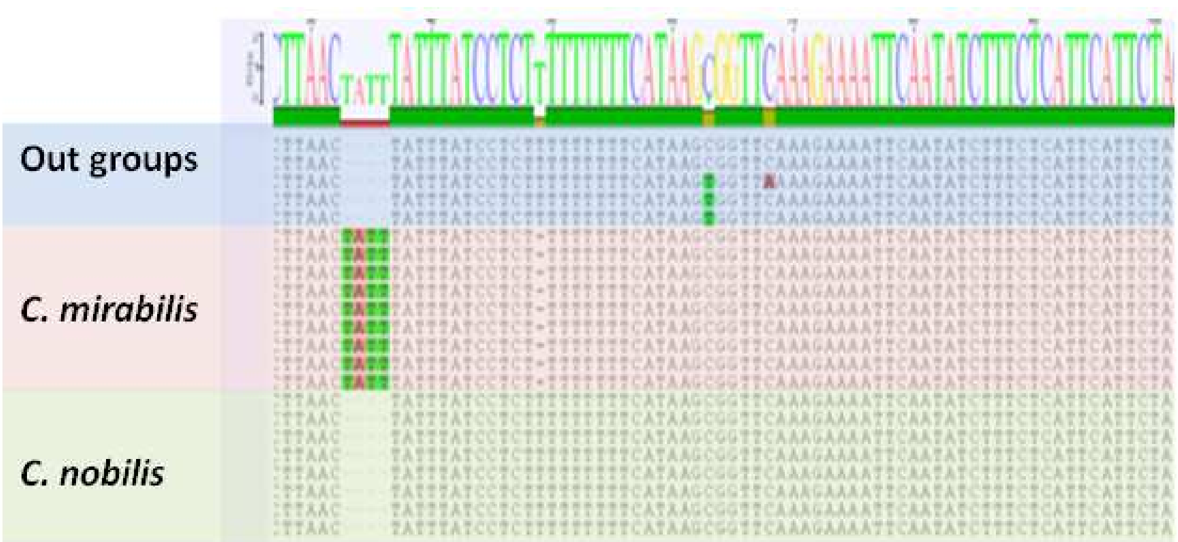

Figure 2.4: A four base pair and a one base pair indel which differentiate between *C. nobilis* and *C. mirabilis* when the *trnL-F* region was analysed (Geneious4.5.1.0).

The number of polymorphic and parsimony informative sites were calculated for each of the seven gene regions used for sequencing. The length of each region was also included in the summary in Table 2.3.

Table 2.3: Summary of the sequencing results of the seven *Clivia* species for the seven gene regions.

Region	Length in bp	Variable (polymorphic) sites	Parsimony informative sites
matK	651	15	8
rpoB	488	3	1
rpoC1	329	1	1
atpH-I	444	0	0
rpl16	916	11	11
trnL-F	725	14	8
ITS1	284	3	3
Total	3837	47	32

2.5. Discussion

All seven regions were easily amplified and could discriminate between the different species, some better than others. Altogether twenty-two variable sites were obtained between *C. nobilis* and *C. mirabilis*. In contrast to the results obtained for all seven species the *atpH-I* and *rpoC1* regions showed one unique difference between *C. nobilis* and *C. mirabilis*. The *matK and ITS1* regions showed four differences between *C. nobilis* and *C. mirabilis*. Six SNPs were observed in the *trnL-F* and *rpl16* regions.

All seven gene regions showed a distinct difference between these two primitive species and the other *Clivia* species used as out-groups. Even the *rpoB* region which showed no differences between *C. nobilis* and *C. mirabilis* did reveal changes between these two primitive species and the species used as out-groups. *Clivia nobilis* and *C. mirabilis* are distinct from the five other Clivia species. All the sequencing results confirmed that *Clivia gardenii* and *C. robusta* are very closely related as well as *C. caulescens* and *C. xnimbicola*.

The sequencing results showed that the *atpH-I*, *rpoB*, *rpoC1* and *ITS1* regions were not as informative as the *matK, rpl16* and *trnL-F* regions. These three regions showed a high number of polymorphic and parsimony informative sites. A site is parsimony informative if it contains at least two types of nucleotides and each of those nucleotides occurs in at least two of the sequences. Chase *et al*. (2007) also reported more sequence variation within the *matK* region than the *rpoB* and *rpoC1* regions. The *rpl16* region could not only reveal interspecific variation between *C. nobilis* and *C. mirabilis* but also intraspecific variation in *C. mirabilis*.

Six of the seven regions proved to be promising barcoding regions for *C. nobilis* and *C. mirabilis*, the *rpoB* region being the exception. This region could only identify variable sites between the two primitive species and the other five *Clivia* species used as out-groups. Five of the remaining regions (*atpH-I, rpoC1, matK*, *trnL-F*, and *ITS1*) all showed variability between different species (Rubinoff *et al*., 2006; Taberlet *et al*., 2007), but were still conserved within species. The *rpl16* chloroplast region showed intraspecific variation in *C. mirabilis* and interspecific variation between *C. nobilis* and *C. mirabilis*.

All seven regions can be used together for barcoding purposes or different combinations can be used. Individually none of the regions provided strong enough species discrimination and, therefore, more than one region should be used. We propose that *matK*, *rpl16* and *trnL-F* are used together as a barcode in the genus *Clivia*. These regions all had conserved flanking regions and could discriminate between the seven *Clivia* species. These three regions also had the highest number of parsimony informative sites.

2.6. Conclusion

The sequencing data obtained from the *atpH-I, rpoC1*, *matK*, *rpl16*, *trnL-F* and *ITS1* regions could be used for species identification in *C. nobilis* and *C. mirabilis* and, therefore, showed great potential as barcoding regions in the genus *Clivia*. These barcodes would be instrumental in the conservation of *C. nobilis* and *C. mirabilis* because mistaken identification would be eliminated.

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Chapter 3:

Genetic variation between and within *Clivia nobilis* and *Clivia mirabilis*

modified from Taxon

H. M. van der Westhuizen, P. Spies and J. J. Spies

3.1. Abstract

Genetic diversity was determined in *C. nobilis* and *C. mirabilis* by sequencing results of six non-coding chloroplast regions and one nuclear region. The regions used for the sequencing analyses were the *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16*, *trnL-F* and *ITS1* regions. The molecular information obtained from the sequencing data could differentiate between the different *Clivia* species with the exception of the *rpoC1* region. A data set combining five of the chloroplast regions resulted in 36 different sequences with 2383 different sites. Thirty-one parsimony informative sites were found in the combined data set and the overall transition/transversion bias was R = 0.743. The *rpl16* region was the only one showing intraspecific variation in *C. mirabilis*. Within the four different populations three distinctive clusters were identified. Although *C. nobilis* and *C. mirabilis* are distinct from the other five *Clivia* species, they are definitely two different species. The *ITS1* nuclear region has the highest rate of variation between *C. nobilis* and *C. mirabilis.*

3.2. Introduction

The genus *Clivia* Lindl. belongs to the family Amaryllidaceae J. St-Hil. (1805). This genus consists of seven different species. The species are *C. nobilis* Lindl., *C. caulescens* R.A.Dyer, *C. miniata* (Lindl.) Regel, *C. gardenii* Hook., *C. mirabilis* Rourke, *C. robusta* Murray, Ran, De Lange, Hammett, Truter & Swanevelder and *C. xnimbicola* Swanevelder, Truter & Van Wyk. Six of these species are found along the Eastern Coast and escarpment of South Africa, whereas *C. mirabilis* grows in the Northern Cape Province. *Clivia mirabilis* is geographically isolated from the other *Clivia* species. There is a distance of 700 kilometres between *C. mirabilis* and spatially the closest other *Clivia* species, *C. nobilis* (Google Earth, 2010). Earlier studies done on *Clivia* proved *C. nobilis* and *C. mirabilis* to be the two most primitive species in this genus (Conrad *et al.,* 2003).

Six chloroplast regions were used for sequencing analysis, namely the *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16,* the *trnL-F* regions and one nuclear region, *ITS1*. During this study the entire gene regions were not sequenced. During this study only variable regions (introns and intergenic spacers) were sequenced.

The *atpI* region facilitates the production of the ATP synthase IV subunit and *atpH* is responsible for the production of the ATP synthase III subunit (Woessner *et al*., 1987; Wakasugi *et al*., 1998). The *atpH-I* region in tobacco is 2146 bp in length (Wakasugi *et al*., 1998). The *matK* region is located within the intron region of the *trnK* gene (Neuhaus & Link, 1987; Wakasugi *et al*., 1998). The *matK* region is about 1500 bp long (Wakasugi *et al*., 1998) and plays an important role in the splicing of group II introns (Neuhaus & Link, 1987). A relatively high substitutions rate were observed in *matK* (Hilu *et al.,* 1999) which makes it ideal for revealing evolutionary history.

The *RNA polymerase B* (*rpoB*) region encodes the β subunit of RNA polymerase (Yepiz-Plascencia *et al*., 1990; Zeltz *et al*., 1993; Wakasugi *et al*., 1998; Drancourt & Raoult, 2002). The length of the *rpoB* region varied between approximately 3212 bp in Tobacco (Wakasugi *et al*., 1998) to 3452 bp - 3845 bp in *Staphylococcus* (Drancourt & Raoult, 2002). The *rpoB* region contains eight introns (Yepiz-Plascencia *et al*., 1990) which makes this region highly discriminative. The *RNA polymerase C1* (*rpoC1*) region is functionally involved in the expression of the β' subunit of chloroplast RNA polymerase (Wakasugi *et al*., 1998; Samigullin *et al*., 1999). The *rpoC1* region is 2804 bp in length and contains a 738 bp intron (Liston, 1992; Wakasugi *et al*., 1998).

The chloroplast gene, *rpl16,* codes for the ribosomal protein L16 (Jordan *et al*., 1996; Campagna & Downie, 1998). A large intron region is reported in most land plants. This intron region varies between 536bp and 1400bp (Campagna & Downie, 1998). The *trnL-F* region, consist of the *trnL* intron, the *trnL* (UAA) exon and the *trnL-F* intergenic spacer (Taberlet *et al.*, 1991). The *trnL-F* region is approximately 1000 bp long, of which the intron comprises 500 bp (Taberlet *et al.,* 1991; Wakasugi *et al*., 1998). Four universal primers (c, d, e and f) are available for the amplification of the *trnL-F* region (Taberlet *et al.,* 1991) and four nested primers for sequencing (Pfosser & Speta, 1999).

The internal transcribed spacer (*ITS*) regions in the nuclear ribosomal DNA have a high evolutionary rate. There are two *ITS* regions which are separated by the 5.8S rDNA. For amplification of the *ITS1* region, ITSL (Hsiao *et al*., 1995) and ITS2 (White *et al*. 1990) were used. The length of this region is approximately 290bp long (White *et al*., 1990).

This paper presents sequencing data obtained for all seven *Clivia* species but focuses mainly on morphologically different specimens of *C. nobilis* and *C. mirabilis.* The *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F and* the *ITS1* regions will be sequenced. Our first objective is to reconstruct the phylogenetic relationships within the genus *Clivia*. The phylogentic tree combining the data of the different chloroplast regions will be used to identify genetic variation between and within *C. nobilis* and *C. mirabilis*.

3.3. Materials and methods

3.3.1. Sample locality and extraction

A total of fifty-one samples were used during this study. All seven species were well represented; twenty-one samples of *C. mirabilis,* eleven *C. nobilis* samples, seven *C. gardenii* samples and six *C. miniata*, three *C. robusta* samples, two *C. caulescens* and one *C. xnimbicola* were used for analysis (Table 3.1). *Cryptostephanus vansonii* was included as an out-group.

Table 3.1: List of the plant specimens used during this study.

Collection Number	**Species**	**Locality**
1	*Cryptostephanus vansonii*	Bvunba mountain, Zimbabwe
90	*C. gardenii*	Ngome
95	*C. gardenii*	Unknown
112	*C. miniata*	Dweza
115	*C. miniata*	Umtamvuna
117	*C. miniata*	Kei Rivier
124	*C. gardenii*	Harburg
129	*C. miniata*	Dweza
131	*C. miniata*	Pedi
139	*C. gardenii*	Ingubevu
142	*C. nobilis*	Qora
144	*C. nobilis*	Unknown
160	*C. nobilis*	Rocklands
164	*C. robusta*	Port Shepstone
166	*C. robusta*	Port St. Johns
168	*C. gardenii*	Port Shepstone
170	*C. nobilis*	Birna River
173	*C. nobilis*	Qora (Cobb Inn)
174	*C. nobilis*	Chulumna (location 1)
175	*C. nobilis*	Keiskamma (location 2)

177	*C. nobilis*	Pirates Creek, Bonza Bay
178	*C. nobilis*	Pirates Creek, Bonza Bay
192	*C. robusta*	Unknown
193	*C. nobilis*	Fish River
210	*C. caulescens*	Gods' Window
221	*C. caulescens*	Pinnacle
240	*C. mirabilis*	Donkerhoek (Population 1)
241	*C. mirabilis*	Donkerhoek (Population 1)
242	*C. mirabilis*	Donkerhoek (Population 1)
247	*C. mirabilis*	Donkerhoek (Population 1)
252	*C. mirabilis*	Donkerhoek (Population 2)
253	*C. mirabilis*	Donkerhoek (Population 2)
254	*C. mirabilis*	Donkerhoek (Population 2)
255	*C. mirabilis*	Donkerhoek (Population 2)
256	*C. mirabilis*	Donkerhoek (Population 2)
257	*C. mirabilis*	Donkerhoek (Population 2)
258	*C. mirabilis*	Donkerhoek (Population 2)
262	*C. mirabilis*	Klein Koebee
264	*C. mirabilis*	Klein Koebee
265	*C. mirabilis*	Klein Koebee
266	*C. mirabilis*	Klein Koebee
267	*C. mirabilis*	Klein Koebee
269	*C. mirabilis*	Klein Koebee
270	*C. mirabilis*	Klein Koebee
271	*C. mirabilis*	Klein Koebee
272	*C. mirabilis*	Klein Koebee
274	*C. gardenii*	Ngome
309	*C. miniata*	Bearded Man
329	*C. xnimbicola*	Bearded Man
449	*C. nobilis*	Qora
533	*C. mirabilis*	Oorlogskloof Nature Reserve

A different number of specimens were used for the different gene regions. Table 3.2 indicates the number of species used for each gene region.

Table 3.2: Number of plants used for each gene region.

Gene region	***C. nobilis***	***C. mirabilis***	***C. miniata***	***C. caulescens***	***C. xnimbicola***	***C. gardenii***	***C. robusta***
atpH-I	8	9	6	2	1	7	3
matK	8	9	6	2	1	7	3
rpoB	8	9	6	2	1	7	3
rpoC1	8	9	6	2	1	7	3
trnL-F	8	9	6	2	1	7	3
rpl16	11	21	-	-	-	-	-
ITS1	9	8	-	-	-	-	-

A modified Cetyltrimethylammonium bromide (CTAB) extraction method was followed (Rogstad, 1992) as mentioned in Chapter 2 (See Appendices A and B).

3.3.2. Sequencing and data analysis

For optimization of PCR programs and concentrations of reactions refer to Chapter 2, section 2.3.2. and Table 2.2. The sequence assembly was done as described in section 2.3.3 (Geneious Pro).

The DnaSP v 5.0 program (Rozas *et al*., 2003) was used to measure the sequence variation found within and between different populations, whereas Network 4.5.1.0 enabled the reconstruction of networks. Maximum parsimony trees were constructed (Polzin *et al.,* 2003), using MEGA 4.1 (Kumar *et al*., 2008).

3.4. Results

3.4.1. Genetic variation

After alignment the consensus sequence obtained for *ITS1* were the shortest of all the gene regions with a length of 284 bp. The *rpoC1*, *atpH-I* and *rpoB* regions were respectively 329, 444 and 488 bp in length. The *matK* region had a length of 651bp. With a length of 916 bp the *rpl16* region were the longest and *trnL-F* region the second longest with a length of 725 bp. The results for *atpH-I, matK*, *rpoB*, *rpoC1* and the *trnL-F* regions were combined and used together for further analysis.

Table 3.3: Summary of the haplotype information obtained when DnaSP and Network 4.5.1.0 were used.

	Number of species analysed	Total number of samples	Different Haplotypes	Haplotype Diversity
Combined data set	7	36	15	0.886
rpl16	2	32	4	0.7238
ITS1	2	17	2	0.5294

The average evolutionary divergence between the different sequences was estimated (MEGA 4.1). The number of base substitutions per site is shown (Table 3.4). All results are based on the pairwise analysis of the respective number of sequences used for each data set. Analyses were conducted using the Maximum Composite Likelihood method in MEGA4 (Tamura *et al*., 2004; Tamura *et al*., 2007). All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

Table 3.4: Summary of the average evolutionary divergence amongst and between the *C. nobilis* and *C. mirabilis* populations.

	Combined Data Set	***rpl16* region**	***ITS1* region**
Overall Mean	0.005	0.004	0.006
Within Group Mean (d)	0.000	0.002	0.000
Between Groups Mean	0.003	0.007	0.011
Net Between Groups Mean	0.003	0.006	0.011
Within Subpopulation	0.000	0.001	0.000
Diversity for Entire Population	0.002	0.004	0.006
Mean Inter Population Diversity	0.002	0.003	0.006
Coefficient of Differentiation Diversity	1.000	0.765	1.000

3.4.2. Evolutionary relationships

The evolutionary history was inferred (MEGA 4.1) using the Neighbor-Joining method (Saitou & Nei, 1987), the Minimum Evolution method (Rzhetsky & Nei, 1992), the Maximum Parsimony method (Eck & Dayhof, 1966) and the UPGMA method (Sneath & Sokal, 1973). The median joining and the reduced median methods were the options used in Network. The different methods used in MEGA 4.1 and those used in Network 4.5.1.0 all produced phylogenies with the same topography.

The combined data set (*atpH-I, matK*, *rpoB*, *rpoC1* and *trnL-F*) were analysed using the Maximum Parsimony method (Eck & Dayhoff, 1966). Out of a 1000 replicates the bootstrap consensus tree in Fig 3.1 is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The final data set contained 2383 positions and 31 of these sites were parsimony informative. The phylogenetic analyses were conducted in MEGA 4.1 (Tamura *et al.*, 2007).

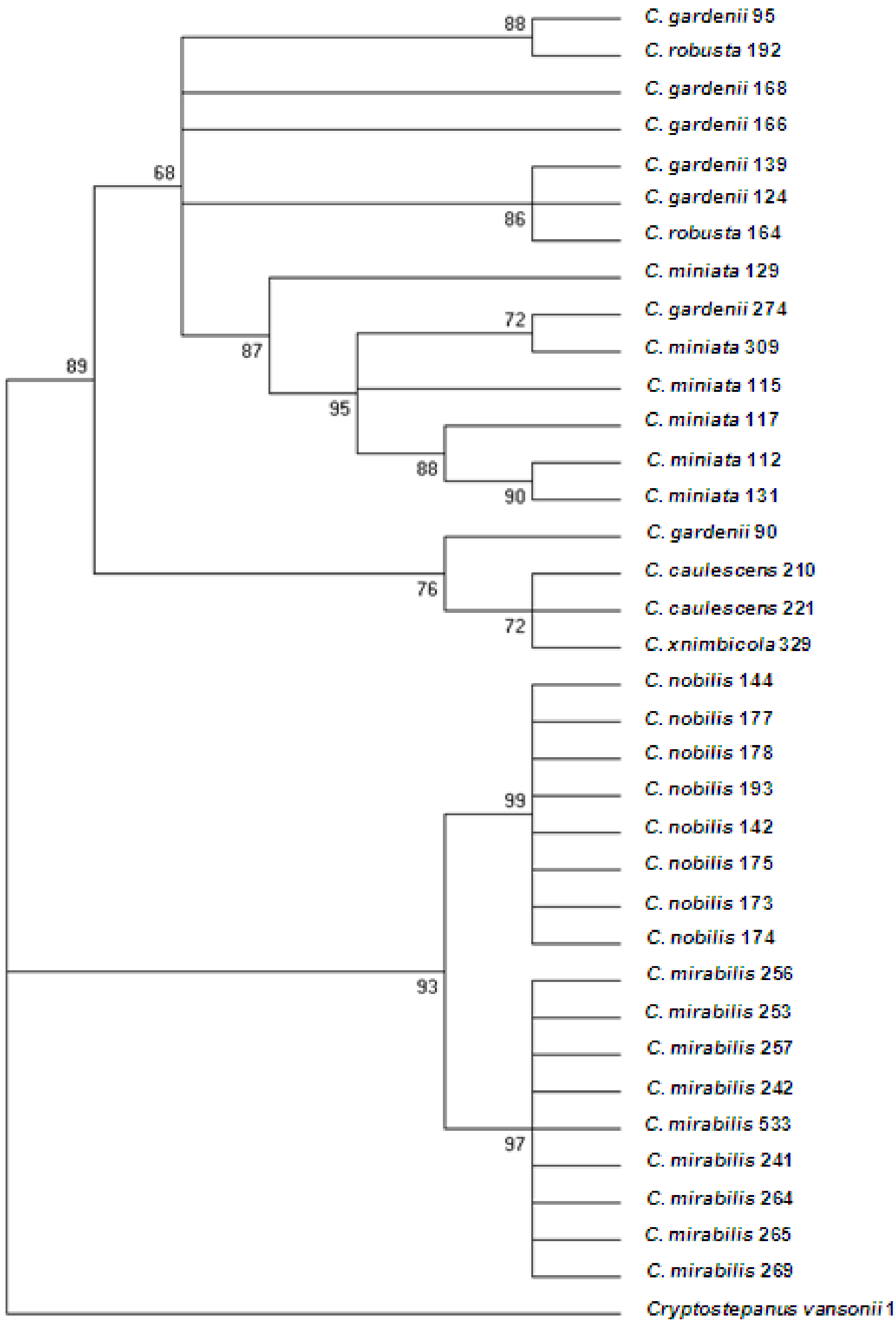

Figure 3.1: A cladogram representing the different *Clivia* species when the Maximum Parsimony method was used (MEGA 4.1) and the combined data set were analysed. The values shown next to each clade are the bootstrap values.

The median joining method was used to construct the evolutionary network (Fig 3.2) (Network 4.5.1.0) for the combined data set (*atpH-I, matK*, *rpoB*, *rpoC1* and *trnL-F*).

