

**PHYLOGENY OF THE GENUS *RAPHIONACME*
(APOCYNACEAE)**

by

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LIST OF CONTENTS

Acknowledgements	i
List of abbreviations	vi
List of tables and figures	ix
List of <i>Raphionacme</i> species	xi
Chapter 1: Introduction	
1.1. General introduction	1
1.2. Aim	2
Chapter 2: Literature review	
2.1. Biogeography of Africa	4
2.2. The family Apocynaceae	17
2.3. The subfamily Periplocoideae	19
2.3.1. Circumscription of the Periplocoideae	19
2.3.2. Distribution and habitat of the Periplocoideae	21
2.4. The genus <i>Raphionacme</i>	22
2.4.1. Historical notes on <i>Raphionacme</i>	22
2.4.2. Sectional classification of <i>Raphionacme</i>	23
2.4.3. Distribution and habitat of <i>Raphionacme</i>	23

3.4.4. Morphology of <i>Raphionacme</i>	25
2.5. Molecular analysis	27
2.5.1. Ribosomal DNA (rDNA)	27
2.5.2. Characterising the Internal Transcribed Spacer (ITS)	27
2.5.3. Complications with ITS	30
2.5.4. The value of the ITS region	31
2.6. Morphological analysis	33
2.6.1. The value and application of morphological data	33
2.6.2. Problems with ad restrictions of morphological data	35
2.7. Phylogenetics	36

Chapter 3: Material and Methods

3.1. Materials	38
3.1.1. Specimens	38
3.1.2. Morphological characters and character states	38
3.1.3. Morphological terminology	38
3.1.4. Biogeography	44
3.2. Methods	44
3.2.1. DNA extraction from dried leaf tissue	44
3.2.2. Sequence analysis of plant specimens	45
3.2.2.1. Amplification of the ITS region	45
3.2.2.2. DNA sequencing of ITS	48
3.2.2.3. Sequence editing and alignment	49

3.2.3. Phylogenetic analyses	49
3.2.3.1. Data	49
3.2.3.2. Parsimony	49
3.2.3.3. Maximum likelihood	51
3.2.3.4. Bayesian analysis	51
3.2.4. Biogeography	51
Chapter 4: Results	
4.1. Biogeography	52
4.1.1. Distribution of <i>Raphionacme</i>	52
4.1.2. <i>Raphionacme</i> species richness	52
4.1.3. Endemism in <i>Raphionacme</i>	55
4.2. Cladistical analyses	55
4.2.1. Morphological data	55
4.2.2. Molecular data	58
4.2.3. Combined data	60
Chapter 5: Discussion and Conclusion	
5.1. Incongruence between the morphological and nuclear datasets	62
5.2. Phylogeny, morphology and biogeography of <i>Raphionacme</i>	62
5.2.1. Phylogenetic relationships and biogeographical affinities in <i>Raphionacme</i>	62
5.3. Cladistical indications for taxonomic questions	77

5.3.1. Forms of <i>Raphionacme hirsuta</i>	77
5.3.2. <i>Raphionacme splendens</i> – unite or divide	77
5.3.3. Sectional classification of <i>Raphionacme</i>	82
5.3.4. Position of <i>Schlechterella abyssinica</i>	82
5.4. Distribution of endemism and species richness	84
5.4.1. Distribution of <i>Raphionacme</i> species within Regions of Endemism	85
5.4.2. Species richness	90
5.5. Possible origin and distribution of <i>Raphionacme</i>	91
5.6. Conclusion	94
References	97
Appendix	116
Summary	121
Opsomming	123

LIST OF ABBREVIATIONS

A

A	Adenine
AIC	Akaike Information Criterion

B

BI	Bayesian Analysis / Bayesian Inference
bp	Base Pairs
BRAHMS	Botanic Research And Herbarium Management System
BS	Bootstrap

C

C	Cytosine
CI	Consistency Index
CTAB	Cetyltrimethylammonium bromide

D

DNA	Deoxyribonucleic acid
-----	-----------------------

E

EDTA	Ethylenediaminetetraacetic acid
------	---------------------------------

G

G	Guanine
---	---------

H

HI Homoplasy Index

I

ICBN International Code of Botanical Nomenclature

ILD Incongruence Length Difference

ITS Internal Transcribed Spacer Region

M

MCMC Markov Chain Monte Carlo

ML Maximum Likelihood

MP Maximum Parsimony

mRNA Messenger RNA

N

nrDNA Nuclear Ribosomal DNA

O

OG Outgroup

ORD Orbitally Forced Range Dynamics

P

PCR Polymerase Chain Reaction

PP Posterior Probability

R

rDNA	Ribosomal DNA
RI	Retention Index
RNase	Ribonuclease A

T

T	Thymine
TAE	Tris-acetate-EDTA
TBR	Tree-Bisection-Reconnection
Tris-HCl	Tris(hydroxymethyl)aminomethane – hydrochloride