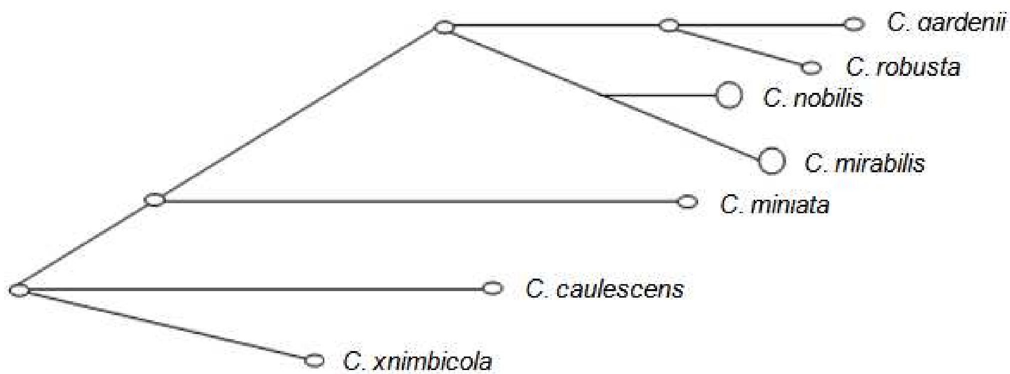

Figure 3.2: The reconstruction of the most parsimonious tree (Network 4.5.1.0) representing the different *Clivia* species when the combined data set were used.

The sequencing results obtained for the *rpl16* region were analysed and the Maximum Parsimony method were used (Eck & Dayhoff, 1966). Tree #1 out of 350 equally parsimonious trees (length = 11) is shown (Fig. 3.3). In the final data set of 884 positions, 11 were parsimony informative. The phylogenetic analyses were conducted in MEGA 4.1 (Tamura *et al.*, 2007).

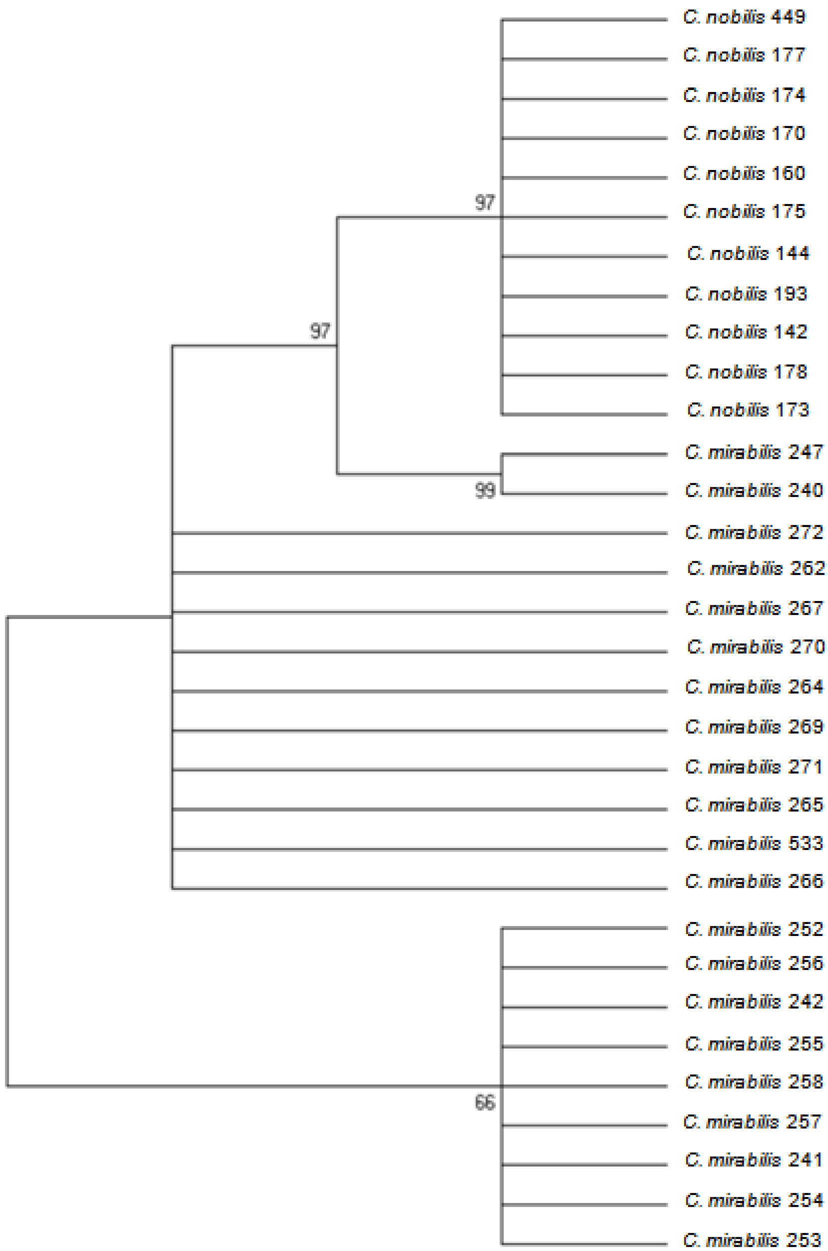

Figure 3.3: A cladogram representing the *rpl16* sequences obtained for *C. nobilis* and *C. mirabilis* when the Maximum Parsimony method was used (MEGA 4.1). The values shown next to each clade are the bootstrap values.

The evolutionary history of the *ITS1* region was inferred using the Maximum Parsimony method (Eck & Dayhoff, 1966). In Fig 3.4 #1 of 270 most parsimonious trees (length = 3) is shown. There were a total of 281 positions in the final dataset, out of which 3 were parsimony informative. Phylogenetic analyses were conducted in MEGA 4.1 (Tamura *et al.*, 2007).

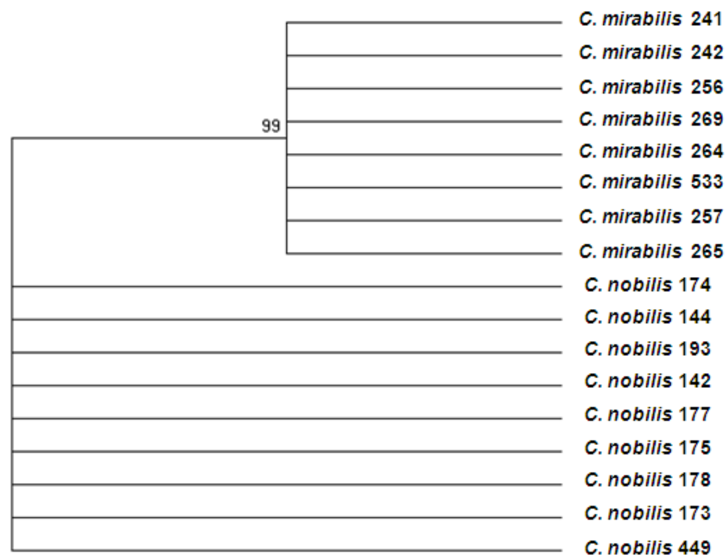

Figure 3.4: An un-rooted cladogram representing the *ITS1* sequences obtained for *C. nobilis* and *C. mirabilis* when the Maximum Parsimony method was used (MEGA 4.1). The values shown next to each clade are the bootstrap values.

3.4.3. Statistical tests

The Tajima' relative rate test (Table 3.5) (MEGA 4.1) was used to test the equality of the evolutionary rate between *C. mirabilis* and *C. nobilis* (Tajima, 1993; Tamura *et al.*, 2007). *Clivia miniata* was used as a outgroup. The χ^2 test statistic was 0.5 (P = 0.47950 with 1 degree[s] of freedom).

Table 3.5: Results from the Tajima test for *C. nobilis*, *C. mirabilis* and *C. miniata*.

Configuration	Count
Identical sites in all sequences (m_{iii})	2363
Divergent sites in all sequences (m_{ijk})	1
Unique differences in *C. mirabilis* (m_{ijj})	3
Unique differences in *C. nobilis* (m_{iji})	5
Unique differences in *C. miniata* (m_{iij})	16

The probability of the substitution from one base (row) to another base (column) is indicated in Table 3.6. The entries within a specific row should be compared. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The nucleotide frequencies are 0.329 (A), 0.32 (T/U), 0.182 (C), and 0.168 (G). The transition/transversion rate ratios are k_1 = 1.521 (purines) and k_2 = 2.7 (pyrimidines). The overall transition/transversion bias is R = 0.743, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$. The final data set contained 2383 positions. All the calculations were conducted in MEGA 4.1 (Tamura *et al*., 2007).

Table 3.6: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution (Tamura *et al*., 2004).

	A	**T**	**C**	**G**
A	-	*7.78*	*4.45*	**6.21**
T	*7.99*	-	**12**	*4.08*
C	*7.99*	**21.02**	-	*4.08*
G	**12.16**	*7.78*	*4.45*	-

The Tajima test statistic (Tajima, 1989) was estimated using MEGA 4.1 (Tamura *et al*., 2007). The abbreviations used in Table 3.7 are as follows: m

= number of sites, S = Number of segregating sites, p_s = S/m, Θ = p_s/a_1, and π = nucleotide diversity. D is the Tajima test statistic (Nei & Kumar, 2000).

Table 3.7: Results from Tajima's Neutrality Test for the 36 sequences used during this study.

m	S	p_s	Θ	π	D
36	53	0.022241	0.005363	0.004565	-0.540529

3.5. Discussion

Of the seven gene regions sequenced all showed variation between *C. nobilis* and *C. mirabilis* but only *rpl16* showed intraspecific variation in *C. mirabilis*. The *atpH-I, rpoB* and *rpoC1* regions showed very little variation between *C. nobilis* and *C. mirabilis*. Intra- and interspecific variation were observed in the other five *Clivia* species for four of the five regions sequenced, the exception being the *rpoC1* region. Fifteen different haplotypes were found within the seven *Clivia* species when the combined data set were analysed (DnaSP and Network 4.5.1.0). The amount of haplotypes observed in the combined data set indicates a high level of intraspecific variation within the five *Clivia* species included in the data set. The *ITS1* results showed that although diversity levels were low within the different populations of a specific species, strong diversity was observed between *C. nobilis* and *C. mirabilis*. The *rpl16* region revealed three haplotypes within *C. mirabilis* and one haplotype for all the *C. nobilis* samples. The *rpl16* region was the best at revealing variation within the two primitive species.

When only the *C. nobilis* and *C. mirabilis* samples were considered, the average evolutionary divergence over sequence pairs within groups proofed that the *rpl16* region had more variation within populations of *C. nobilis* and *C. mirabilis* than the combined data set or the *ITS1* nuclear region. The variation between *C. nobilis* and *C. mirabilis*, however, were the highest in the *ITS1* region.

The bootstrap test estimates the reliability of a particular grouping. Most of the bootstrap values obtained were high and, therefore, the phylogentic trees were considered to be reliable. The tree obtained from the combined data set showed that *C. nobilis* and *C. mirabilis* forms monophyletic groups and *C. caulescens* and *C. xnimbicola* forms a monophyletic group. Amongst *C. miniata*, *C. gardenii* and *C. robusta* more variation were observed and therefore no distinct groups were identified. Although *C. nobilis* and *C. mirabilis* is distinct from the other five *Clivia* species these two species is distinct from one another.

The *rpl16* chloroplast region showed intraspecific variation in *C. mirabilis* and inter-specific variation between *C. nobilis* and *C. mirabilis*. The cladogram representing the sequencing results of the *rpl16* region showed that within the four different *C. mirabilis* populations, three distinctive groups can be identified. Two plants within one of the Donkerhoek populations were different from the other plants in this population.

3.6. Conclusion

The five genes used in the combined data set were unable to show intraspecific variation in *C. nobilis* and *C. mirabilis* but four of these regions (*atpH-I, matK*, *rpoB*, and *trnL-F*) did show intraspecific variation within *C. miniata*, *C. gardenii* and *C. robusta*. The *rplI6* region was the only region where intraspecific variation was observed within *C. mirabilis* and even variation within a specific population. Genetic variation was strong between *C. nobilis* and *C. mirabilis* in six of the seven gene region sequenced. These two primitive species were distinct from the other *Clivia* species in the results of all seven regions.

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Chapter 4:

Testing for intraspecific variation in *Clivia nobilis* and *Clivia mirabilis*

Preliminary study

H. M. van der Westhuizen

4.1. Abstract

Seven gene regions were sequenced. In six of these regions interspecific variation were observed (*atpH-I*, *matK*, *rpoB*, *rpoC1, trnL-F* and *ITS1*). The chloroplast region *rpl16* showed intraspesific variation within the different *C. mirabilis* populations. Microsatellite regions contain a high frequency of mono-, di-, tri- or tetra nucleotide repeats. These markers contain a high level of mutations and could reveal genetic variation easily even in small populations sizes. Microsatellites had been used across species with success. *Clivia nobilis* and *C. mirabilis* were tested with primers designed originally for *Phaedranassa tunguraguae, Hymenocallis coronia* and *Clivia miniata*. The cross-species microsatellite makers were evaluated and although amplification was obtained, the amplified regions were not microsatellites in the studied species. The unsuccessful attempts could be due to the fact that the microsatellite region stabilized in *C. nobilis* and *C. mirabilis*. All contributing factors were taken into consideration, including mutations in the flanking regions of the microsatellites and ancient polypoidy found in *Clivia*. In future the other five *Clivia* species should also be tested with these primers since *C. nobilis* and *C. mirabilis* are distinct from the other species. Specific primers for the genus *Clivia* should be designed in future.

4.2. Introduction

Clivia nobilis Lindl. and *C. mirabilis* Rourke belong to the family Amaryllidaceae J. St-Hil. (1805). The genus *Clivia* is comprised of seven different species of which *C. nobilis* and *C. mirabilis* are the two most primitive (Conrad *et al.,* 2003). The other *Clivia* species are *C. caulescens* R.A.Dyer, *C. miniata* (Lindl.) Regel, *C. gardenii* Hook., *C. robusta* Murray, Ran, De Lange, Hemmett, Truter & Swanevelder and *C. xnimbicola* Swanevelder, Truter & Van Wyk. *Clivia mirabilis* is found in the Northern Cape Province whereas *C. nobilis* grows along the Eastern Coast and escarpment of South Africa. Google Earth shows a distance of 700 kilometres that separates *C. mirabilis* and *C. nobilis* which is spatially the closest other *Clivia* species. *Clivia mirabilis* can therefore be seen as being geographically isolated from the other *Clivia* species.

Gene regions which reveal intraspecific variation should contain non-coding regions like introns and intergenic spacers. Non-coding regions are more prone to mutations because selection against mutations is not as strong as in the regions essential for gene function (Taberlet *et al.,* 1991; Hamilton, 1999). By sequencing introns and intergenic spacers, it would be possible to study intraspecific variation if the genes sequenced is informative enough (Hamilton, 1999).

A microsatellite region has high frequencies of mono-, di-, tri- or tetra-nucleotide repeats (Fairbanks & Andersen, 1999). These markers are also called variable-number of tandem repeats (VNTR), short tandem repeats (STR) or simple sequence repeats (SSR) (Selkoe & Toonen, 2006). Microsatellite markers had been successfully used in a wide variety of plant species. These markers had been employed for the detection of genomic instability (Leroy *et al*., 2000), estimation of divergence time (Zhivotovsky, 2001) and resolving the taxonomy (Jøgensen *et al*., 2008).

The popularity of microsatellites is brought about by their cost effectiveness and time efficiency (Selkoe & Toonen, 2006). Furthermore high mutation rates observed in the microsatellite regions would result in high allelic diversity. Microsatellites would, therefore, be ideal for analysing species with small population sizes (Hendrick, 1999; Selkoe & Toonen, 2006). Cross-contamination is reduced because of the species specificity of the primer pairs (Selkoe & Toonen, 2006). This advantage could prevent microsatellite primers from binding and amplifying across species. The low mutation rate observed at the flanking regions, however, could result in different species of the same genus or family having the same nucleotide sequence (Selkoe & Toonen, 2006). Cross-species amplification would, therefore, increase as the evolutionary distance between species decreases (Steinkellner *et al*., 1997).

Microsatellite makers had been used effectively across different species (Rossetto *et al*., 2001; Gupta *et al*., 2003; Eujayl *et al*., 2004; Saha *et al*., 2004; Markwith & Scanlon, 2006; Cotrim *et al*., 2009). In some cases the transferability of these markers between different species and genera were unsuccessful or very low (White & Powell, 1997; Peakall *et al*., 1998; Roa *et al*., 2000).

Six chloroplast regions were used for sequencing analysis, namely the *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F* regions and one nuclear region, *ITS1*. The seven regions will be tested for intraspecific variation within *C. nobilis* and *C. mirabilis.* *Phaedranassa tunguraguae, Hymenocallis coronia* and *Clivia miniata* all belongs to the family Amaryllidaceae J. St-Hil. (1805). Our main objective is to evaluate the use of cross-species markers and then to test for intraspecific variation using sequencing and microsatellites.

4.3. Materials and methods

4.3.1. Sample locality and extraction

Four different *C. mirabilis* populations were sampled. Ten to thirteen plants from each population were sampled with the exception of the Oorlogskloof Nature Reserve, where only one sample was obtained. Due to the relatively small population sizes of this species, these samples represented *C. mirabilis* well. *Clivia nobilis* were well represented; samples from twenty different populations were used for microsatellite analysis. Three *C. nobilis* samples, collected at the mouth of the Qora River in the Eastern Cape Province, were included in the preliminary study. This population is known for its wide range of phenotypic diversity.

Table 4.1: List of *C. nobilis* plant samples used.

Number	Species	Locality	Number	Species	Locality
52	*C. nobilis*	Unknown	171	*C. nobilis*	Trenneries
141	*C. nobilis*	Qora	172	*C. nobilis*	Trenneries
142	*C. nobilis*	Horseshoe Valley	173	*C. nobilis*	Qora (Cobb Inn)
143	*C. nobilis*	Bonza Bay	174	*C. nobilis*	Chulumna, location 1
144	*C. nobilis*	Unknown	175	*C. nobilis*	Keiskamma, location 2
145	*C. nobilis*	Ncera	176	*C. nobilis*	Pirates Creek, Bonza Bay
146	*C. nobilis*	Beacon Bay	177	*C. nobilis*	Pirates Creek, Bonza Bay
147	*C. nobilis*	Gonubie	178	*C. nobilis*	Pirates Creek, Bonza Bay
148	*C. nobilis*	Fish River	179	*C. nobilis*	Pirates Creek, Bonza Bay
149	*C. nobilis*	Kei River	180	*C. nobilis*	Pirates Creek, Bonza Bay
150	*C. nobilis*	Nahoon River	181	*C. nobilis*	Pirates Creek, Bonza Bay
151	*C. nobilis*	Keimouth	182	*C. nobilis*	Bonza Bay
152	*C. nobilis*	Riet River	183	*C. nobilis*	Shadow Park
153	*C. nobilis*	Ndweza	184	*C. nobilis*	Qugara
156	*C. nobilis*	Qaxa	185	*C. nobilis*	Horseshoe Valley
157	*C. nobilis*	Witriver	186	*C. nobilis*	Qugara
159	*C. nobilis*	Ncera	187	*C. nobilis*	Needscamp
160	*C. nobilis*	Rocklands	188	*C. nobilis*	Keimouth
161	*C. nobilis*	Birna River	191	*C. nobilis*	Boesmans River
162	*C. nobilis*	Qora	193	*C. nobilis*	Fish River
163	*C. nobilis*	Chalumna, location 5	194	*C. nobilis*	Bonza Bay
169	*C. nobilis*	Chalumna, location 3	196	*C. nobilis*	Shadow Park
170	*C. nobilis*	Birna River	197	*C. nobilis*	Shadow Park

Table 4.2: List of the *C. mirabilis* plant samples used.

Number	Species	Locality	Number	Species	Locality
239	*C. mirabilis*	Donkerhoek (Population 1)	257	*C. mirabilis*	Donkerhoek (Population 2)
240	*C. mirabilis*	Donkerhoek (Population 1)	258	*C. mirabilis*	Donkerhoek (Population 2)
241	*C. mirabilis*	Donkerhoek (Population 1)	259	*C. mirabilis*	Donkerhoek (Population 2)
242	*C. mirabilis*	Donkerhoek (Population 1)	260	*C. mirabilis*	Donkerhoek (Population 2)
243	*C. mirabilis*	Donkerhoek (Population 1)	261	*C. mirabilis*	Donkerhoek (Population 2)
244	*C. mirabilis*	Donkerhoek (Population 1)	262	*C. mirabilis*	Klein Koebee
245	*C. mirabilis*	Donkerhoek (Population 1)	263	*C. mirabilis*	Klein Koebee
246	*C. mirabilis*	Donkerhoek (Population 1)	264	*C. mirabilis*	Klein Koebee
247	*C. mirabilis*	Donkerhoek (Population 1)	265	*C. mirabilis*	Klein Koebee
248	*C. mirabilis*	Donkerhoek (Population 1)	266	*C. mirabilis*	Klein Koebee
249	*C. mirabilis*	Donkerhoek (Population 1)	267	*C. mirabilis*	Klein Koebee
250	*C. mirabilis*	Donkerhoek (Population 1)	268	*C. mirabilis*	Klein Koebee
251	*C. mirabilis*	Donkerhoek (Population 1)	269	*C. mirabilis*	Klein Koebee
252	*C. mirabilis*	Donkerhoek (Population 2)	270	*C. mirabilis*	Klein Koebee
253	*C. mirabilis*	Donkerhoek (Population 2)	271	*C. mirabilis*	Klein Koebee
254	*C. mirabilis*	Donkerhoek (Population 2)	272	*C. mirabilis*	Klein Koebee
255	*C. mirabilis*	Donkerhoek (Population 2)	273	*C. mirabilis*	Klein Koebee
256	*C. mirabilis*	Donkerhoek (Population 2)	533	*C. mirabilis*	Oorlogskloof Nature Reserve

Clivia nobilis DNA was extracted from fresh leaf material, whereas the *C. mirabilis* samples were stored in saturated Sodium chloride-Cetyltrimethylammonium bromide (NaCl-CTAB) for five months before DNA extraction. A modified Cetyltrimethylammonium bromide (CTAB) extraction method was followed as proposed by Rogstad (1992). (Results are shown in Appendices A and B).

4.3.2. Amplification, sequencing and data analysis

The optimization of the sequencing reactions were described in Chapter 2, section 2.3.2. and Table 2.2. For data analysis refer to section 2.3.3 (Geneious Pro).

For microsatellites the 10 µl reaction mixture consisted of: 2 µl 5x Buffer (10 µl 10 mM dNTP, 500 µl 10x Buffer, 0.001 g Gelatine, 455 µl dH_2O, 5 µl 100x Triton), 0.1 µl 5 units/µl Super-Therm Taq Polymerase, 0.5 µl 25 mM $MgCl_2$, 0.5 µl 10 µM of each Primer Pair (14 different primer pairs were tested), 4.9 µl dH_2O, 2 µl 20ng/µl Template DNA. The Taguchi optimisation (Cobb & Clarkson, 1994) method was used to find the correct concentration of each component.

The following program for DNA amplification was used: 3 min at 94ºC; 30 s at 94ºC, 30 s at XºC, 30 s at 72ºC repeated 35 times; 60 min at 72ºC and stored at 4ºC. X represents the optimum annealing temperature (T_a) established for each primer set.

Table 4.3: Primer pairs used for cross-species microsatellite analysis**.**

Locus	Primer Sequence 5'-3'	Repeat motif	T_a(ºC)	Allele size range (bp)[1]	Dye label[2]
[3]**Pt 4**	F: TCCTTGTATCGTATGCTCCC R: CAAACGCTGTATCCCCTTC	$(CT)_{23}$	57	105-250	NED
[3]**Pt 9**	F: AAAACCCTAAGGAGAGAGGAG R: GAAATTTGACGATGAACGGAC	$(GA)_{17}$	56	87-125	VIC

[3]**Pt 14**	F: GGAGGATGGTAGTACCATGAAC R: TGTATGGTTGGGTATGGGAAC	$(GA)_{14}$	55	153-191	6FAM
[3]**Pt 36**	F: AGAGAATGTGATGGGAGAGAG R: TCTTCCTTATCCCCTCCACC	$(GA)_{22}$	52	178-199	NED
[3]**Pt 39**	F: TCAAAACACTCATACCAACACC R: CCTCTCTCTCCAAACTCTCTC	$(CA)_{10}$	52	232-264	6FAM
[4]**HcoA1**	F: TCTTACATTCAGGAAAGCAA R: TCTTAGGATTCATCTTGTGA	$i(AGA)_5AAC(AGA)_4$	47	185-206	6FAM
[4]**HcoA10**	F: TATGAGTTGAAGTGGAGTTGCA R: ATCCTCCATGATGAGACCCAA	$i(TGA)_6$	55	214-235	PET
[4]**HcoB1**	F: CTTCTACAAAACTACAGAGAGTCCA R: GTTGCATGAGATATGCCATAGG	$(AGA)_8$	50	280-316	6FAM
[4]**HcoD7**	F: AAGCTATGGATCGAAGTAGGCCTG R: CCCTAGAAGGTTATGCTTCCCACA	$(TGA)_3N_{42}(TGA)_4$	52	189-195	VIC
[4]**HcoD9**	F: CCACAGAGAATCCAGGTTCCTA R: ACATTCACACACTCACGCCTA	$(CA)_3N_{14}(CA)_4N_{29}(CA)_5$	58	245-257	6FAM
[5]**CLV 1**	F: CAATAATGTGGCTAATGGGTTG R: CTCAAGCTATGCATCCAACG	T_4AT_6	53	±200	VIC
[5]**CLV 2**	F: CTTGTTGTAGCTTGTAATAGC R: CTGAACGGCAGAGGAGTTG	$(GT)_9$	53	±225	6FAM
[5]**CLV 3**	F: ACAACTCCTCTGCCGTTCAG R: GGGTGCAGTGCACTAGTGC	A_{11}	53	±246	PET
[5]**CLV 4**	F: GCATCCCTTGCTCCTCTAC R: CTCAAGCTATGCATCCAACG	$(CCT)_2TCT(CCT)_2CGT$	55	±210	NED

[1] The allele size range was determined from the analysis on the respective plants for which it was designed.

[2] The forward primer of each marker was labelled at the 5’ end.

[3] Primer pair developed for *Phaedranassa tunguraguae* (Oleas, 2005).

[4] Primer pair developed for *Hymenocallis coronaria* (Markwith & Scalon, 2006).

[5] Primer pair developed for *Clivia miniata* (Swanevelder, 2003).

After amplification the PCR product was diluted 1 to 3. The diluted PCR product and Hi-Di Formamide was loaded onto an ABI3130 DNA analyser. LIZ was used as an internal size standard. GeneMaker software (Anonymous, 2010) was used for fragment sizing.

4.4. Results

4.4.1. DNA amplification and visualization

For the amplification and visualization of the sequencing results, refer to section 2.4.1. Genetic variation results were specified in section 3.3.1. The microsatellite PCR products were subjected to electrophoresis. The separation was done on a 5% polyacrylamide gel or a 2% agarose gel (Appendix F). Silver staining (Creste *et al.*, 2001) and ethidium bromide were respectively used to enable visualization of the amplified DNA fragments. If the amplification were inadequate, the Taguchi optimisation (Cobb & Clarkson, 1994) method was used for optimization. The equation $E=2k+1$ were used to determine the number of reactions needed. Nine reactions were used during this study and three different concentrations of three different variable components (DNA, $MgCl_2$ and Primer) were tested (Table 4.4).

Table 4.4: The concentrations of the different variable components used during the Taguchi method is summarized below. The different volumes are represented by **A**, **B** and **C**. The last column is the volume in µl of sterile water which would make-up the final reaction volume to 10 µl.

	DNA	**Primer**	**$MgCl_2$**	**Buffer**	**Taq Polymerase**	**H_2O**
1.	A (1)	A (0.3)	A (0.3)	A (2)	A (0.1)	6.3
2.	B (1.5)	A (0.3)	B (0.5)	A (2)	A (0.1)	5.6
3.	C (2)	A (0.3)	C (0.7)	A (2)	A (0.1)	4.9
4.	B (1.5)	B (0.5)	A (0.3)	A (2)	A (0.1)	5.6
5.	C (2)	B (0.5)	B (0.5)	A (2)	A (0.1)	4.9
6.	A (1)	B (0.5)	C (0.7)	A (2)	A (0.1)	5.7
7.	C (2)	C (0.8)	A (0.3)	A (2)	A (0.1)	4.8
8.	A (1)	C (0.8)	B (0.5)	A (2)	A (0.1)	5.6

9.	B (1.5)	C (0.8)	C (0.7)	A (2)	A (0.1)	4.9

The fragments visualized on the gels were scored with a number between 1 and 5. If there were no visible fragment a score of 1 were awarded and the brightest fragment received the score of 5. The signal-to-noise equation ($SNL=-log[1/n\ \Sigma\ 1/y^2]$) would indicate the effect of each individual component on the amplification of a reaction. The letter *n* represents the number of different concentrations and *y* the score given for each reaction (yield) (Cobb & Clarkson, 1994). A graph representing the SNL values for each variable component would indicate the optimum concentration for that particular element.

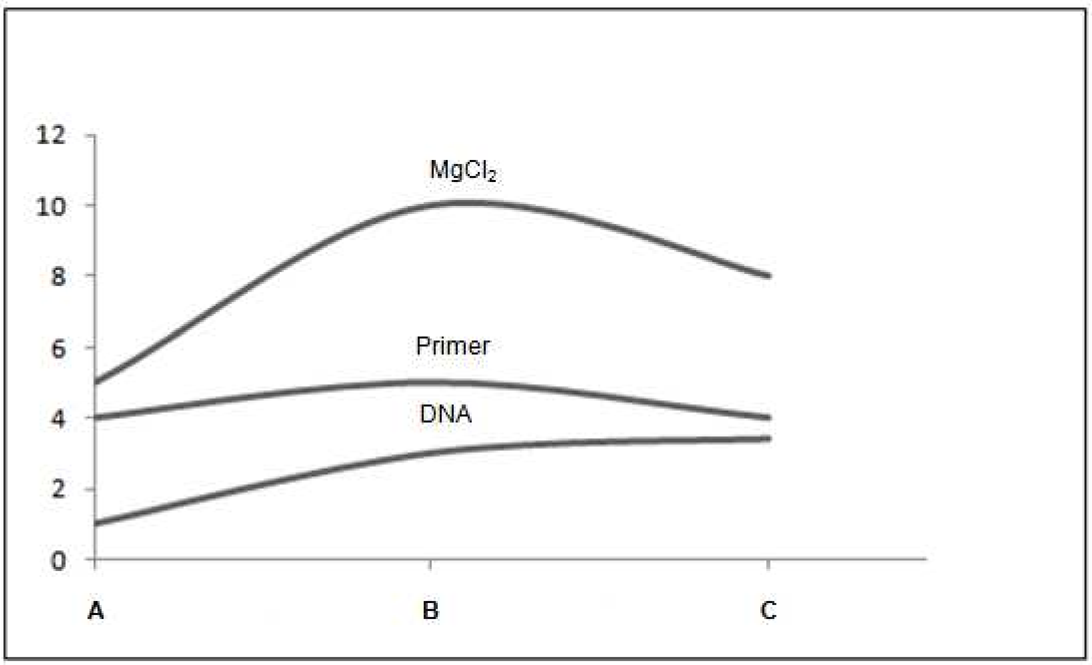

Figure 4.1: Graph obtained from the SNL values. The maximum of the curve is the optimum concentration for the respective variable component.

Not only amplification, but also variation between *C. nobilis* and *C. mirabilis* were seen after the optimization of the initial PCR reaction. For some of the primer sets only one of the two species had a visible fragment and for some pairs the amplified regions were at different size ranges.

The microsatellite data were analysed with GeneMarker software, after analysis on the ABI3130 DNA sequencer. Some of the electropherograms obtained showed peaks within the theoretical allele size rages (Appendix G). No more than two alleles were obtained for a specific primer set but mostly only one allele was observed. To confirm the occurrence of null alleles the reactions were repeated a few times. Different annealing temperatures were used and information obtained from studies focusing on optimization was incorporated into our methods (Brownie *et al*., 1997; Rahman *et al*., 2002; Niens *et al*., 2005). We focused on the optimization of the reactions for several months and then concluded that the quality of the peaks shown on the electropherograms were not sufficient for further analysis.

4.5. Discussion

The sequencing results could differentiate between different species, thus, showed interspecific variation but no intraspecific variation. The *rpl16* region showed intraspecific variation only within *C. mirabilis*. Three distinct groups were observed within the four different populations of *C. mirabilis*. The specimen from the Oorlogskloof Nature Reserve formed a monophyletic group with the samples from the Klein Koebee location. These two locations are geographically very close to each other. Two plants within one of the Donkerhoek populations showed more variation than the rest of the populations. Although variation was obtained between the different populations there was very little variation within populations. Microsatellites were tested in order to reveal more intraspecific variation and also variation within populations.

The success of cross-species markers rely on the nucleotide sequences of the flanking regions being conserved (Selkoe & Toonen, 2006). Cross-species amplification would be stronger when the evolutionary distance between the two species is smaller (Steinkellner *et al*., 1997; White & Powell, 1997). *Phaedranassa tunguraguae* and *Hymenocallis coronia* belongs to the same family as *Clivia*, but naturally the primers designed by Swanevelder (2003) would have a better chance of amplifying a microsatellite region because it is within the same genus.

Mostly only one allele was observed. The other alleles can be regarded as null alleles. Variation in the nucleotide sequence of the flanking region, will lead to the occurrence of a null allele (Smulders *et al*., 1997). Mutations are usually the primary cause of nucleotide variation observed at the flanking regions (Pemberton *et al*., 1995). Although amplification was obtained, in most cases the amplified product could not be optimized enough for reliable results.

An organism with a big genome size would experience difficulty amplifying with a PCR based analysis (Garner, 2001). The nuclear DNA content of the genus *Clivia* was measured by flow cytometry. The 2C DNA value for *C. mirabilis* was 31.3pg and for *C. nobilis* 34.5pg (Spies, P. personal communication, 2010). *Clivia miniata* had a 2C value of 39pg (Zonneveld *et al*., 2005). The nuclear 2C value of *Phaedranassa tunguraguae* and *Hymenocallis coronia* has not been measured. The primer pairs designed for *Hymenocallis coronia* were tested on *Zephyranthes candida* (Markwith & Scanlon, 2006) which indicated that these primers could be used as cross-species makers. *Zephyranthes candida* has a 2C value of 38pg (Zonneveld *et al*., 2005).

The genus *Clivia* has a chromosome number of 22 (Inariyama, 1937). *Hymenocallis coronia* has a chromosome number of 44 (Flory, 1976) whereas the chromosome number of *Phaedranassa tunguraguae* has not been established.

4.6. Conclusion

The *rpl16* region should be the region of choice to reveal intraspecific variation. The other six regions (*atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F* and *ITS1*) were unable to show intraspecific variation within *C. nobilis* and *C. mirabilis*. The development of species specific microsatellite primers for *C. nobilis* and *C. mirabilis* should be the route taken in future. Sequencing information would reveal microsatellite regions and primers amplifying these regions would be designed. Species specific primers would eliminate the problems brought about by differences in chromosome number and ancient polyploidy.

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Chapter 5:

Summary

H. M. van der Westhuizen

The genus *Clivia* Lindl., which belongs to the family Amaryllidaceae J. St-Hil. (1805), is comprised out of seven different species. *Clivia nobilis, C. caulescens*, *C. miniata*, *C. gardenii*, *C. mirabilis*, *C. robusta* and the natural hybrid *Clivia xnimbicola* all forms part of this genus. *Clivia mirabilis* is found in the Northern Cape Province and is geographically isolated from the six other species which grows along the Eastern Coast and escarpment of South Africa. Conrad *et al.* (2003) proved that *C. mirabilis* and *C. nobilis* were the two most primitive species in the genus *Clivia*.

During this study sequencing results were used to detect barcodes/SNPs for *C. nobilis* and *C. mirabilis* and to reveal genetic variation between the *Clivia* species. *Clivia nobilis* and *C. mirabilis* were tested with cross-species microsatellite makers to reveal intraspecific variation. Seven different gene regions were sequenced. Six were chloroplast regions, namely the *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16,* the *trnL-F* regions and one was a nuclear region, *ITS1*. The regions used for sequencing were evaluated as potential barcoding/SNP regions for future use. They were also used to infer the evolutionary development of *C. nobilis* and *C. mirabilis*. All seven *Clivia* species were analysed but this study focused mainly on morphologically different specimens of *C. nobilis* and *C. mirabilis.* The sequences were aligned and edited with Geneious Pro. A total of forty-seven polymorphic sites were observed between al seven species. Within the *rpl16* region eleven parsimony informative sites were observed. The *matK* and *trnL-F* regions each had eight parsimony informative sites. *ITS1* had three sites and *rpoB* and *rpoC1,* one parsimony informative site each. Within the *atpH-I* region no parsimony informative sites were observed. The sequencing data obtained could be used for species identification and, therefore, showed great potential as barcoding regions. We propose that *matK*, *rpl16* and *trnL-F* are used as a barcode in *C. nobilis* and *C. mirabilis* because they had the most parsimony informative sites.

The cladogram obtained from the combined data set (*atpH-I, rpoB, rpoC1, matK* and *trnL-F*) confirmed that *C. nobilis* and *C. mirabilis* are two separate species. *Clivia caulescens* and *C. xnimbicola* forms a monophyletic group. Within the *rpl16* chloroplast region intraspecific variation in *C. mirabilis* and interspecific variation between *C. nobilis* and *C. mirabilis* were observed. The phylogentic tree representing the sequencing results of the *rpl16* region revealed three distinctive groups within the four different *C. mirabilis* populations. Two plants within one of the Donkerhoek populations showed more variation than the rest of the population. The *rpl16* gene region proved to be ideal in order to test intraspecific variation in *C. nobilis* and *C. mirabilis*.

To evaluate the use of cross-species markers, microsatellite makers designed for *Phaedranassa tunguraguae, Hymenocallis coronia* and *Clivia miniata* were tested on *C. nobilis* and *C. mirabilis*. Although amplification was obtained, in most cases the results could not be optimized in order to provide reliable analysis. In future species specific primers for *C. nobilis* and *C. mirabilis* will be developed.

This study undoubtedly identified barcodes/SNPs for *C. nobilis* and *C. mirabilis* which can be used to eliminated mistaken identity. Gene regions specific for intra- and interspecific variation were identified and can be used in future for population studies.

Keywords: *atpH-I*, barcoding, *Clivia*, *matK*, microsatellite markers, phylogenetic relationship, *rpoB*, *rpoC1, rpl16, trnL-F*

Chapter 6:

Samevatting

H. M. van der Westhuizen

Die genus *Clivia* Lindl., behoort aan die familie Amaryllidaceae J. St-Hil (1805) en bestaan uit sewe verskillende spesies. Die spesies is *C. nobilis*, C. *caulescens*, *C. miniata*, *C. gardenii*, *C. mirabilis*, *C. robusta* en die natuurlike baster *Clivia xnimbicola*. *Clivia mirabilis* word gevind in die Noord Kaap Provinsie en is geografies geïsoleer van die ses ander *Clivia* spesies wat groei langs die Oos-Kus en platorand van Suid-Afrika. Conrad *et al.* (2003) het bevind dat *C. mirabilis* en *C. nobilis* die twee primitiefste spesies in die genus *Clivia* is*.*

DNA volgordebepalings is gebruik om strepieskodes vir *C. nobilis* en *C. mirabilis* te bepaal en om genetiese variasie tussen die verskillende *Clivia* spesies te ondersoek. Nie-spesie-spesifieke mikrosatelliet merkers is gebruik om intraspesifieke variasie in *C. nobilis* en *C. mirabilis* te ondersoek. DNA volgordes van sewe verskillende geengebiede is bepaal. Ses was chloroplasgebiede, naamlik *atpH-I*, *matK*, *rpoB*, *rpoC1, rpl16, trnL-F* streke en een kerngebied, naamlik *ITS1*. Die DNA volgordebepalings is ook geëvalueer as potensiële toekomstige strepieskode/SNP gebiede. Al sewe *Clivia* spesies is geanaliseer, maar die studie het gefokus op morfologiese verskillende eksemplare van *C. nobilis* en *C. mirabilis*. Die volgordes is saamgevoeg en gewysig met Geneious Pro. ʼn Totaal van sewe-en-veertig verskillende polimorfiese posisies is in die sewe spesies waargeneem. Binne die *rpl16* gebied is elf parsinomies informatiewe posisies waargeneem. Die *matK* en *trnL-F* streke het elk agt informatiewe posisies getoon. *ITS1* het drie posisies en *rpoB* en *rpoC1,* elk een parsinomies informatiewe posisie. Binne die *atpH-I* gebied was daar geen parsinomies informatiewe posisies nie. Die DNA volgordebepalings kan dus gebruik word om spesies te identifiseer, en is daarom potensiële strepieskode gebiede. Ons stel voor dat ʼn kombinasie van *matK*, *rpl16* en *trnL-F* gebruik word as ʼn strepieskode vir *C. nobilis* en *C. mirabilis,* weens hul hoë aantal parsinomies informatiewe posisies.

Die kladogram wat deur die saamgestelde datastel (*atpH-I, rpoB, rpoC1, matK* and *trnL-F*) verkry is, het bevestig dat *C. nobilis* en *C. mirabilis* twee verskillende spesies is. *Clivia caulescens* en *C. xnimbicola* vorm 'n monofiletiese groep. Alhoewel *C. nobilis* en *C. mirabilis* merkbaar verskil van die ander *Clivia* spesies, verskil hul steeds van mekaar. Die *rpl16* chloroplasgebied toon intraspesifieke variasie aan in *C. mirabilis* en interspesifieke variasie tussen *C. nobilis* en *C. mirabilis*. Die filogram gebaseer op volgordebepalings van die *rpl16* gebied, toon aan dat binne die vier *C. mirabilis* populasies drie kenmerkende groepe geïdentifiseer kan word. Twee plante binne een van die Donkerhoek populasies vertoon meer variasie as die res van die populasie. Die *rpl16* geen gebied blyk die beste te wees om intraspesifieke variasie tussen *C. nobilis* and *C. mirabilis* aan te toon.

Om die gebruik van kruis spesies mikrosatelliet merkers te evalueer is merkers gebruik wat oorspronklik ontwerp is vir *Phaedranassa tunguraguae, Hymenocallis coronia* en *Clivia miniata.* Alhoewel amplifisering verkry is, kon die resultate meestal nie genoegsaam geoptimaliseer word om betroubare analise te verseker nie. In die toekoms sal die ontwikkeling van spesies spesifieke inleiers vir *C. nobilis* en *C. mirabilis* noodsaaklik wees.

Die studie het ongetwyfeld strepieskodes/SNPs geïdentifiseer vir *C. nobilis* en *C. mirabilis* wat gebruik kan word om verkeerdelike identifisering te elimineer. Geenstreke, spesifiek vir intra- en interspesifieke variasie, is geïdentifiseer en kan in die toekoms gebruik word vir populasie studies.

Sleutelwoorde: *atpH-I*, strepieskode, *Clivia*, *matK*, mikrosatelliet merkers, filogenetiese verwantskappe, *rpoB*, *rpoC1, rpl16, trnL-F*

Appendix A: Photographs of agarose gel electrophoresis used to determine the DNA quality.

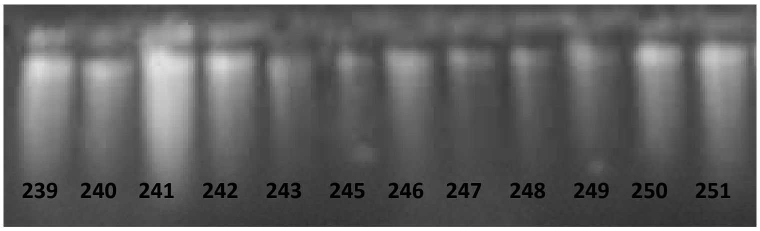

Figure A1: Photograph of agarose gel used for determining DNA quality for *C. mirabilis* specimens

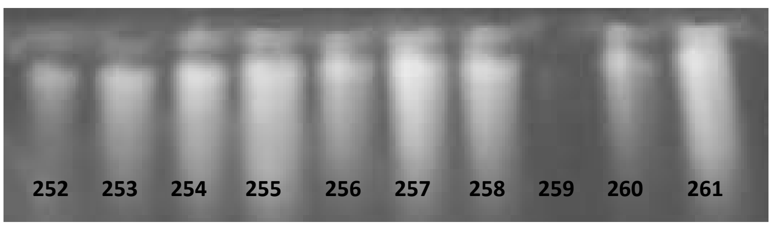

Figure A2: Photograph of agarose gel used for determining DNA quality for *C. mirabilis* specimens

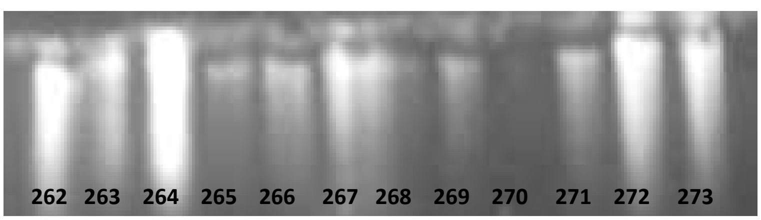

Figure A3: Photograph of gel used for determining DNA quality for *C. mirabilis* specimens 262 to 273.