U

UV	Ultraviolet
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Y

Y	Pyrimidine (C and T)
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LIST OF TABLES AND FIGURES

Table 2.1. Classification of the main phytochoria of Africa (White, 1983).	10
Table 2.2. <i>Raphionacme</i> species within Venter and Verhoeven's (1988) four sections.	24
Table 3.1. List of morphological characters with character states as used in the data-matrix.	39
Table 3.2. Nucleotide sequences of ITS primers used in this study.	47
Table 3.3. <i>Raphionacme</i> and outgroup species with their respective accession numbers for which ITS sequences were obtained from GenBank.	50
Table 4.1. <i>Raphionacme</i> species endemic to White's (1983) phytochoria.	56
Fig. 2.1. White's (1983) vegetation map of Africa indicating the main phytochoria.	11
Fig. 2.2. Schematic representation of the universal structure of the rDNA region in plants as adopted from Poczai and Hyvönen (2010).	28
Fig. 3.1. Schematic representation of a generalized <i>Raphionacme</i> flower.	42
Fig. 3.2. Schematic representation of ITS regions 1 and 2 as adopted from Blattner (1999).	46
Fig. 4.1. Known distribution of <i>Raphionacme</i> species in Africa.	53
Fig. 4.2. Map indicating <i>Raphionacme</i> species richness in Africa.	54

Fig. 4.3. Morphological data cladogram of <i>Raphionacme</i> species.	57
Fig. 4.4. ITS cladogram of <i>Raphionacme</i> species.	59
Fig. 4.5. Cladogram based on combined data of ITS and morphology of <i>Raphionacme</i> species.	61
Fig. 5.1. Distribution of <i>Raphionacme</i> species within clade A.	64
Fig. 5.2. Distribution of <i>Raphionacme</i> species within sub-clade A1.	66
Fig. 5.3. Distribution of <i>Raphionacme</i> species within sub-clade A2.	67
Fig. 5.4. Distribution of <i>Raphionacme</i> species within sub-clade A3.	69
Fig. 5.5. Distribution of <i>Raphionacme</i> species within sub-clade A4.	70
Fig. 5.6. Distribution of <i>Raphionacme</i> species within clade B.	71
Fig. 5.7. Distribution of <i>Raphionacme</i> species within clade C.	73
Fig. 5.8. Distribution of <i>Raphionacme</i> species within sub-clade C1.	75
Fig. 5.9. Distribution of <i>Raphionacme</i> species within clade D.	76
Fig. 5.10. Voucher specimens of the two <i>Raphionacme hirsuta</i> forms.	78
Fig. 5.11. ITS sequence alignment of the two <i>Raphionacme hirsuta</i> forms.	79
Fig. 5.12. Specimens of (i) <i>Raphionacme splendens</i> subsp. <i>bingeri</i> and (ii) <i>R. splendens</i> subsp. <i>splendens</i> .	80
Fig. 5.13. Sections of <i>Raphionacme</i> plotted onto the combined data cladogram.	83

LIST OF *RAPHIONACME* SPECIES NAMES

Raphionacme arabica A.G.Mill. & Biagi

Raphionacme angolensis (Baill.) N.E.Br. = *Raphionacme kubangensis*

Raphionacme bingeri (A.Chev.) Lebrun & Stork = *Raphionacme splendens* subsp. *bingeri*

Raphionacme borenensis Venter & M.G.Gilbert

Raphionacme brownii Scott-Elliott

Raphionacme caerulea E.A.Bruce

Raphionacme chimanimaniana Venter & R.L.Verh.

Raphionacme dyeri Retief & Venter

Raphionacme elsana Venter & R.L.Verh.

Raphionacme excisa Schltr. = *Raphionacme splendens* subsp. *splendens*

Raphionacme flanaganii Schltr.

Raphionacme galpinii Schltr.

Raphionacme globosa K.Schum.

Raphionacme grandiflora N.E.Br.

Raphionacme haeneliae Venter & R.L.Verh.

Raphionacme hirsuta (E.Mey.) R.A.Dyer

Raphionacme inconspicua H.Huber

Raphionacme keayii Bullock

Raphionacme kubangensis S.Moore

Raphionacme lanceolata Schinz

Raphionacme linearis K.Schum.