Appendix B: Sample number and quantity of DNA obtained for the *Clivia nobilis* samples.

Sample ID	ng/µl	A260	A280	260/280	260/230
52	27.715	0.555	0.342	1.63	1.04
141	614.855	12.297	6.167	1.995	2.095
142	1306.37	26.128	12.88	2.03	2.18
143	1394.14	27.883	13.76	2.025	2.035
144	1007.705	20.139	10.154	1.98	2.205
145	1806.125	36.123	18.155	1.99	2.155
146	2185.59	43.712	21.475	2.035	2.095
147	1250.755	25.016	12.383	2.02	3.265
148	2485.90	49.718	24.540	2.03	2.15
149	2313.055	46.261	22.95	2.015	2.125
159	771.72	15.435	7.824	1.97	2.17
160	1213.815	24.277	12.324	1.97	2.135
161	2436.48	48.730	24.193	2.01	2.17
162	400.735	8.015	3.976	2.015	2.12
163	313.07	6.262	3.149	1.99	2.03
169	262.23	5.245	2.707	1.935	1.59
170	358.96	7.179	3.755	1.915	1.98
171	510.725	10.215	5.108	2.00	1.735
172	1320.91	26.419	13.607	1.94	1.905
173	1670.435	33.409	17.161	1.955	2.00
174	2138.235	42.765	21.323	2.005	2.11
175	4370.39	87.408	45.439	1.92	2.045
176	3494.165	69.884	35.800	1.955	1.995
177	1754.235	35.085	17.723	1.98	2.05
178	2415.585	68.312	34.751	1.965	2.075
179	1007.435	37.149	18.774	1.98	2.065
180	2317.76	46.355	23.926	1.94	1.86
193	3906.13	78.123	40.194	1.94	2.045

Sample number and quantity of DNA obtained for the *Clivia mirabilis* samples.

Sample ID	ng/µl	A260	A280	260/280	260/230
239	542.24	10.845	6.077	1.78	1.08
240	308.105	6.163	3.515	1.75	1.20
241	650.375	13.008	6.851	1.90	1.915
242	530.61	10.612	5.897	1.80	1.185
243	131.005	2.32	1.257	1.85	1.915
244	176.07	3.522	2.091	1.685	0.93
245	110.365	2.208	1.184	1.865	1.79
246	129.97	2.599	1.39	1.87	1.68
247	422.56	8.451	4.7	1.80	1.24
248	483.09	9.662	5.43	1.78	1.155
249	764.64	15.293	9.751	1.57	0.775
250	8.878	8.878	4.745	1.87	1.835
251	289.255	5.785	17.158	1.87	1.76
252	332.225	6.645	3.564	1.865	1.62
253	494.045	9.921	5.657	1.75	1.355
254	616.885	12.338	6.699	1.84	1.7
256	760.365	15.208	8.073	1.88	1.24
257	1012.925	20.259	10.705	1.89	1.795
258	475.07	9.502	5.421	1.755	1.375
259	23.38	0.468	0.264	1.78	1.375
260	480.96	9.619	5.415	1.78	1.125
261	950.13	19.003	10.303	1.845	1.61
262	718.525	14.371	8.197	1.755	1.11
263	706.175	14.124	7.639	1.845	1.08
264	2346.64	46.933	25.744	1.82	1.70

265	262.31	5.246	2.833	1.865	1.05
266	346.56	6.931	3.749	1.845	1.335
267	483.735	9.675	5.328	1.815	1.97
268	4.255	0.085	0.07	1.245	0.68
269	65.54	1.311	0.732	1.79	1.68
270	17.525	0.351	0.202	1.745	1.445
271	140.745	2.815	1.551	1.815	1.89
272	618.335	12.367	6.620	1.865	2.025
273	392.525	7.851	4.315	1.82	1.865

Appendix C: Photograph of agarose gel electrophoresis was used to test for amplification after the initial sequencing reactions.

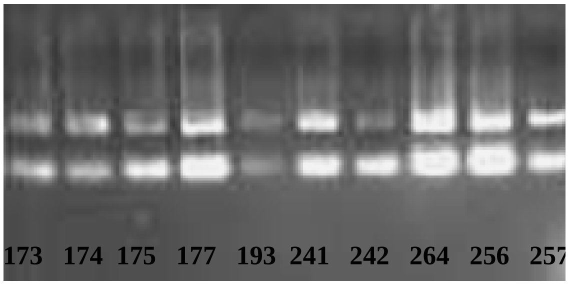

Figure C1: Amplification results for the *atpH-I* region.
The specimen numbers are indicated on the photo.

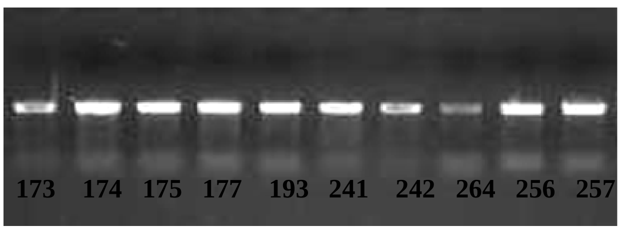

Figure C2: Amplification results for the *matK* region.
The specimen numbers are indicated on the photo.

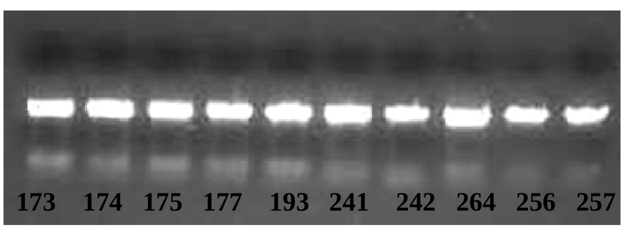

Figure C3: Amplification results for the *rpoB* region.
The specimen numbers are indicated on the photo.

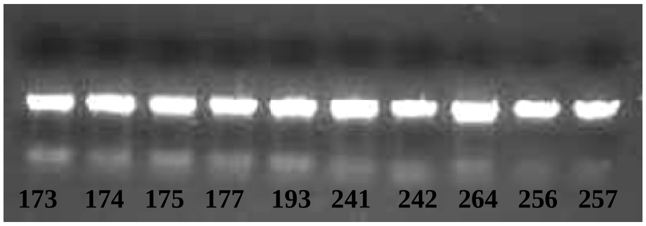

Figure C4: Amplification results for the *rpoC1* region.
The specimen numbers are indicated on the photo.

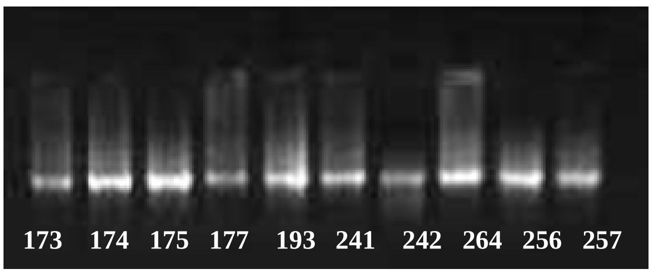

Figure C5: Amplification results for the *trnL-F(c-d)* region.
The specimen numbers are indicated on the photo.

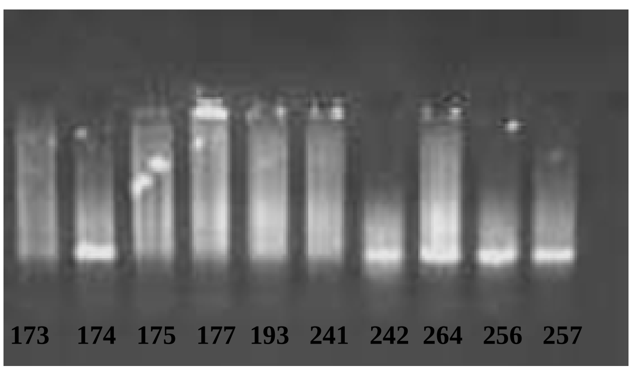

Figure C6: Amplification results for the *trnL-F(e-f)* region.
The specimen numbers are indicated on the photo.

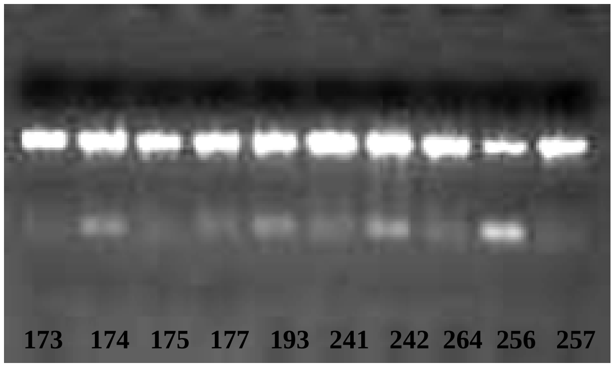

Figure C7: Amplification results for the *rpl16* region.
The specimen numbers are indicated on the photo.

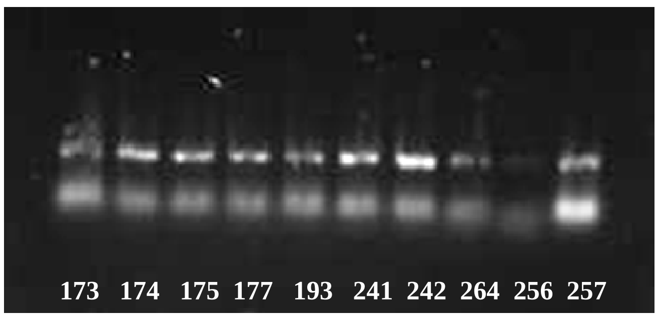

Figure C8: Amplification results for the *ITS* region.
The specimen numbers are indicated on the photo.

Appendix D: The nucleotide differences observed within the different gene regions (*atpH-I*, *matK*, *rpoB*, *rpoC1, trnL-F, ITS1* and *rpl16* regions).

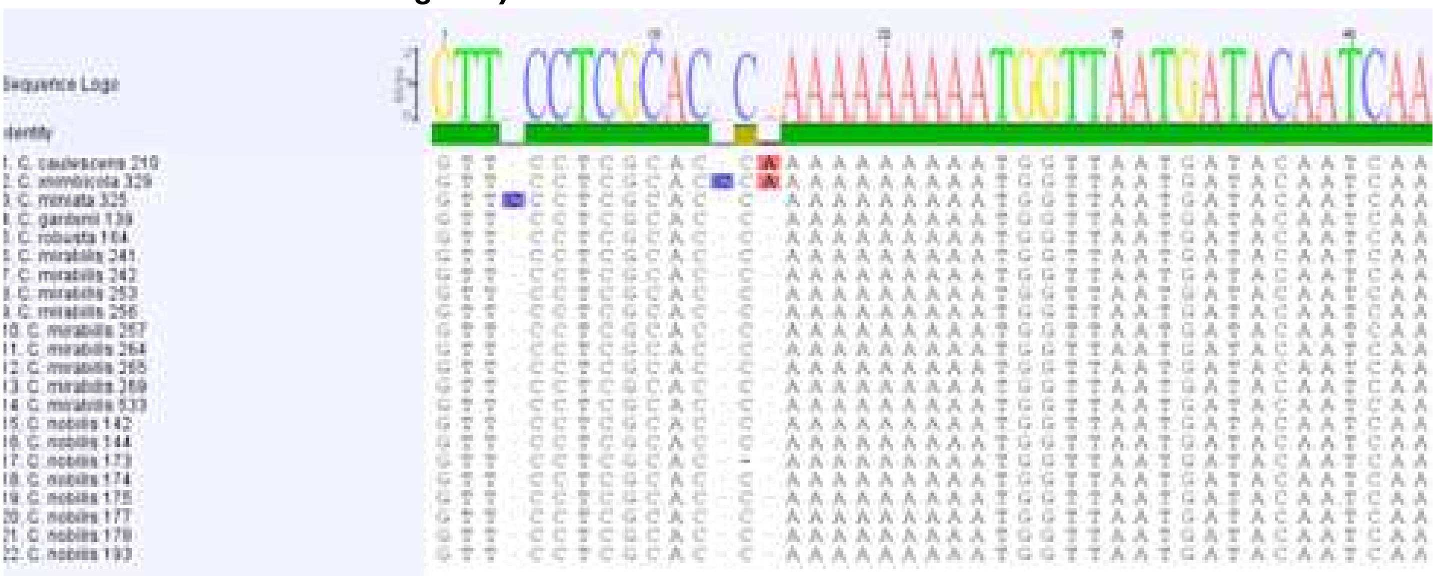

Figure D1: Nucleotide differences for *C. caulescens*, *C. xnimbicola* and *C. miniata* within the *atpH-I* region.

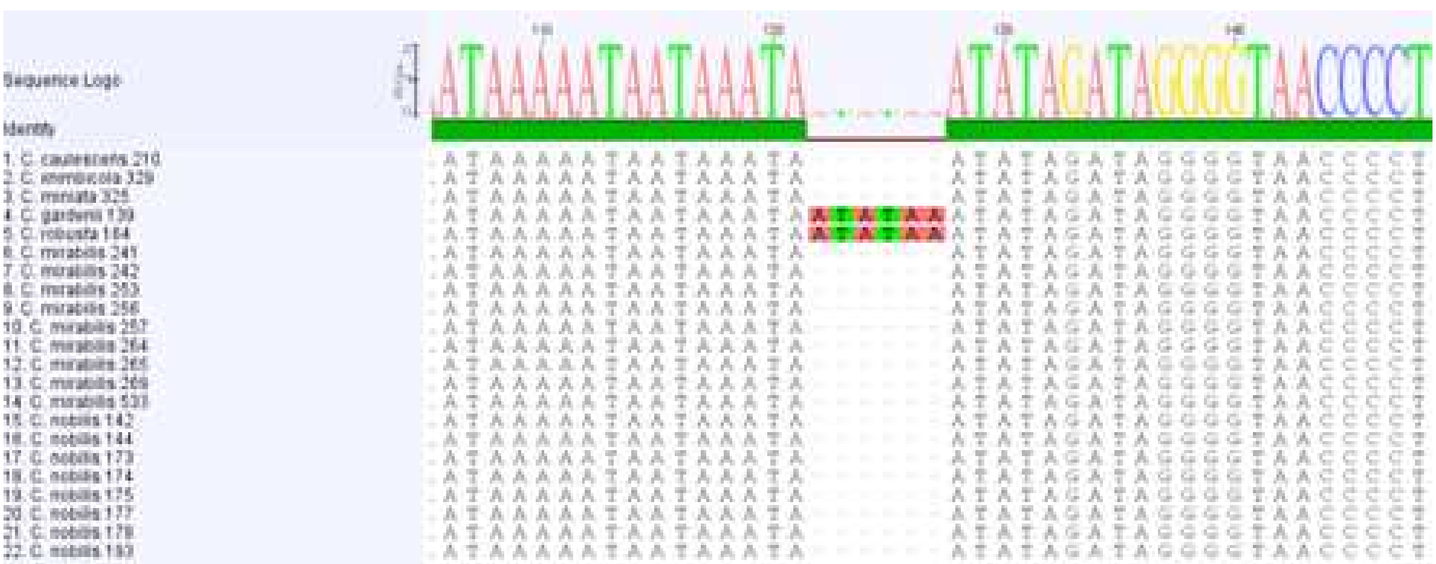

Figure D2: A six base pair indel obtained which differentiate *C. robusta* and *C. gardenia* from the other *Clivia* species when the *atpH-I* region was analysed.

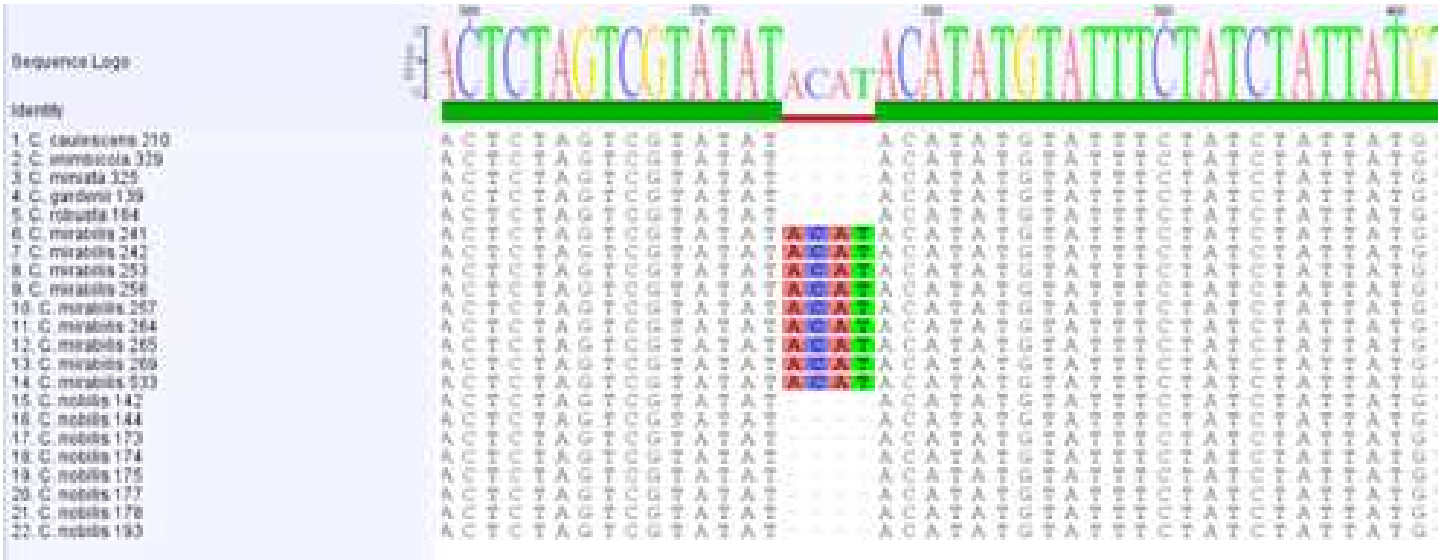

Figure D3: A four base pair indel obtained which differentiate between *C. nobilis* and *C. mirabilis* when the *atpH-I* region was analysed.

Figure D4: A nucleotide difference for *C. caulescens* within the *matK* region.

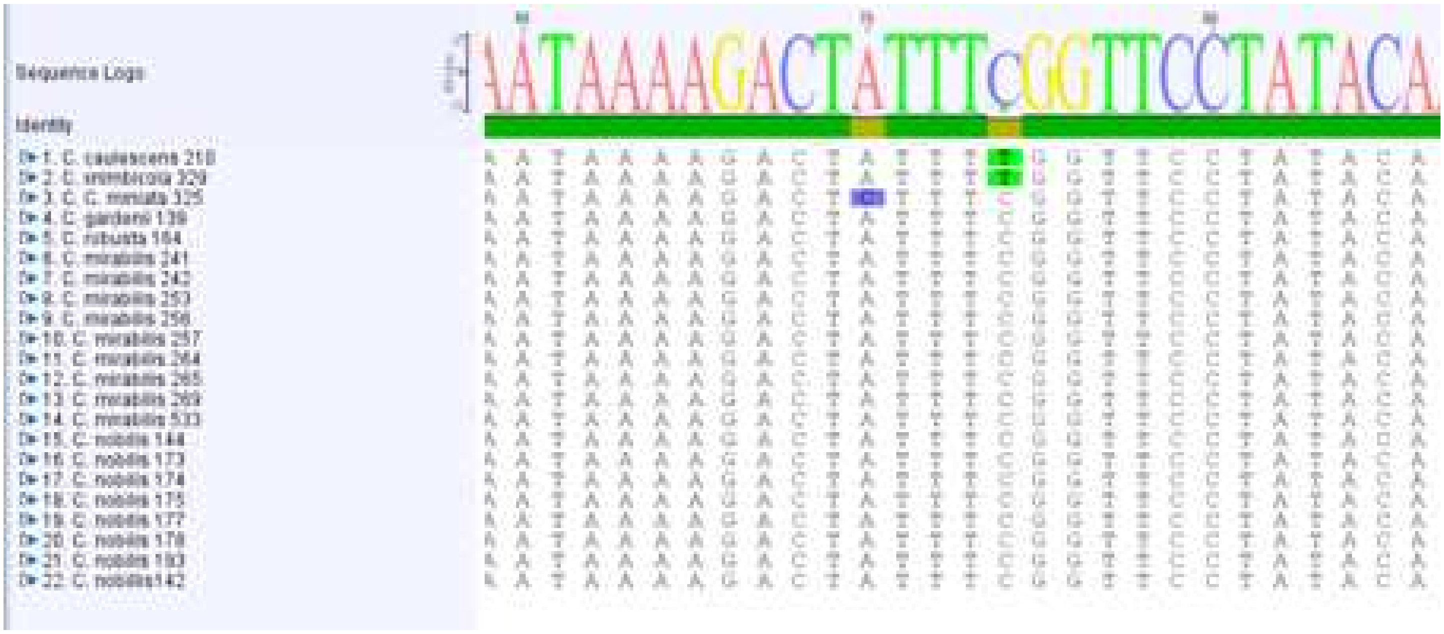

Figure D5: Nucleotide differences for *C. caulescens*, *C. xnimbicola* and *C. miniata* within the *matK* region.

Figure D6: A one base pair difference for *C. caulescens* within the *matK* region.

Figure D7: A unique one base pair difference within the *matK* region for all *C. nobilis* samples.

Figure D8: Nucleotide differences for *C. caulescens*, *C. xnimbicola*, *C. miniata, C. gardenii* and *C. robusta* within the *matK* region.

Figure D9: A nucleotide difference for *C. caulescens* and a unique one base pair difference within the *matK* region for all *C. mirabilis* samples.

Figure D10: Two unique one base pair barcodes/SNPs obtained respectively for *C. nobilis* and *C. mirabilis* within the *matK* region. A one base pair difference obtained for *C. caulescens* and *C. xnimbicola.*

Figure D11: Two one base pair differences obtained for *C. miniata* within the *rpoB* region.

Figure D12: Nucleotide differences for *C. caulescens*, *C. xnimbicola*, *C. miniata, C. gardenii* and *C. robusta* within the *rpoB* region.

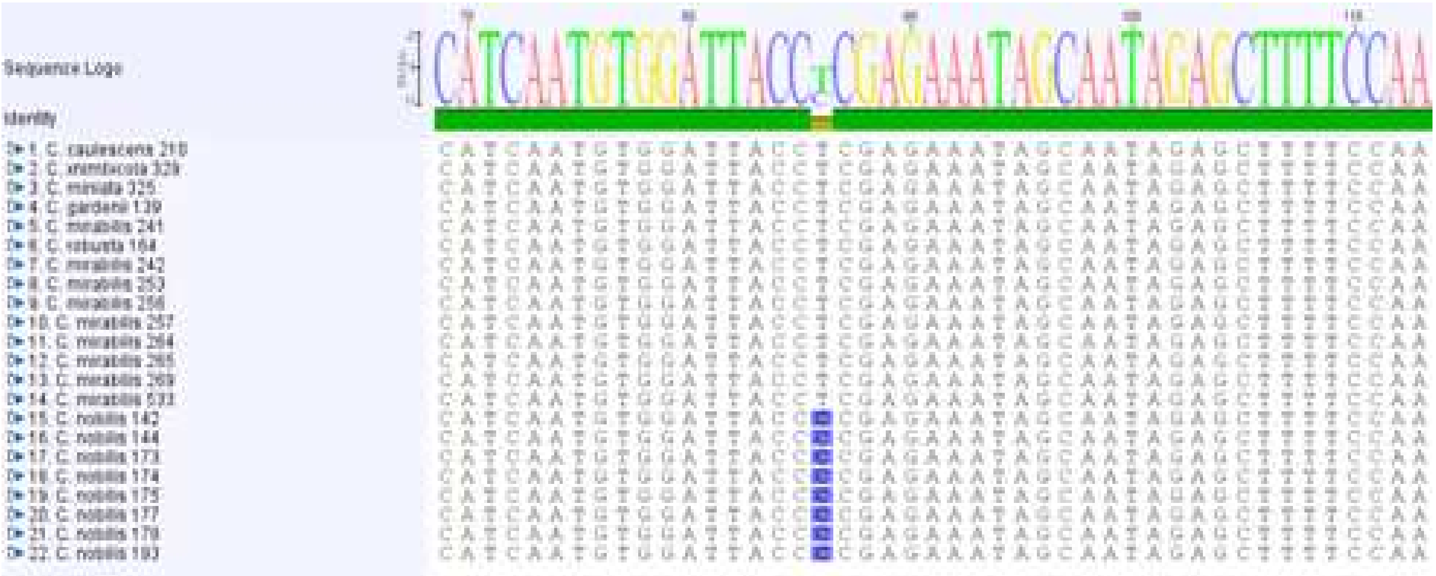

Figure D13: A unique one base pair difference within the *rpoC1* region for all *C. nobilis* samples.

Figure D14: To one nucleotide differences for *C. xnimbicola* and a unique one base pair difference within the *trnL-F* region for all *C. nobilis* samples.

Figure D15: Nucleotide differences for *C. caulescens*, *C. xnimbicola*, *C. miniata, C. gardenii* and *C. robusta* within the *trnL-F* region.

Figure D16: Nucleotide differences for *C. caulescens* and *C. xnimbicola* within the *trnL-F* region.

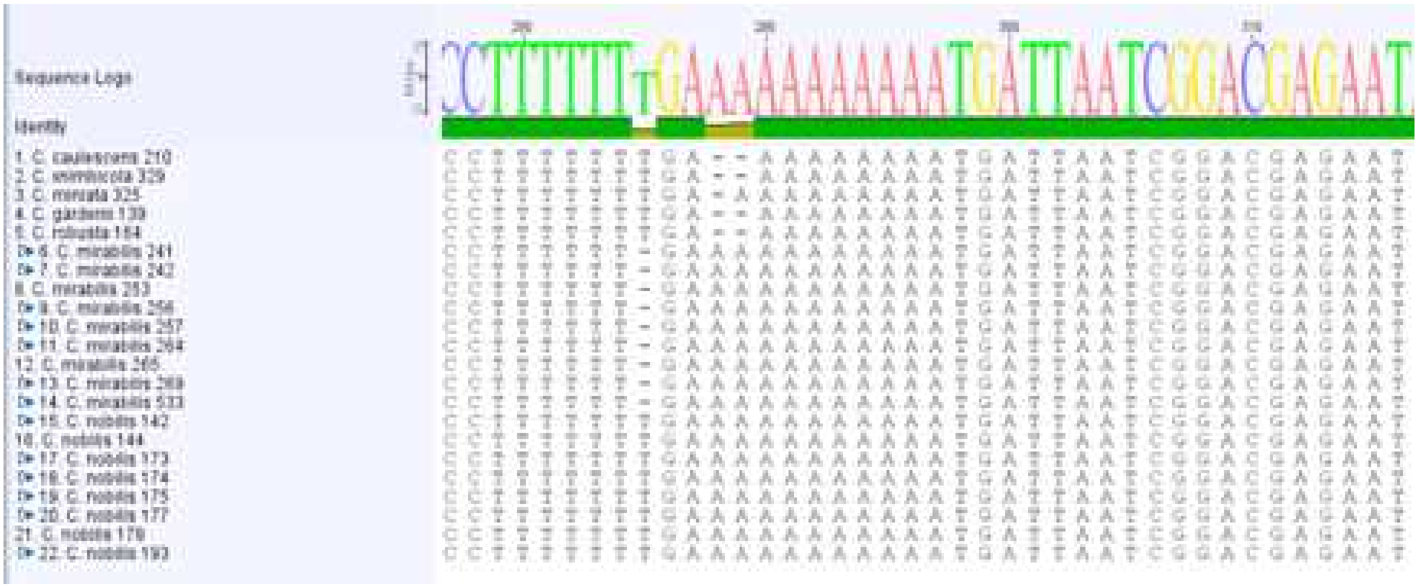

Figure D17: Indels differentiating between the different *Clivia* species within the *trnL-F* region.

Figure D18: A four base pair and a one base pair indel which differentiate between *C. nobilis* and *C. mirabilis* when the *trnL-F* region was analysed. Nucleotide differences obtained for *C. miniata, C. gardenii* and *C. robusta*.

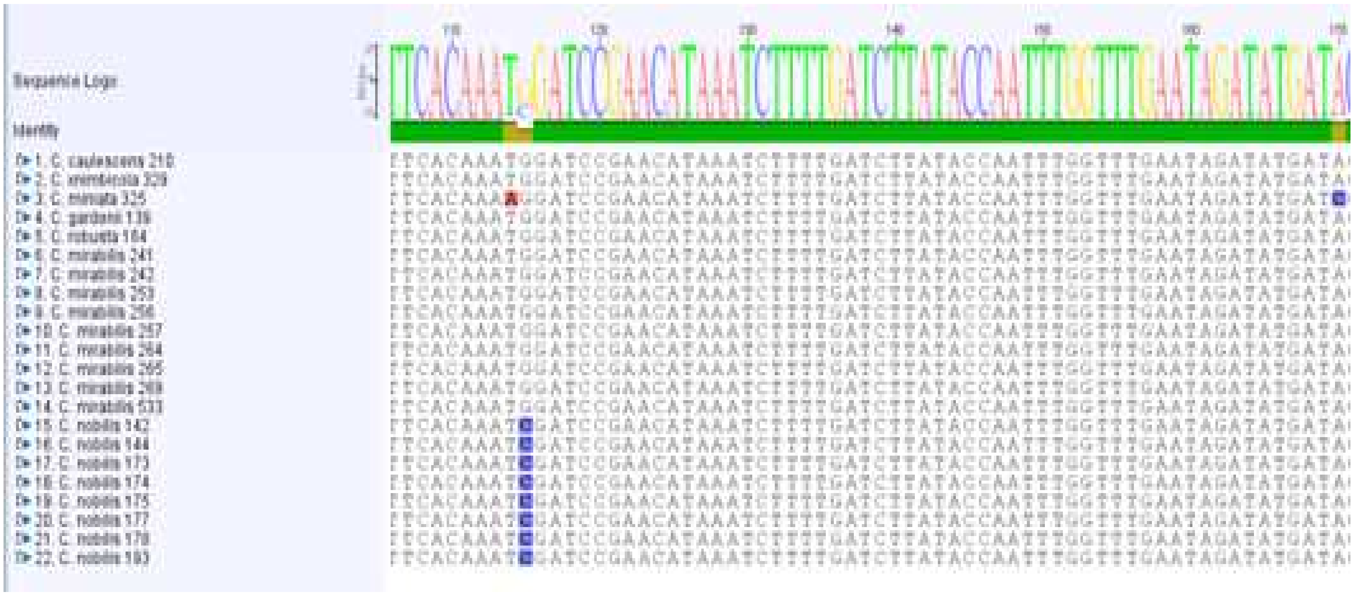

Figure D19: A unique one base pair difference within the *trnL-F* region for all *C. nobilis* samples and two one base pair differences in *C. miniata.*

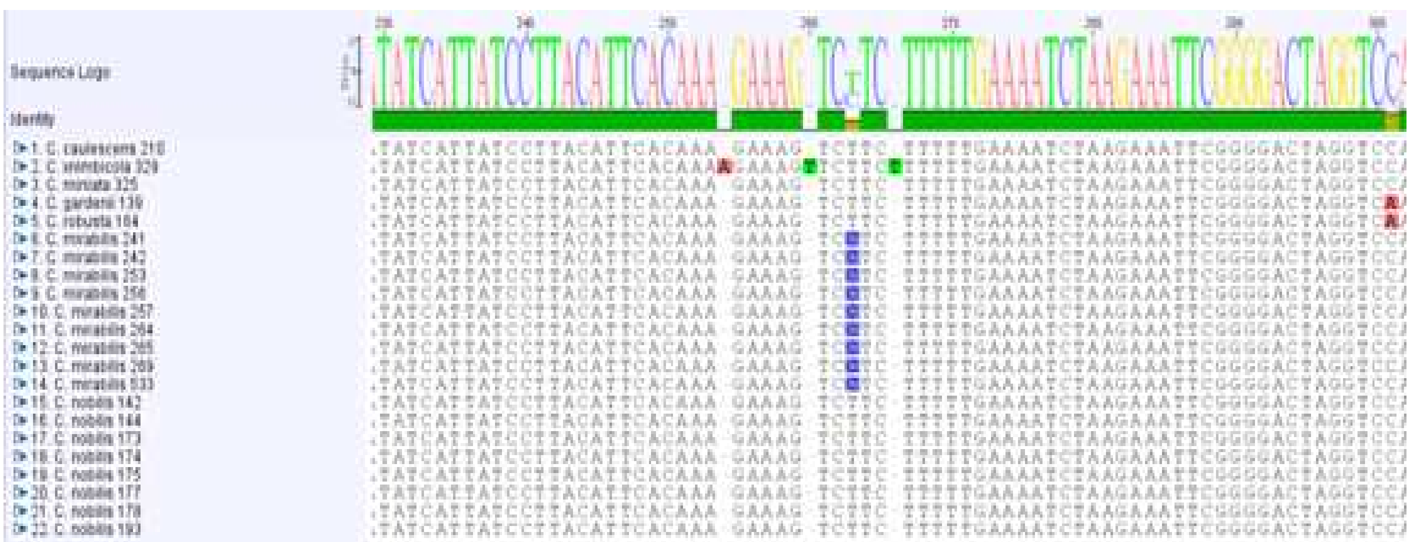

Figure D20: A unique one base pair difference within the *trnL-F* region for all *C. mirabilis* samples and three one base pair differences in *C. miniata.* One nucleotide differences in *C. gardenii* and *C. robusta.*

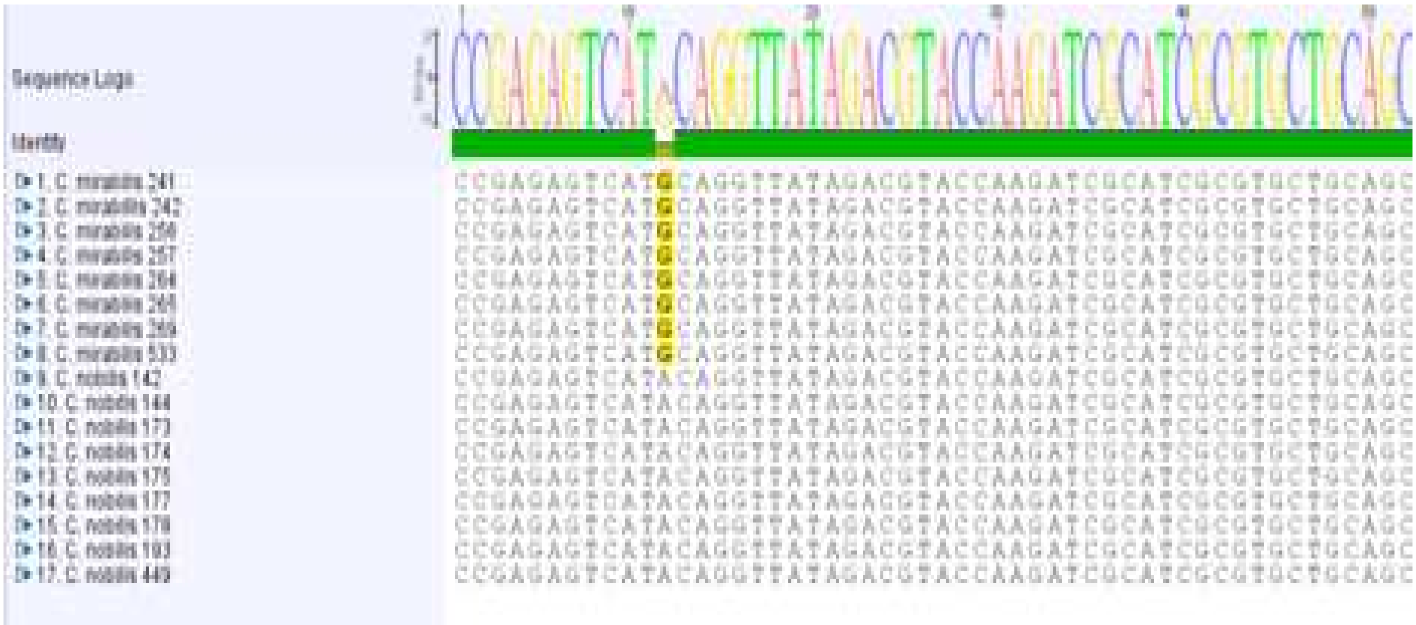

Figure D21: A unique one base pair difference within the *ITS* region for all *C. mirabilis* samples.

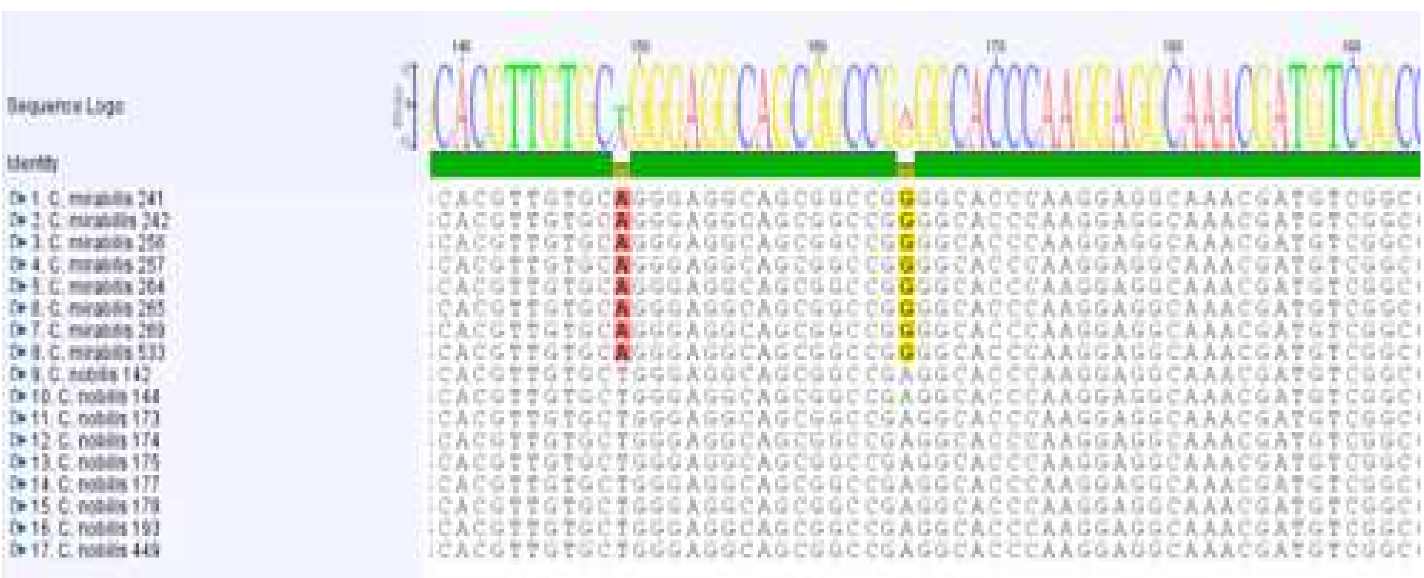

Figure D22: Two unique one base pair differences within the *ITS* region for all *C. mirabilis* samples.

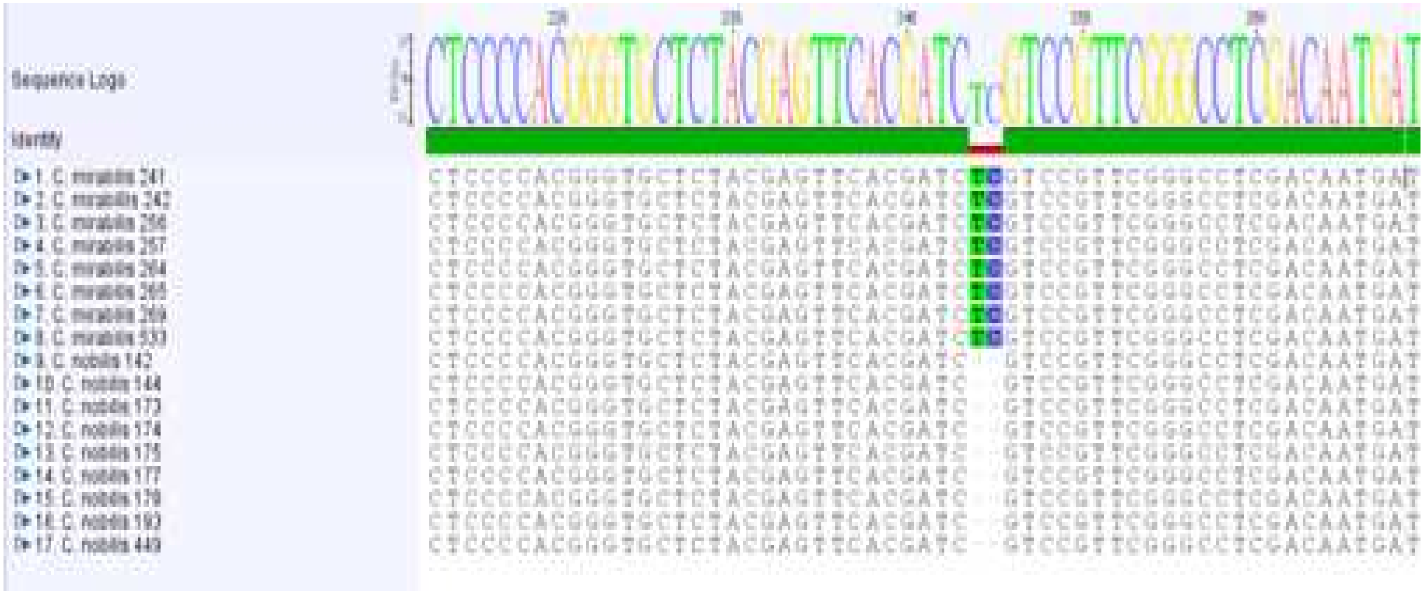

Figure D23: A unique two base pair difference within the *ITS* region for all *C. mirabilis* samples

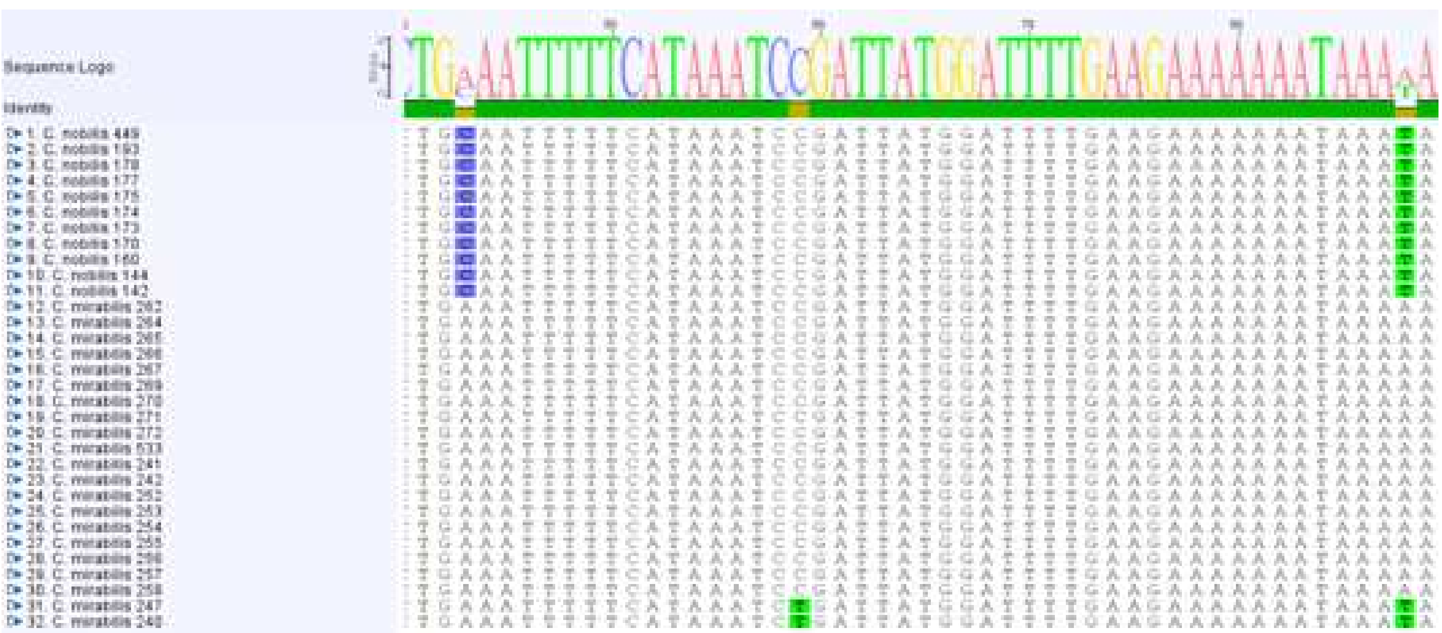

Figure D24: Two unique one base pair differences within the *rpl16* region for all *C. nobilis* samples. Two C. mirabilis samples were deferent from all the other *C. mirabilis* samples.