Raphionacme longifolia N.E.Br.

Raphionacme longituba E.A.Bruce

Raphionacme lucens Venter & R.L.Verh.

Raphionacme madiensis S.Moore
Raphionacme michelii De Wild.
Raphionacme monteiroae (Oliv.) N.E.Br. = *Chlorocyathus monteiroae* Oliv.
Raphionacme moyalica Venter & R.L.Verh.
Raphionacme namibiana Venter & R.L.Verh.
Raphionacme palustris Venter & R.L.Verh.
Raphionacme procumbens Schltr.
Raphionacme pulchella Venter & R.L.Verh.
Raphionacme splendens Schltr.
Raphionacme splendens Schltr. subsp. *splendens*
Raphionacme splendens subsp. *bingeri* (A.Chev.) Venter
Raphionacme sylvicola Venter & R.L.Verh.
Raphionacme utilis N.E.Br. & Stapf
Raphionacme velutina Schltr.
Raphionacme vignei E.A.Bruce
Raphionacme villicorona Venter
Raphionacme welwitschii Schltr. & Rendle
Raphionacme zeyheri Harv.

CHAPTER 1

INTRODUCTION



1.1. General introduction

The Gentianales, originally described by Jussieu (1789), consists of the families Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae and Rubiaceae. Several common vegetative, floral and phytochemical characteristics are shared by these families (APG, 2009). The vegetative forms range from small alpine herbs to large, woody, rainforest trees with opposite, entire leaves, often with stipules and colleters.

Jussieu (1789) was also the author of the family name Apocineae (now known as the Apocynaceae) as part of his new order. Brown (1810) divided the Apocynaceae and named his new family the Asclepiadeae (currently known as Asclepiadaceae). However, the Apocynaceae and Asclepiadaceae share more similarities with each other than with the rest of the Gentianales families. In a number of characters there are gradations of characteristics from the Apocynaceae to the Asclepiadaceae (Endress, 2001). This made the delimitation of the Apocynaceae/Asclepiadaceae problematic and the status of these families has been the subject of on-going controversy. The most compelling evidence for uniting the Apocynaceae and Asclepiadaceae was obtained from detailed and extensive morphological studies as well as the rapidly growing body of molecular information (Judd et al., 1994; Civeyrel, 1996; Endress et al., 1996; Sennblad and Bremer, 2000; Endress, 2001; Endress and Stevens, 2001). At present the combined Apocynaceae, second largest family in the Gentianales (APG, 2009), consists of approximately 5100 species distributed among 357 genera (Nazar et al., 2013). The Apocynaceae is one of four families occurring in South Africa that are characterised by the presence of latex, the others being Euphorbiaceae, Moraceae and Sapotaceae (Leistner, 2005) and one of over 40 plant families worldwide containing latex (Hunter, 1994). The primary function of latex is protection against browsing (Hunter, 1994).

The subfamily name Periplocoideae was established when Brown (1810) divided his Asclepiadeae and named one of the groups Periploceae (Endress, 2001; 2004; Endress and Bruyns, 2000). Distribution of this subfamily is entirely Old World, in Madagascar, Asia, Australia, but is most diverse in Africa (Venter, 2009). It has been estimated that the Periplocoideae diversified in the middle Eocene (53 – 33.7 million years ago).

This estimated age of biogeographical diversification of Asclepiadoideae was based on the plastid sequences *trnL* intron and *trnL*-F intergenic spacer (*trnL*-F). This study included one Periplocoideae species (Rapini et al., 2007).

Raphionacme Harv., comprising 36 species, is the largest genus in the Periplocoideae and is distributed throughout Africa with one species occurring in Arabia (Dyer, 1975; Venter, 2009). Economically *Raphionacme* does not make any significant contribution, the exception being *R. utilis* from which rubber was extracted commercially in the early 1900's. However, members of this genus have numerous ethnobotanical applications. The tuber is mostly used as a source of water, medicine and food, but if applied incorrectly, this could be poisonous (Venter, 2009). Some examples are the tubers of *R. hirsuta* of southern Africa, which have been used to treat cancer (Graham et al., 2000) and a decoction of *R. splendens* leaves which are used to treat conjunctivitis (Venter, 2009). *Raphionacme* species of which tubers are eaten raw by locals include *R. arabica*, *R. brownii*, *R. longifolia*, *R. madiensis*, *R. splendens*, *R. velutina* and *R. vignei* (Miller and Biagi, 1988; Venter, 2009).