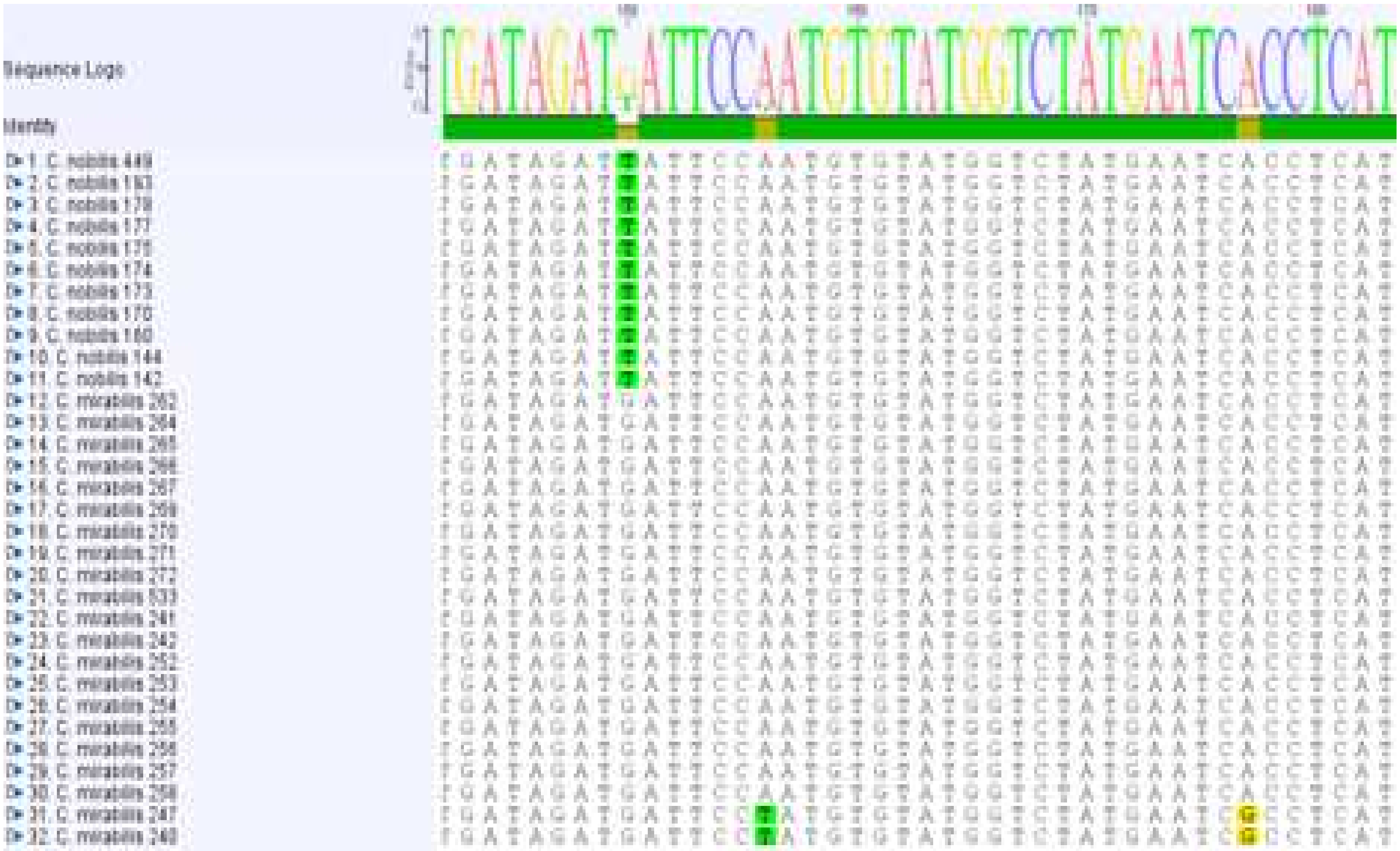

Figure D25: A unique one base pair differences within the *rpl16* region for all *C. nobilis* samples. Two C. mirabilis samples were deferent from all the other *C. mirabilis* samples.

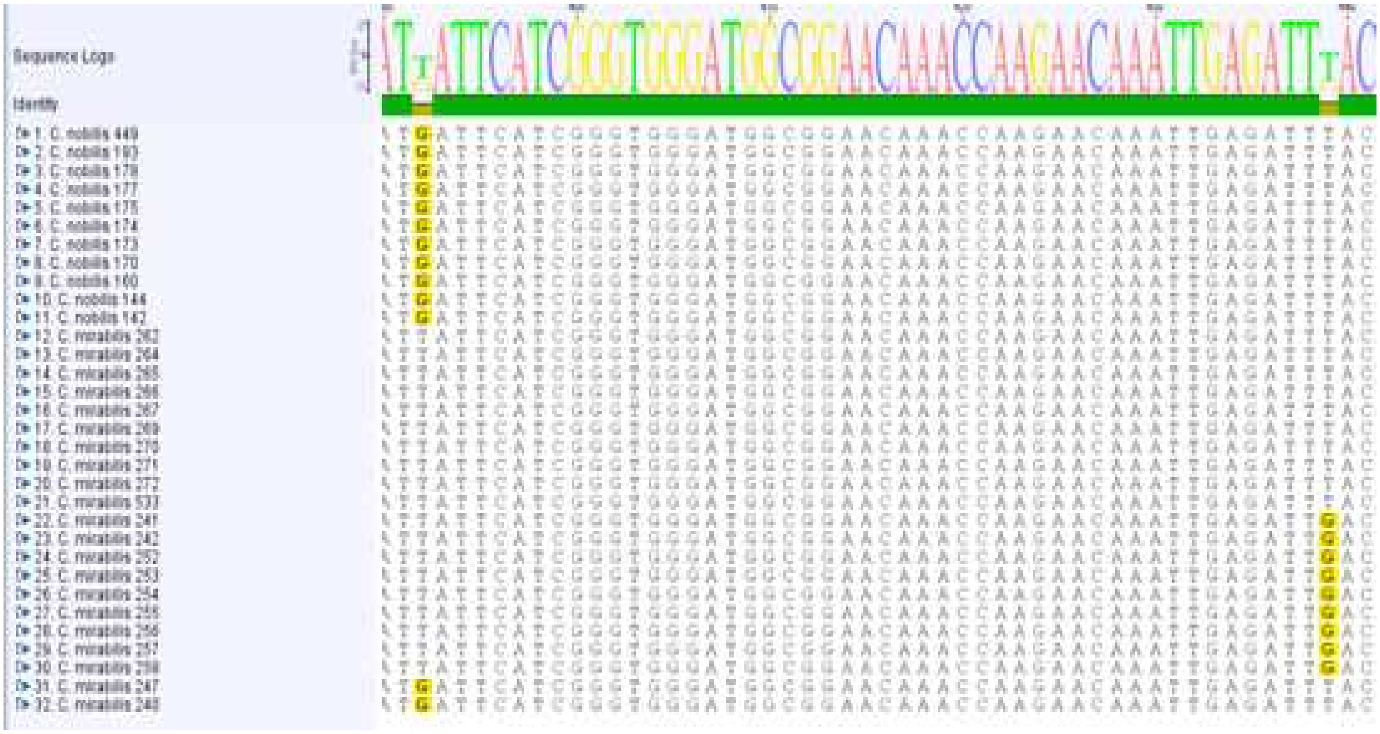

Figure D26: A unique one base pair differences within the *rpl16* region for all *C. nobilis* samples. Two C. mirabilis samples were deferent from all the other *C. mirabilis* samples. A one nucleotide difference was obtained for one of the *C. mirabilis* populations.

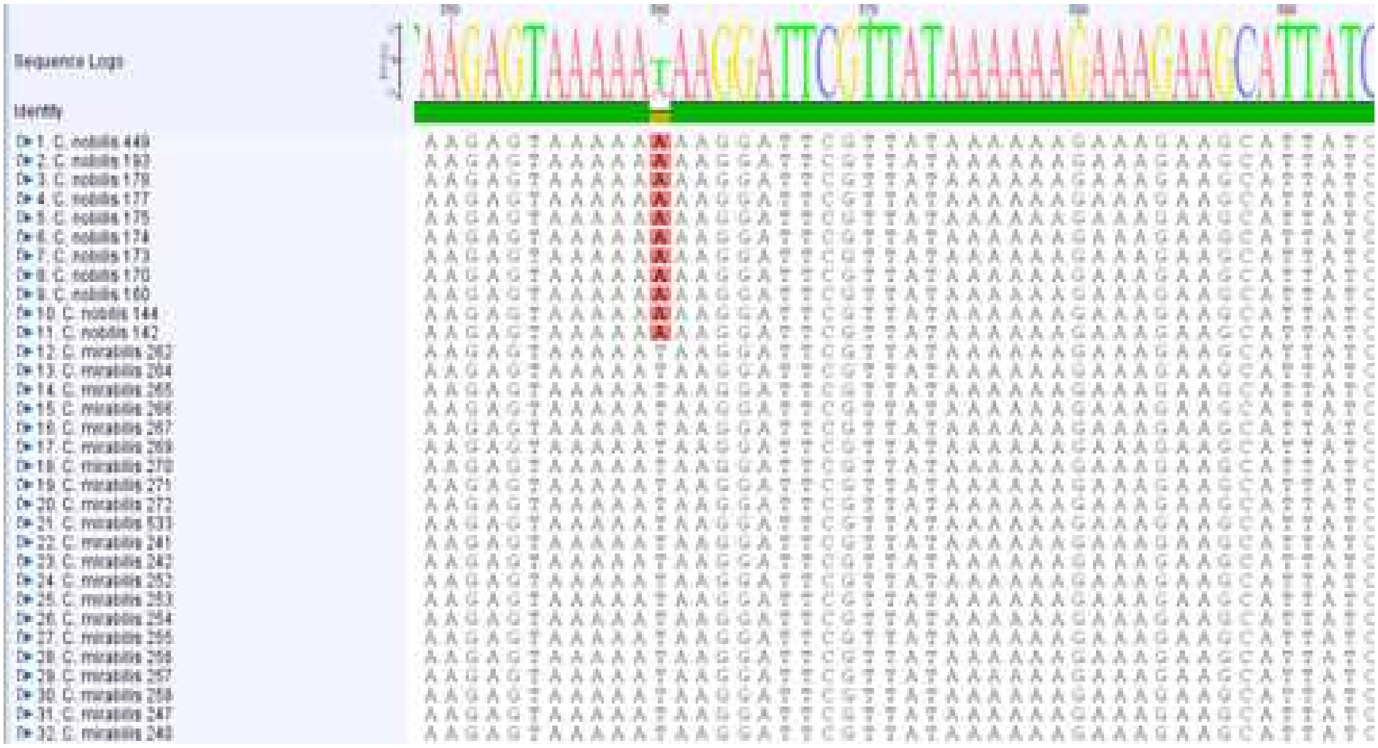

Figure D27: A unique one base pair differences within the *rpl16* region for all *C. nobilis* samples.

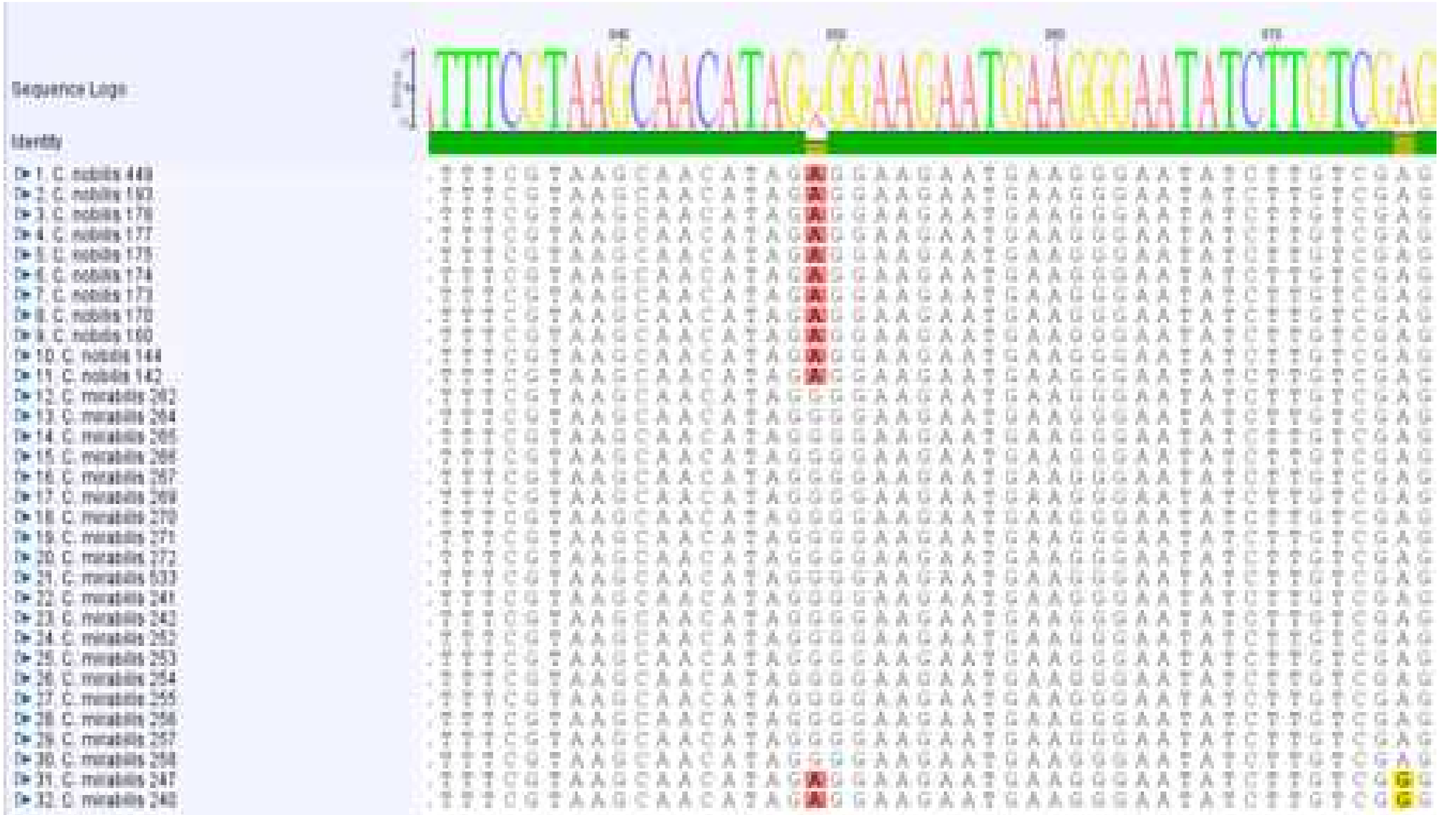

Figure D28: A unique one base pair differences within the *rpl16* region for all *C. nobilis* samples. Two C. mirabilis samples were deferent from all the other *C. mirabilis* samples.

Appendix E: Aligned sequences of the *atpH-I*, *matK*, *rpoB*, *rpoC1, trnL-F, ITS1* and *rpl16* regions).

The *atpH-I* region:

```
                      1        10        20        30        40        50        60
                      |        |         |         |         |         |         |
C. caulescens 210     GTT-CCTCGCAC-CAAAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. xnimbicola 329     GTT-CCTCGCACCCAAAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. miniata 325        GTTCCCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. gardenii 139       GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. robusta 164        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 241      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 242      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 253      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 256      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 257      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 264      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 265      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 269      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. mirabilis 533      GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 142        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 144        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 173        GTT-CCTCGCAC---AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 174        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 175        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 177        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 178        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT
C. nobilis 193        GTT-CCTCGCAC-C-AAAAAAAAATGGTTAATGATACAATCAACCAATGAATTATTACTT

C. caulescens 210     ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. xnimbicola 329     ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. miniata 325        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. gardenii 139       ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. robusta 164        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 241      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 242      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 253      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 256      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 257      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 264      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 265      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 269      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. mirabilis 533      ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 142        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 144        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 173        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 174        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 175        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 177        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 178        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT
C. nobilis 193        ATTTGATCACTAAAATATATCGAGTCGAAGTAACTAAAACTTCGAATAAAAATAATAAAT

C. caulescens 210     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. xnimbicola 329     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. miniata 325        A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. gardenii 139       AATATAAATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. robusta 164        AATATAAATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 241      A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 242      A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
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C. mirabilis 253     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 256     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 257     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 264     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 265     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 269     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. mirabilis 533     A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 142       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 144       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 173       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 174       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 175       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 177       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 178       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA
C. nobilis 193       A------ATATAGATAGGGGTAACCCCTATATAACTAGTATATATCTAATATCACATATA

C. caulescens 210    CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. xnimbicola 329    CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. miniata 325       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. gardenii 139      CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. robusta 164       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 241     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 242     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 253     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 256     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 257     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 264     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 265     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 269     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. mirabilis 533     CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 142       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 144       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 173       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 174       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 175       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 177       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 178       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA
C. nobilis 193       CATTTGTTTCTTTCATAACGTAAACCGCGCGCATTTTCTATTGAATCGGATTCTAGAATA

C. caulescens 210    ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. xnimbicola 329    ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. miniata 325       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. gardenii 139      ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. robusta 164       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 241     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 242     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 253     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 256     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 257     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 264     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 265     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 269     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. mirabilis 533     ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 142       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 144       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 173       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 174       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 175       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 177       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
C. nobilis 178       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT
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C. nobilis 193       ATTCTTCGAAAGATATACATACAGGGGCTGTGGCTGGACTTATAGACATTACATATATCT


C. caulescens 210    AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. xnimbicola 329    AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. miniata 325       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. gardenii 139      AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. robusta 164       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 241     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 242     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 253     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 256     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 257     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 264     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 265     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 269     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. mirabilis 533     AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 142       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 144       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 173       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 174       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 175       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 177       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 178       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC
C. nobilis 193       AGTGTGACCCCCCTAACCCATCCACCATTTCGTAATATCGTCTATCTTTCCTCCTACAAC

C. caulescens 210    TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. xnimbicola 329    TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. miniata 325       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. gardenii 139      TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. robusta 164       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 241     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 242     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 253     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 256     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 257     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 264     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 265     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 269     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. mirabilis 533     TCTAGTCGTATATACATACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 142       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 144       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 173       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 174       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 175       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 177       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 178       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA
C. nobilis 193       TCTAGTCGTATAT----ACATATGTATTTCTATCTATTATGTTCCCCAACTAAGAACCAA

C. caulescens 210    ATAGATTTTCCTGAACCACGCATT
C. xnimbicola 329    ATAGATTTTCCTGAACCACGCATT
C. miniata 325       ATAGATTTTCCTGAACCACGCATT
C. gardenii 139      ATAGATTTTCCTGAACCACGCATT
C. robusta 164       ATAGATTTTCCTGAACCACGCATT
C. mirabilis 241     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 242     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 253     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 256     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 257     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 264     ATAGATTTTCCTGAACCACGCATT
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C. mirabilis 265     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 269     ATAGATTTTCCTGAACCACGCATT
C. mirabilis 533     ATAGATTTTCCTGAACCACGCATT
C. nobilis 142       ATAGATTTTCCTGAACCACGCATT
C. nobilis 144       ATAGATTTTCCTGAACCACGCATT
C. nobilis 173       ATAGATTTTCCTGAACCACGCATT
C. nobilis 174       ATAGATTTTCCTGAACCACGCATT
C. nobilis 175       ATAGATTTTCCTGAACCACGCATT
C. nobilis 177       ATAGATTTTCCTGAACCACGCATT
C. nobilis 178       ATAGATTTTCCTGAACCACGCATT
C. nobilis 193       ATAGATTTTCCTGAACCACGCATT
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The *matK* region:

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                    1        10        20        30        40        50        60
                    |        |         |         |         |         |         |
C. caulescens 210   TAATTGGTATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. xnimbicola 329   TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. miniata 325      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. gardenii 139     TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. robusta 164      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 241    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 242    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 253    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 256    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 257    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 264    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 265    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 269    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. mirabilis 533    TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 144      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 173      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 174      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 175      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 177      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 178      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 193      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA
C. nobilis 142      TAATTGGAATAGTCTTCTCATTACTCAGAAGAAATCCATTTACGTTTTTTCAAAAGAAAA

C. caulescens 210   TAAAAGACTATTTTGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. xnimbicola 329   TAAAAGACTATTTTGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. miniata 325      TAAAAGACTCTTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTGT
C. gardenii 139     TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTT-T
C. robusta 164      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTT-T
C. mirabilis 241    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 242    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 253    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 256    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 257    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 264    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 265    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 269    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. mirabilis 533    TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 144      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 173      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 174      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 175      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 177      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 178      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
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C. nobilis 193      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-
C. nobilis 142      TAAAAGACTATTTCGGTTCCTATACAATTTTTATGTGTTTGAATGTGAATTTTTATTTG-

C. caulescens 210   ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. xnimbicola 329   ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. miniata 325      TTTTAGTTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. gardenii 139     ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. robusta 164      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 241    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 242    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 253    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 256    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 257    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 264    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 265    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 269    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. mirabilis 533    ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 144      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 173      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 174      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 175      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 177      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 178      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 193      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC
C. nobilis 142      ------TTTTTATTCGTAAACAATCTTCTTATTTACGATTAACATCTTTTGGAACTTTTC

C. caulescens 210   TTGAGCGAACACATTTCTATGGAAAAATAAAACATCTTCAAATAGAAAAATTTATAGTAA
C. xnimbicola 329   TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. miniata 325      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. gardenii 139     TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. robusta 164      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 241    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 242    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 253    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 256    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 257    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 264    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 265    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 269    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. mirabilis 533    TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 144      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 173      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 174      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 175      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 177      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 178      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 193      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA
C. nobilis 142      TTGAGCGAACACATTTCTATGGAAAAATAGAACATCTTCAAATAGAAAAATTTATAGTAA

C. caulescens 210   TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. xnimbicola 329   TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. miniata 325      TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. gardenii 139     TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. robusta 164      TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 241    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 242    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 253    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 256    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 257    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 264    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
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C. mirabilis 265    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 269    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. mirabilis 533    TATGTCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 144      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 173      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 174      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 175      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 177      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 178      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 193      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG
C. nobilis 142      TATGCCGTAACAATTTTCATAGGACCTTATGGTTCTTCAAGGATCCTTTTATGCATTATG

C. caulescens 210   TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTTTCTGATGACGAAA
C. xnimbicola 329   TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. miniata 325      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. gardenii 139     TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. robusta 164      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 241    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 242    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 253    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 256    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 257    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 264    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 265    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 269    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. mirabilis 533    TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 144      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 173      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 174      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 175      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 177      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 178      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 193      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA
C. nobilis 142      TTCGATATCAAGGAAAAGCAATTCTTGCTTCAAAGGGGACTCATCTT-CTGATGACGAAA

C. caulescens 210   TGGAAATATCATTTTGTCAATTTTCTGGCAATATTATTTTCACTT-TGGTCTCA-CCGTA
C. xnimbicola 329   TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. miniata 325      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. gardenii 139     TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. robusta 164      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 241    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 242    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 253    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 256    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 257    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 264    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 265    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 269    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. mirabilis 533    TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 144      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 173      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 174      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 175      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 177      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 178      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 193      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA
C. nobilis 142      TGGAAATATCATTTTGTCAA-TTTCTGGCAATATTATTTTCACTTTTGGTCTCAACCGTA

C. caulescens 210   CAGGATCCCATATAAATAAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCA-
C. xnimbicola 329   CAGGAT-CCATATAAATAAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
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C. C. miniata 325   CAGGAT-CCATATAAATAAATTCTCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. gardenii 139     CAGGAT-CCATATAAATAAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. robusta 164      CAGGAT-CCATATAAATAAATTATCAAACT-TTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 241    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 242    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 253    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 256    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 257    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 264    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 265    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 269    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. mirabilis 533    CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 144      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 173      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 174      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 175      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 177      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 178      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 193      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA
C. nobilis 142      CAGGAT-CCATATAAATCAATTATCAAACTATTCTTTCTATTTTCTGGGTTATCTTTCAA

C. caulescens 210   GTCTACTAATAAATCTTCCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. xnimbicola 329   GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. miniata 325      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. gardenii 139     GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. robusta 164      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 241    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 242    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 253    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 256    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 257    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 264    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 265    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 269    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. mirabilis 533    GTCTACTAAGAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 144      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 173      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 174      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 175      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 177      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 178      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 193      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG
C. nobilis 142      GTCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGTTAGAGAATTCATTTCTAATAG

C. caulescens 210   ATACCATTACTAAGAAATTTGATACCATAGTCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. xnimbicola 329   ATACCATTACTAAGAAATTTGATACCATAGTCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. miniata 325      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. gardenii 139     ATACCGTTACTAAGAAATTTGATACCATAGTCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. robusta 164      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 241    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 242    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 253    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 256    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 257    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 264    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 265    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 269    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. mirabilis 533    ATACCGTTACTAAGAAATTTGATACCATAGCCCCAGTTATTCTTCTTATTGGGTCCTTGT
C. nobilis 144      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 173      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
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C. nobilis 174      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 175      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 177      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 178      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 193      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT
C. nobilis 142      ATACCGTTACTAAGAAATTTGATACCATAGTCCCAATTATTCTTCTTATTGGGTCCTTGT

C. caulescens 210   CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. xnimbicola 329   CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. miniata 325      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. gardenii 139     CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. robusta 164      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 241    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 242    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 253    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 256    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 257    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 264    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 265    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 269    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. mirabilis 533    CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 144      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 173      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 174      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 175      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 177      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 178      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 193      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
C. nobilis 142      CTAAAGCGAAATTTTGTACCGTATCGGGCCATCCTATTAGTAAGCCGATCT
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The *rpoB* region:

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                     1        10        20        30        40        50        60
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C. caulescens 210    AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. xnimbicola 329    AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. miniata 325       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. gardenii 139      AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 241     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. robusta 164       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 242     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 253     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 256     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 257     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 264     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 265     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 269     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. mirabilis 533     AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 142       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 144       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 173       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 174       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 175       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 177       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 178       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT
C. nobilis 193       AAGTGCATTGTTGGAACTGGGCTGGAACGCCAAACGGCTCTAGATTCGGGGGTTTCCGTT

C. caulescens 210    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
C. xnimbicola 329    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
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 C. miniata 325      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTTACAAGATCATTTTATCAAGT
 C. gardenii 139     ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 241    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. robusta 164      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 242    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 253    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 256    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 257    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 264    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 265    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 269    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. mirabilis 533    ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 142      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 144      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 173      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 174      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 175      ATAGCTGAACGCGAAGG-AAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 177      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 178      ATAGCTGAACGCGAAGG-AAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT
 C. nobilis 193      ATAGCTGAACGCGAAGGAAAGATCATTTATACTGATACTCACAAGATCATTTTCTCAAGT

C. caulescens 210    AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. xnimbicola 329    AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. miniata 325       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. gardenii 139      AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 241     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. robusta 164       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 242     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 253     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 256     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 257     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 264     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 265     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 269     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. mirabilis 533     AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 142       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 144       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 173       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 174       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 175       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 177       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 178       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT
C. nobilis 193       AATGGGGACACTATAAGCATTCCATTAGTTATGTATCAACGTTCCAACAAAAATACTTGT

C. caulescens 210    ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. xnimbicola 329    ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. miniata 325       ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. gardenii 139      ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 241     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. robusta 164       ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 242     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 253     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 256     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 257     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 264     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 265     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 269     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. mirabilis 533     ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 142       ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 144       ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
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C. nobilis 173	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 174	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 175	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 177	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 178	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG
C. nobilis 193	ATGCATCAAAAACCTCAGGTTCGGCAGGGTAAATGCATTAAAAAGGGACAAATTTTAGCG

C. caulescens 210	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGCAGCTTAT
C. xnimbicola 329	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGCAGCTTAT
C. miniata 325	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGCAGCTTAT
C. gardenii 139	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGCAGCTTAT
C. mirabilis 241	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. robusta 164	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGCAGCTTAT
C. mirabilis 242	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 253	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 256	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 257	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 264	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 265	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 269	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. mirabilis 533	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 142	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 144	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 173	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 174	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 175	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 177	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 178	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT
C. nobilis 193	GATGGTGCGGCTACTGTTGGTGGGGAACTCGCTTTAGGAAAAAACGTATTAGTAGCTTAT

C. caulescens 210	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. xnimbicola 329	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. miniata 325	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. gardenii 139	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 241	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. robusta 164	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 242	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 253	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 256	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 257	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 264	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 265	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 269	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. mirabilis 533	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 142	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 144	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 173	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 174	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 175	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 177	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 178	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT
C. nobilis 193	ATGCCATGGGAAGGTTACAATTCTGAAGACGCAGTACTAATTAGCGAACGTCTGGTGTAT

C. caulescens 210	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. xnimbicola 329	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. miniata 325	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. gardenii 139	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 241	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. robusta 164	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 242	GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC

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C. mirabilis 253     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 256     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 257     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 264     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 265     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 269     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. mirabilis 533     GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 142       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 144       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 173       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 174       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 175       GAAGATATTTATACTTCTTTTCACATACGGAAATATG-AATTCAGACTCATGTGACAAGC
C. nobilis 177       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC
C. nobilis 178       GAAGATATTTATACTTCTTTTCACATACGGAAATATG-AATTCAGACTCATGTGACAAGC
C. nobilis 193       GAAGATATTTATACTTCTTTTCACATACGGAAATATGAAATTCAGACTCATGTGACAAGC

C. caulescens 210    CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. xnimbicola 329    CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. miniata 325       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. gardenii 139      CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 241     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. robusta 164       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 242     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 253     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 256     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 257     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 264     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 265     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 269     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. mirabilis 533     CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 142       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 144       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 173       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 174       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 175       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 177       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 178       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT
C. nobilis 193       CAAGGTCCCGAAAGAATCACTAAGGAAATACCGCATTTAGAGGCTTATTTACTCCGAAAT

C. caulescens 210    TTAGACAG
C. xnimbicola 329    TTAGACAG
C. miniata 325       TTAGACAG
C. gardenii 139      TTAGACAG
C. mirabilis 241     TTAGACAG
C. robusta 164       TTAGACAG
C. mirabilis 242     TTAGACAG
C. mirabilis 253     TTAGACAG
C. mirabilis 256     TTAGACAG
C. mirabilis 257     TTAGACAG
C. mirabilis 264     TTAGACAG
C. mirabilis 265     TTAGACAG
C. mirabilis 269     TTAGACAG
C. mirabilis 533     TTAGACAG
C. nobilis 142       TTAGACAG
C. nobilis 144       TTAGACAG
C. nobilis 173       TTAGACAG
C. nobilis 174       TTAGACAG
C. nobilis 175       TTAGACAG
C. nobilis 177       TTAGACAG
C. nobilis 178       TTAGACAG
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C. nobilis 193       TTAGACAG
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The *rpoC1* region:

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                      1       10        20        30        40        50        60
                      |        |         |         |         |         |         |
C. caulescens 210     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. xnimbicola 329     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. miniata 325        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. gardenii 139       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 241      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. robusta 164        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 242      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 253      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 256      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 257      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 264      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 265      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 269      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 533      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 142        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 144        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 173        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 174        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 175        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 177        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 178        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 193        CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC

C. caulescens 210     TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. xnimbicola 329     TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. miniata 325        TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. gardenii 139       TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 241      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. robusta 164        TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 242      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 253      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 256      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 257      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 264      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 265      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 269      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 533      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 142        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 144        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 173        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 174        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 175        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 177        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 178        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 193        TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG

C. caulescens 210     TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. xnimbicola 329     TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. miniata 325        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. gardenii 139       TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 241      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. robusta 164        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 242      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
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C. mirabilis 253      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 256      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 257      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 264      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 265      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 269      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 533      TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 142        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 144        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 173        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 174        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 175        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 177        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 178        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 193        TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA

C. caulescens 210     TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. xnimbicola 329     TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. miniata 325        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. gardenii 139       TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 241      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. robusta 164        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 242      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 253      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 256      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 257      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 264      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 265      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 269      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 533      TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 142        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 144        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 173        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 174        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 175        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 177        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 178        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 193        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG

C. caulescens 210     TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. xnimbicola 329     TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. miniata 325        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. gardenii 139       TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 241      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. robusta 164        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 242      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 253      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 256      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 257      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 264      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 265      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 269      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 533      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 142        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 144        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 173        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 174        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 175        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 177        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 178        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
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C. nobilis 193       TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT

C. caulescens 210    TAGTGGAGGGACGCGCTATTTGTTTACAC
C. xnimbicola 329    TAGTGGAGGGACGCGCTATTTGTTTACAC
C. miniata 325       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. gardenii 139      TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 241     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. robusta 164       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 242     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 253     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 256     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 257     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 264     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 265     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 269     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. mirabilis 533     TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 142       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 144       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 173       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 174       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 175       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 177       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 178       TAGTGGAGGGACGCGCTATTTGTTTACAC
C. nobilis 193       TAGTGGAGGGACGCGCTATTTGTTTACAC
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The *trnL-F* region:

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                     1        10        20        30        40        50        60
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C. caulescens 210    CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. xnimbicola 329    CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. miniata 325       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. gardenii 139      CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 241     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. robusta 164       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 242     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 253     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 256     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 257     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 264     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 265     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 269     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. mirabilis 533     CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 142       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 144       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 173       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 174       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 175       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 177       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 178       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC
C. nobilis 193       CTCTGCTTGGTAAACGGGTTGATTATTCGGGACGTTCCGTCATTGTCGTGGGTCCTTCGC

C. caulescens 210    TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. xnimbicola 329    TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. miniata 325       TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. gardenii 139      TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 241     TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. robusta 164       TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 242     TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
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C. mirabilis 253	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 256	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 257	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 264	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 265	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 269	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. mirabilis 533	TTTCATTACATCAATGTGGATTACCTCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 142	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 144	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 173	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 174	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 175	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 177	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 178	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG
C. nobilis 193	TTTCATTACATCAATGTGGATTACCCCGAGAAATAGCAATAGAGCTTTTCCAAACATTTG

C. caulescens 210	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. xnimbicola 329	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. miniata 325	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. gardenii 139	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 241	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. robusta 164	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 242	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 253	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 256	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 257	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 264	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 265	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 269	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. mirabilis 533	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 142	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 144	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 173	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 174	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 175	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 177	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 178	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA
C. nobilis 193	TAATTCGTGGTCTAATCAGACAACATATTGCTTCTAACATAGGGATTGCGAAAAGTAAAA

C. caulescens 210	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. xnimbicola 329	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. miniata 325	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. gardenii 139	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 241	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. robusta 164	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 242	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 253	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 256	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 257	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 264	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 265	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 269	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. mirabilis 533	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 142	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 144	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 173	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 174	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 175	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 177	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG
C. nobilis 178	TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG

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C. nobilis 193        TTCGGGAAAAAGAACCGATTGTATGGGAAATACTTCAAGAAGTTATGCAGGGGCATCCTG

C. caulescens 210     TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. xnimbicola 329     TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. miniata 325        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. gardenii 139       TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 241      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. robusta 164        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 242      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 253      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 256      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 257      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 264      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 265      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 269      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. mirabilis 533      TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 142        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 144        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 173        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 174        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 175        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 177        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 178        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT
C. nobilis 193        TATTGTTGAATAGAGCACCCACCCTGCATAGATTGGGCATACAGGCGTTCCAACCCATTT

C. caulescens 210     TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. xnimbicola 329     TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. miniata 325        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. gardenii 139       TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCAAAAT
C. mirabilis 241      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCAAAAT
C. robusta 164        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 242      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 253      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 256      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 257      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 264      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 265      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 269      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. mirabilis 533      TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 142        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 144        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 173        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 174        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 175        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 177        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 178        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT
C. nobilis 193        TAGTGGAGGGACGCGCTATTTGTTTACACAATCTAAGAAATTCGGGGACTAGGTCCAAAT

C. caulescens 210     TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. xnimbicola 329     TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. miniata 325        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. gardenii 139       TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. robusta 164        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. mirabilis 241      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 242      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 253      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 256      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 257      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 264      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
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C. mirabilis 265      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 269      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. mirabilis 533      TCCCCAGTAAAAAGCCCATTTCACTTCTTAACTATTTATTTATCCTCT-TTTTTTTCATA
C. nobilis 142        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 144        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 173        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 174        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 175        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 177        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 178        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA
C. nobilis 193        TCCCCAGTAAAAAGCCCATTTCACTTCTTAAC----TATTTATCCTCTTTTTTTTTCATA

C. caulescens 210     AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. xnimbicola 329     AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. miniata 325        AGTGGTTAAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAAAGGATCC
C. gardenii 139       AGTGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. robusta 164        AGTGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 241      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 242      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 253      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 256      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 257      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 264      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 265      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 269      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. mirabilis 533      AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATGGATCC
C. nobilis 142        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 144        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 173        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 174        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 175        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 177        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 178        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC
C. nobilis 193        AGCGGTTCAAAGAAAATTCAATATCTTTCTCATTCATTCTACTCTTTCACAAATCGATCC

C. caulescens 210     GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. xnimbicola 329     GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. miniata 325        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATCCCCGTACAAA
C. gardenii 139       GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. robusta 164        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 241      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 242      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 253      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 256      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 257      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 264      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 265      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 269      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. mirabilis 533      GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 142        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 144        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 173        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 174        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 175        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 177        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 178        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA
C. nobilis 193        GAACATAAATCTTTTGATCTTATACCAATTTGGTTTGAATAGATATGATACCCGTACAAA

C. caulescens 210     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. xnimbicola 329     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
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C. miniata 325       TGAACATATATGGTCAAGGGATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. gardenii 139      TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. robusta 164       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 241     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 242     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 253     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 256     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 257     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 264     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 265     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 269     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. mirabilis 533     TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 142       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 144       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 173       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 174       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 175       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 177       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 178       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC
C. nobilis 193       TGAACATATATGGTCAAGGAATTCCCATTATTGAATCATTCACAGTCCATATCATTATCC

C. caulescens 210    TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. xnimbicola 329    TTACATTCACAAAAGAAAGTTCTTCTTTTTTGAA
C. miniata 325       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. gardenii 139      TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. robusta 164       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. mirabilis 241     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 242     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 253     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 256     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 257     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 264     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 265     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 269     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. mirabilis 533     TTACATTCACAAA-GAAAG-TCCTC-TTTTTGAA
C. nobilis 142       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 144       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 173       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 174       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 175       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 177       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 178       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
C. nobilis 193       TTACATTCACAAA-GAAAG-TCTTC-TTTTTGAA
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The *ITS1* region:

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                    1        10         20         30         40         50         60
                    |        |          |          |          |          |          |
C. mirabilis 241    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 242    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 256    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 257    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 264    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 265    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 269    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. mirabilis 533    CCGAGAGTCATGCAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
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C. nobilis 142      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 144      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 173      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 174      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 175      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 177      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 178      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 193      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG
C. nobilis 449      CCGAGAGTCATACAGGTTATAGACGTACCAAGATCGCATCGCGTGCTGCAGCACAAAGCG

C. mirabilis 241    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 242    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 256    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 257    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 264    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 265    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 269    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. mirabilis 533    TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 142      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 144      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 173      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 174      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 175      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 177      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 178      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 193      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG
C. nobilis 449      TGCCGCACACTCCGCGCTCCAAATGGGTCTGCTCCTTGGCGCACGCCGCGCCGGAAATTG

C. mirabilis 241    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 242    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 256    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 257    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 264    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 265    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 269    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. mirabilis 533    TTCCCACTGCTCGTCCCGCACGTTGTGCAGGGAGGCAGCGGCCGGGGCACCCAAGGAGGC
C. nobilis 142      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 144      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 173      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 174      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 175      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 177      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 178      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 193      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC
C. nobilis 449      TTCCCACTGCTCGTCCCGCACGTTGTGCTGGGAGGCAGCGGCCGAGGCACCCAAGGAGGC

C. mirabilis 241    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 242    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 256    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 257    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 264    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 265    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 269    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. mirabilis 533    AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 142      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 144      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 173      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 174      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 175      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 177      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
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C. nobilis 178      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 193      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG
C. nobilis 449      AAACGATGTCGGCCATCGCCTCCCCCCTTCTCCTCCCCACGGGTGCTCTACGAGTTCACG

C. mirabilis 241    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 242    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 256    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 257    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 264    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 265    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 269    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. mirabilis 533    ATCTCGTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 142      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 144      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 173      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 174      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 175      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 177      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 178      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 193      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
C. nobilis 449      ATC--GTCCGTTCGGGCCTCGACAATGATCCTTCCGCAGGTTC
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The *rpl16* region:

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                  1        10        20        30        40        50        60
                  |        |         |         |         |         |         |
 C. nobilis 449   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 193   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 178   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 177   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 175   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 174   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 173   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 170   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 160   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 144   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. nobilis 142   AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGCAATTTTTCATAAATCCG
 C. mirabilis 533 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 272 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 271 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 270 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 269 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 267 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 266 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 265 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 264 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 262 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 258 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 257 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 256 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 255 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 254 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 253 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 252 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 242 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 241 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCCG
 C. mirabilis 247 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCTG
 C. mirabilis 240 AGTGACTATATGACTGGATCAATCATATAGTTGTAACAACTGAAATTTTTCATAAATCTG
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C. nobilis 449     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 193     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 178     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 177     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 175     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 174     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 173     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 170     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 160     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 144     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. nobilis 142     ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 533   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 272   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 271   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 270   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 269   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 267   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 266   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 265   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 264   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 262   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 258   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 257   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 256   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 255   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 254   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 253   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 252   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 242   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 241   ATTATGGATTTTGAAGAAA-AAAATAAAAAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 247   ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG
C. mirabilis 240   ATTATGGATTTTGAAGAAA-AAAATAAATAAAGGGATGCGGATAAATGGAAAGGTGATAG

C. nobilis 449     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 193     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 178     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 177     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 175     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 174     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 173     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 170     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 160     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 144     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. nobilis 142     AAAGAGAGAACAAAAATATCAATGATAGATTATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 533   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 272   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 271   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 270   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 269   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 267   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 266   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 265   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 264   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 262   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 258   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 257   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 256   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 255   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 254   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 253   AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
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C. mirabilis 252	AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 242	AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 241	AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-AATGTGTATGGTCTATGAATCAC
C. mirabilis 247	AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-TATGTGTATGGTCTATGAATCGC
C. mirabilis 240	AAAGAGAGAACAAAAATATCAATGATAGATGATTCC-TATGTGTATGGTCTATGAATCGC

C. nobilis 449	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 193	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 178	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 177	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 175	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 174	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 173	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 170	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 160	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 144	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. nobilis 142	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 533	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 272	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 271	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 270	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 269	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 267	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 266	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
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C. mirabilis 264	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 262	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 258	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 257	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 256	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 255	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 254	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 253	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 252	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 242	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 241	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 247	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA
C. mirabilis 240	CTCATAA-AAGGCAGTGTGATAAAGCATTAATATTGATATAAATAATAATAAATAATAAA

C. nobilis 449	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 193	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 178	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 177	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 175	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 174	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 173	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 170	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 160	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 144	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. nobilis 142	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 533	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 272	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 271	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 270	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 269	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 267	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 266	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 265	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 264	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 262	GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC

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C. mirabilis 258   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 257   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 256   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 255   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 254   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 253   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 252   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 242   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 241   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 247   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC
C. mirabilis 240   GAGCCTCGGGTTAATAGAAACT-GAGGAGATTGACTCGGGAACAA-ATTTTGTTGGGAGC

C. nobilis 449     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 193     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 178     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 177     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 175     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 174     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 173     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 170     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 160     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 144     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. nobilis 142     TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 533   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 272   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 271   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 270   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 269   TCCATTGCAGAGTTTCGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 267   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 266   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 265   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 264   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 262   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 258   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 257   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 256   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 255   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 254   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 253   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 252   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 242   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 241   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 247   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG
C. mirabilis 240   TCCATTGCAGAGTT-CGGGCCTAACCATTCATGGAGAAGCTATAGGAACGACGAAACCTG

C. nobilis 449     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 193     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 178     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 177     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 175     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 174     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 173     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 170     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 160     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 144     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. nobilis 142     TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. mirabilis 533   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 272   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 271   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 270   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
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C. mirabilis 269   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 267   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 266   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 265   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 264   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 262   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 258   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 257   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 256   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 255   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 254   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 253   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 252   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 242   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 241   TGACTATATAAGATTCTATTAAAAACGAATCCTAATTATTCATCGGGT-GGGATGGCG-G
C. mirabilis 247   TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G
C. mirabilis 240   TGACTATATAAGATTCTATTAAAAACGAATCCTAATGATTCATCGGGT-GGGATGGCG-G

C. nobilis 449     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 193     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 178     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 177     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 175     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 174     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 173     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 170     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 160     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 144     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. nobilis 142     AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 533   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 272   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 271   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 270   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 269   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 267   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 266   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 265   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 264   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 262   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 258   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 257   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 256   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 255   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 254   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 253   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 252   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 242   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 241   AACAAACC-AAGAACAAA-TTGAGATTGACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 247   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT
C. mirabilis 240   AACAAACC-AAGAACAAA-TTGAGATTTACTCTGAGAGATCATGAATTGATCCTACGAAT

C. nobilis 449     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 193     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 178     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 177     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 175     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 174     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 173     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 170     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 160     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
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C. nobilis 144     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. nobilis 142     AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 533   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 272   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 271   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 270   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 269   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 267   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 266   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 265   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 264   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 262   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 258   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 257   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 256   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 255   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 254   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 253   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 252   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 242   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 241   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 247   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA
C. mirabilis 240   AAAGAAAGCAGGAAAGAGTCAATATTCGCCC-GCG-AAACCCTTATTTATTGTATTCCAA

C. nobilis 449     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 193     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 178     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 177     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 175     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 174     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 173     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 170     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 160     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 144     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. nobilis 142     TATTGTCGCTTGATTTAATAAGAGTAAAAAAAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 533   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 272   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 271   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 270   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 269   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 267   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 266   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 265   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 264   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 262   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 258   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 257   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 256   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 255   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 254   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 253   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 252   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 242   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 241   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 247   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA
C. mirabilis 240   TATTGTCGCTTGATTTAATAAGAGTAAAAATAA-GGATTCG-TTATAAA-AAAGAA-AGA

C. nobilis 449     AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 193     AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 178     AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
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C. nobilis 177	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 175	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 174	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 173	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 170	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 160	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 144	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. nobilis 142	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 533	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 272	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 271	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 270	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 269	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 267	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 266	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 265	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 264	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 262	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 258	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 257	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 256	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 255	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 254	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 253	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 252	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 242	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 241	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 247	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A
C. mirabilis 240	AGCATTATCTATAAATATACACATCTAGCCGTATATACAACACAGCTTCCTATGTAAT-A

C. nobilis 449	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 193	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 178	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 177	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 175	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 174	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 173	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 170	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 160	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 144	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. nobilis 142	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 533	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 272	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 271	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 270	AATGAAATATCAATAAAT-CCCATTACTTAGTTTTAGTGTATTAGTTATTA-AATACATG
C. mirabilis 269	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 267	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 266	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 265	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 264	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 262	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 258	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 257	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 256	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 255	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 254	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 253	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 252	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 242	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 241	AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG

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C. mirabilis 247   AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG
C. mirabilis 240   AATGAAATATCAATAAAT-CCCATTACTTAGTT-TAGTGTATTAGTTATTA-AATACATG

C. nobilis 449     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 193     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 178     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 177     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 175     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 174     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 173     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 170     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 160     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 144     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. nobilis 142     TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 533   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 272   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 271   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 270   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 269   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 267   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 266   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 265   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 264   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 262   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 258   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 257   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 256   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 255   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 254   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 253   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 252   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 242   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 241   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 247   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT
C. mirabilis 240   TGTATATGTAATATTATCGAAT-CCTTTCAT-TCG-CGAGGAGCTGGATGAGAAGAAACT

C. nobilis 449     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 193     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 178     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 177     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 175     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 174     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 173     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 170     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 160     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 144     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. nobilis 142     CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 533   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 272   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 271   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 270   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
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C. mirabilis 267   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 266   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 265   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 264   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
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C. mirabilis 255   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
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C. mirabilis 242   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
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C. mirabilis 247   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC
C. mirabilis 240   CTCATGTCCGGTTCTG-TAGTAGAGATG-GGATTCAGAAAAAACCATCAACTATAACCCC

C. nobilis 449     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 193     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 178     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 177     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 175     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 174     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 173     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 170     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 160     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 144     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. nobilis 142     AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 533   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 272   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 271   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 270   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 269   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 267   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 266   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 265   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 264   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 262   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 258   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 257   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 256   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 255   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 254   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 253   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 252   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 242   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 241   AAAAGAACCAGATT-TCGTAAGCAACATAGGGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 247   AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG
C. mirabilis 240   AAAAGAACCAGATT-TCGTAAGCAACATAGAGG-AAGAATGAAGGGA-ATATCTTG-TCG

C. nobilis 449     AG-GTAATCATA--TT
C. nobilis 193     AG-GTAATCATA--TT
C. nobilis 178     AG-GTAATCATA--TT
C. nobilis 177     AG-GTAATCATA--TT
C. nobilis 175     AG-GTAATCATA--TT
C. nobilis 174     AG-GTAATCATA--TT
C. nobilis 173     AG-GTAATCATA--TT
C. nobilis 170     AG-GTAATCATA--TT
C. nobilis 160     AG-GTAATCATA--TT
C. nobilis 144     AG-GTAATCATA--TT
C. nobilis 142     AG-GTAATCATA--TT
C. mirabilis 533   AG-GTAATCATA--TT
C. mirabilis 272   AG-GTAATCATA--TT
C. mirabilis 271   AG-GTAATCATA--TT
C. mirabilis 270   AG-GTAATCATA--TT
C. mirabilis 269   AG-GTAATCATA--TT
C. mirabilis 267   AG-GTAATCATA--TT
C. mirabilis 266   AG-GTAATCATA--TT
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C. mirabilis 265   AG-GTAATCATA--TT
C. mirabilis 264   AG-GTAATCATA--TT
C. mirabilis 262   AG-GTAATCATA--TT
C. mirabilis 258   AG-GTAATCATA--TT
C. mirabilis 257   AG-GTAATCATA--TT
C. mirabilis 256   AG-GTAATCATA--TT
C. mirabilis 255   AG-GTAATCATA--TT
C. mirabilis 254   AG-GTAATCATA--TT
C. mirabilis 253   AG-GTAATCATA--TT
C. mirabilis 252   AG-GTAATCATA--TT
C. mirabilis 242   AG-GTAATCATA--TT
C. mirabilis 241   AG-GTAATCATA--TT
C. mirabilis 247   GG-GTAATCATA--TT
C. mirabilis 240   GG-GTAATCATA--TT
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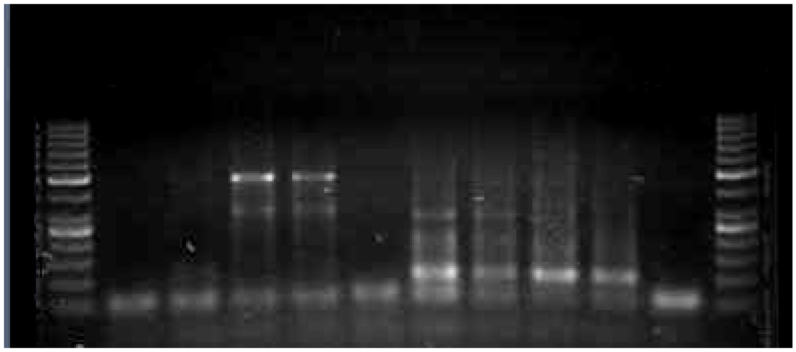
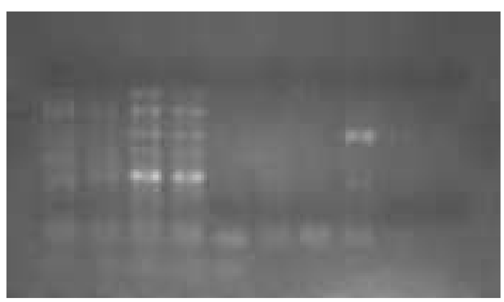
Appendix F: The photographs of agarose gels used for optimization of microsatellite primers.



1 2 3 4 5 6 7 8 9 10

Figure F1: Photograph of gel after initial amplification. The DNA ladder shown in the first and last lanes were a 100bp ladder. (1) *C. nobilis* 142 with primer Pt14, (2) *C. nobilis* 144 with primer Pt14, (3) *C. mirabilis* 257 with primer Pt14, (4) *C. mirabilis* 245 with primer Pt14, (5) negative control, (6) *C. nobilis* 142 with primer HA10, (7) *C. nobilis* 144 with primer HA10, (8) *C. mirabilis* 257 with primer HA10, (9) *C. mirabilis* 245 with primer HA10, (10) negative control.



1 2 3 4 5 6 7 8 9 10

Figure F2: Photograph of gel after initial amplification. (1) *C. nobilis* 142 with primer HD12, (2) *C. nobilis* 144 with primer HD12, (3) *C. mirabilis* 257 with primer HD12, (4) *C. mirabilis* 245 with primer HD12, (5) negative control, (6) *C. nobilis* 142 with primer HB1, (7) *C. nobilis* 144 with primer HB1, (8) *C. mirabilis* 257 with primer HB1, (9) *C. mirabilis* 245 with primer HB1, (10) negative control.

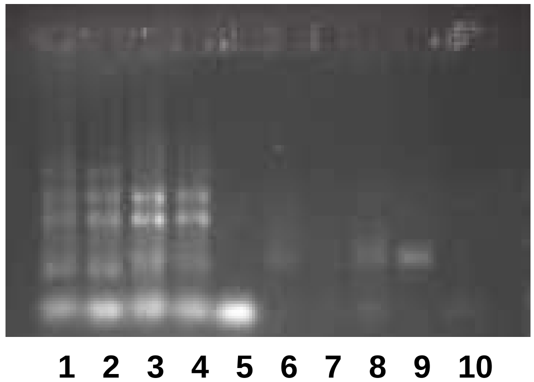


Figure F3: Photograph of gel after initial amplification. (1) *C. nobilis* 142 with primer HD7, (2) *C. nobilis* 144 with primer HD7, (3) *C. mirabilis* 257 with primer HD7, (4) *C. mirabilis* 245 with primer HD7, (5) negative control, (6) *C. nobilis* 142 with primer Pt36, (7) *C. nobilis* 144 with primer Pt36, (8) *C. mirabilis* 257 with primer Pt36, (9) *C. mirabilis* 245 with primer Pt36, (10) negative control.

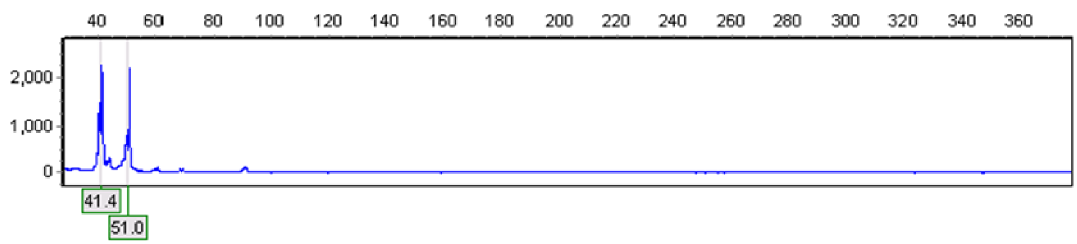
Appendix G: Electropherograms obtained from the microsatellite results with the GeneMarker software.

SoftGenetics **Allele Report** 9/1/2010 3:41:23 PM

GeneMarker V1.60 Page 1

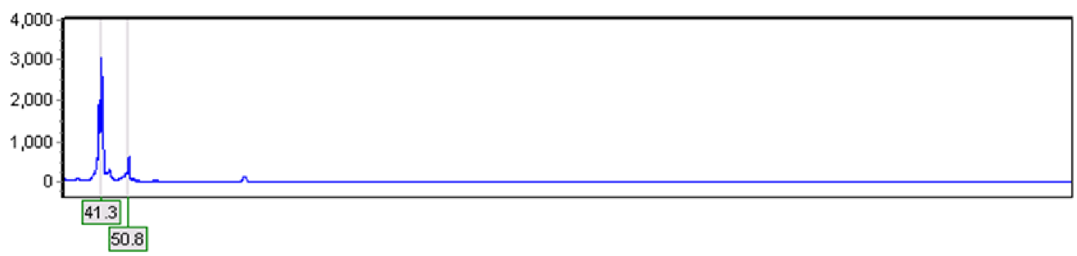
Sample 1

Dye: Blue - 2 peak -Pt 14



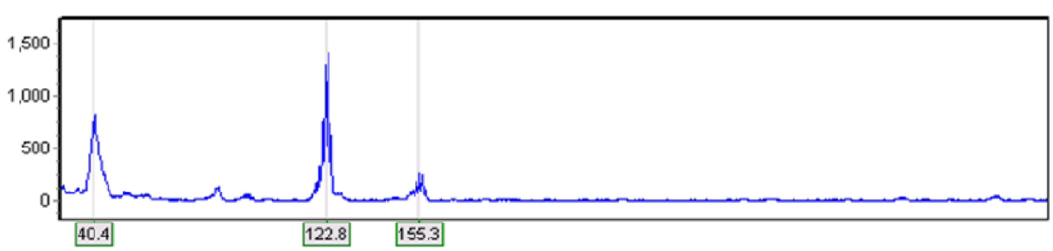
Sample 2

Dye: Blue - 2 peaks -Pt 14



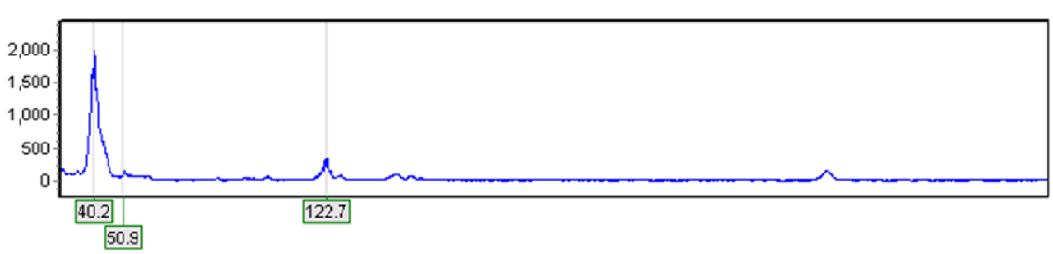
Sample 3

Dye: Blue - 3 peaks -Pt 14



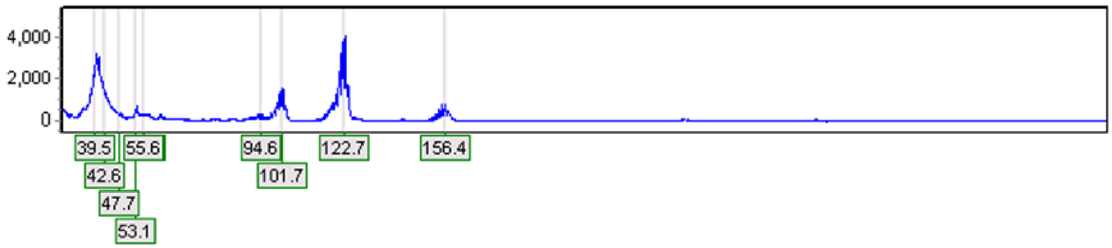
Sample 4

Dye: Blue - 3 peaks -Pt 14



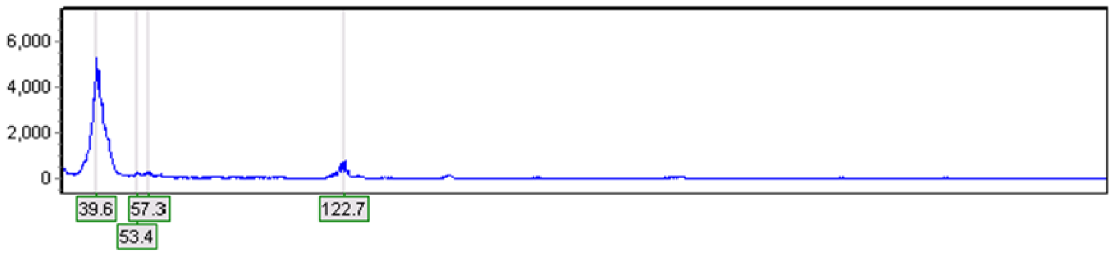
Sample 5

Dye: Blue - 9 peaks -HcoD9



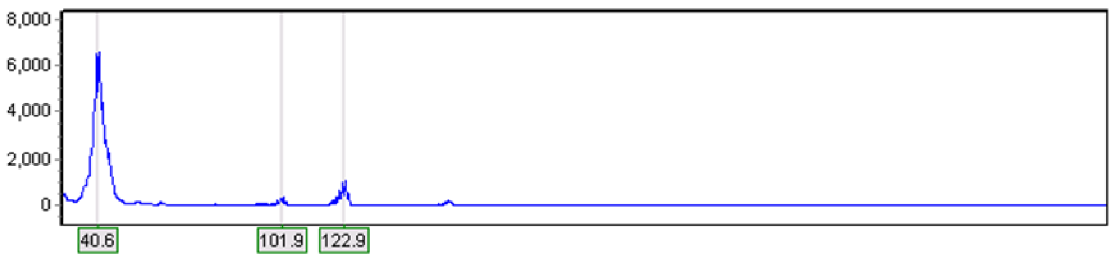
Sample 6

Dye: Blue - 4 peaks -HcoD9



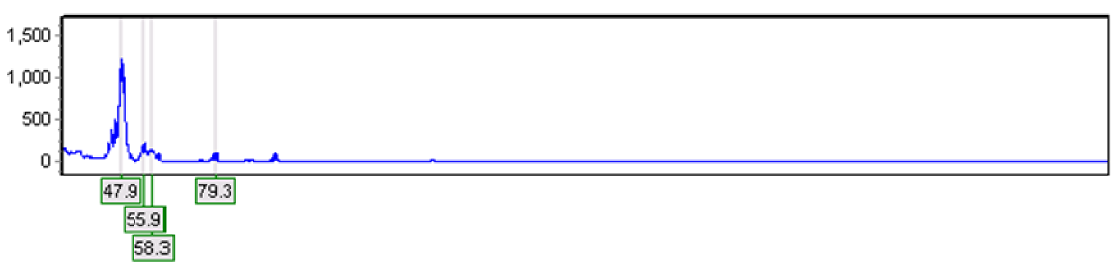
Sample 7

Dye: Blue - 3 peaks -HcoD9



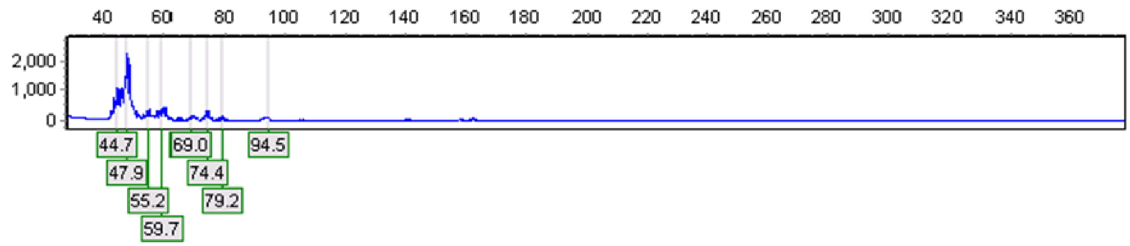
Sample 8

Dye: Blue - 4 peaks -HcoD9



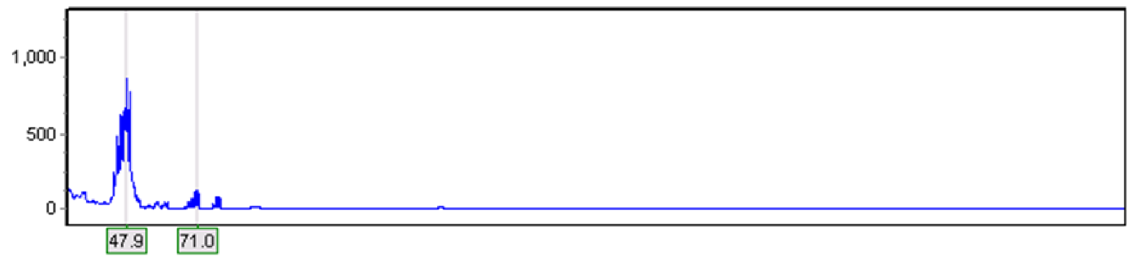
Sample 9

Dye: Blue - 8 peaks -HcoB1



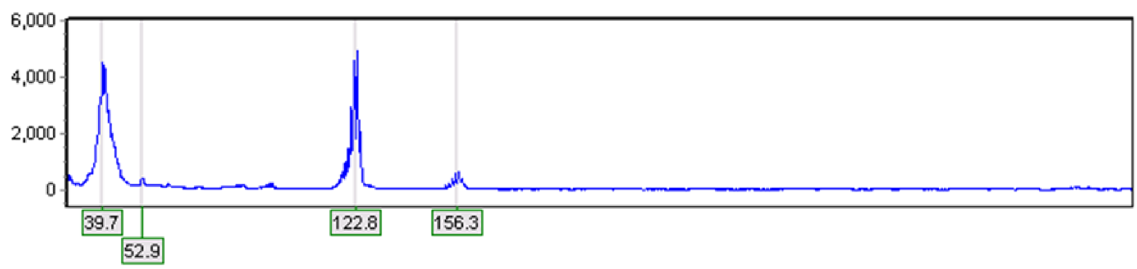
Sample 10

Dye: Blue - 2 peaks -HcoB1



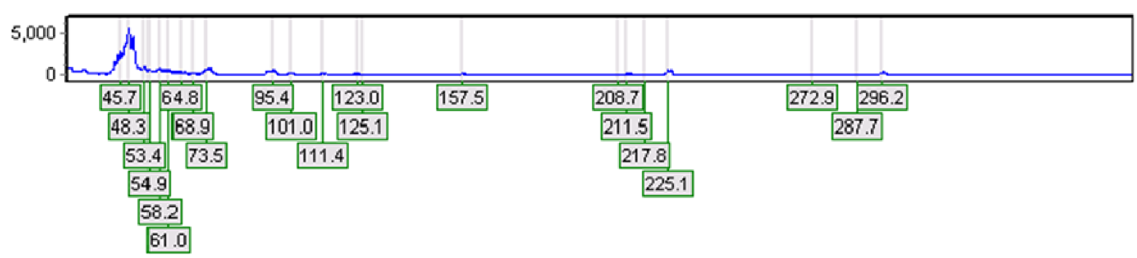
Sample 11

Dye: Blue - 4 peaks -HcoB1



Sample 12

Dye: Blue - 22 peaks -HcoB1



Sample 13

Dye: Blue - 7 peaks -HcoB1