1.2. Aim

The African genera of the Periplocoideae were revised by Venter and Verhoeven (2001) and have been the subject of a number of publications. These publications included the revision of *Ectadium* E.Mey. (Venter et al., 1990b), *Tacazzea* Decne. (Venter et al., 1990a), *Stomatostemma* N.E.Br. (Venter and Verhoeven, 1993), *Buckollia* Venter & R.L.Verh. (Venter and Verhoeven, 1994a), *Maclaudia* Venter & R.L.Verh. (Venter and Verhoeven, 1994b), *Periploca* L. (Venter, 1997), *Schlechterella* K.Schum. (Venter and Verhoeven, 1998), *Chlorocyathus* Oliv. (Venter, 2008), *Baseonema* Schltr. & Rendle (Venter and Verhoeven, 2009), *Batesanthus* N.E.Br. (Venter and Verhoeven, 2009) and *Raphionacme* (Venter, 2009).

Regarding *Raphionacme* itself, no publication on the phylogeny, which includes all the species of this genus, has appeared. A small number of *Raphionacme* species were incorporated in some phylogenetic studies on the Apocynaceae and Periplocoideae.

Analyses published by Lahaye et al. (2007) contained only one *Raphionacme* species, Ionta and Judd (2007) included three species, Meve and Liede (2004) seven species and Ionta (2009) sixteen species.

Because of the complexity of the flower, especially the androecium-gynoecium complex or gynostegium, morphological studies and deductions based on a single or a few selected characteristics led to misconceptions. Unnatural grouping (Venter and Verhoeven, 1988) and circumscription of genera and species thus occurred, as was found by García et al. (2009) in their study of the *Malva* L. alliance.

The only way to resolve these problems of relationships between species and related genera and to divide the genus into natural groups is to add molecular data to the cladistical analyses. The phylogenetic study of the genus *Raphionacme* presented here complements the revision of the genus (Venter, 2009) and attempts to determine the relationships between species, evolutionary trends, distribution patterns and habitat preferences and infer a possible classification of the species.

The knowledge of biodiversity is necessary in order to make informed decisions to protect habitat and biodiversity against human exploitation and destruction. Sustainable use of natural resources also depends on the knowledge of local biodiversity. Taxonomy thus provides a basic understanding of the components of biodiversity which is necessary for effective decision making about conservation and sustainable use.

Theodosius Dobzhansky stated that, "Nothing in biology makes sense except in the light of evolution". Thus, in arrangement with explicit methods for phylogenetic analysis, molecular data have reformed concepts of relationships and limitations at all levels of the taxonomic hierarchy.

A close-up photograph of a plant stem with several green, fuzzy flower buds. The buds are covered in fine, light-colored hairs and have a slightly curled, pointed shape. The background is a soft, out-of-focus green, suggesting more of the plant or foliage.

CHAPTER 2

LITERATURE REVIEW

2.1. Biogeography of Africa

Africa is the second largest continent on earth (White, 1983), with many features in plant life that combine to make the continent enthralling and puzzling. The current landscape of Africa is the result of tectonic movement, since the far distant breakup of Gondwana. Another influence is the incidence of on-going episodes of rifting and volcanism since the establishment of Africa as a distinct continent. These tectonic and thermal mechanisms clarify certain specific characteristics of present-day Africa (Summerfield, 1996).

Since the early Permian, 295 to 250 million years ago, Africa has experienced no less than seven major rifting episodes (Lambiase, 1989). The first of the rifting episodes preceded the breakup of Gondwana and was the most noteworthy, relating to the Karoo Rift and Basin development. Sedimentation in the Karoo Basin of southern Africa was nearly continuous from 280 to 100 million years ago, while the uplift of the Cape Fold Belt to the south was coexisting with the early stages of the basin subsidence (Summerfield, 1996).

Rifts and basins of the early Permian originated over a wide area of Africa, ranging from the Benue Trough in the west, to Sudan in the east and the Sirte Basin in Libya to the north. Due to the permanency of these rift systems, it has been collectively termed the Central African Rift System (Fairhead, 1986). A linear feature is shaped by the rift structures, but individual basins are located at highly variable angles to the overall inclination of the rift system. The early Miocene (approximately 23.5 million years ago) involved the start of the present rifting episode (Summerfield, 1996), while the East African Rift System continues to develop to the southwest towards the Kalahari Craton as shown by seismic data (Fairhead and Reeves, 1977).

Associated with the East African Rift System are the most prominent upwarps, which itself, appears to signify the site of developing continental rifting. One such example is the series of broad upwarps rising to altitudes of several hundred metres or more above the adjacent terrain that occur over much of the continent. These are mostly evident as long-recognized marginal upwarps which run parallel to the coastline in numerous areas and which are bound on their seaward side by a sharp topographic gap in the form of a major escarpment, or series of escarpments (Summerfield, 1996).

Elevation distribution across the African continent is of considerable importance. A height-frequency distribution assessment for the whole African continent indicates a concentration of elevation between 400 and 600 m, with a secondary peak in distribution between 800 and 1000 m. The consequence of such height-area distribution lies in the way they reflect, at an essential level, the tectonic and landscape evolution of the continent (Summerfield, 1996).

First-order landscape morphological distribution within the African continent is between the broadly elevated south and east, and the lower-lying north and west (King, 1967). Africa can be divided into high and low parts when a line is drawn across the map of Africa, from Angola to western Ethiopia. Low Africa, in the north-west, consists of sedimentary basins and upland plains mostly between 150 and 600 m above sea level, comprising the Sahara and the catchments of the lower Nile, Senegal, Niger, Chad and Zaire rivers. Land rising above 1000 m is restricted mainly to the Atlas Mountains in the Maghreb (northern part of Morocco, Algeria and Tunisia), the Sahara massifs, Ahaggar and Tibetsi, Jebel Marra in the Sudan Republic, the headwaters of the Niger, the Jos Plateau in Nigeria and the Cameroon highlands. To the south and east, almost all of High Africa rises above 1000 m, with the exception of Somalia, the broad lowlands of Mozambique as well as moderately narrow coastal plains and valley strips elsewhere. Even the Kalahari basin is about 1000 m above the sea and in east Africa the surface of Lake Victoria stands 1130 m above sea-level (White, 1983).

Africa's tectonic history did not result in the formation of great cordillera that acted as a major climatic divide, in contrast to the situation on the American continent with the Rocky and Andes Mountains and Eurasia with the Alps and Himalayas. The lack of widespread cordillera means that the climatic pattern of Africa, in many respects, resembles that of the 'ideal' or 'hypothetical' continent of many textbooks (Goudie, 1996).

A large part of Africa consists of elevated plateaux and these high altitudes play a very important role in decreasing temperatures over extensive areas. The high zonal index of Africa is the key feature resulting in temperature changes. A number of additional factors also play significant roles in temperature changes of the African continent.

Firstly, the symmetrical positioning of Africa with regard to latitude, extend almost equidistant from the Equator with its northern extremity at Cape Blanc (37° N) and southern extremity at Cape Agulhas (35° S). Roughly comparable series of climates can be traced northward and southward from the hot, moist Equatorial belt. Sudan has its complement in Zimbabwe, the Sahara in the arid tracts of the Namib and the Kalahari and the Mediterranean coast in the southwest of South Africa. Secondly, Africa is the most tropical of all the continents. Only the extremes reach far enough pole-wards to be directly influenced by the mid-latitude westerly winds and their accompanying instabilities. The Sahara and the belt of country to the south are warmer than any other part of the world of similar size. Thirdly, Africa is strongly influenced by the subtropical anticyclonic belts of both hemispheres and as a result retains widespread areas of dry climate to both the south and north (Goudie, 1996).

However, Africa has hemispherical asymmetry in climate, attributable to the fact that Africa north of the Equator is not only broader than Africa to the south of the Equator, but it also lacks a true ocean boundary to the north and northeast, being bordered instead by the great Eurasian continent. Another factor is the small annual variety of temperatures prevalent in Africa. Approximately one-third of Africa experiences an annual range lower than 6 °C. In the equatorial region there are particular areas where the range is lower than 3 °C. Mean monthly maximum temperatures are widespread and more than 32 °C, while approximately 30% of the area is subject to temperatures exceeding 38 °C. The highest temperatures occur in the Sahara. The average monthly minimum temperatures indicate the effects of latitude, where only relatively small areas in the interior of southern African and the northern Sahara and its margins have values lower than 5 °C. It is consequently clear that a large part of the continent experiences very high temperatures (Goudie, 1996).

Africa has an annual rainfall of approximately 725 mm, though there is a great range in precipitation with as little as 1 – 2 mm in the driest parts of the Libyan Desert to about 10 000 mm in the Mount Cameroon foothills. Africa exhibits significant deviancies from the standard world pattern of climatic and rainfall distribution (Trewartha, 1981). This can firstly be attributed to the uneven low rainfalls that occur over a large area of eastern tropical-equatorial Africa (especially in Somalia and Kenya) where, on the basis of a windward position and of uplands rising suddenly, one might have anticipated much wetter environments.

Secondly is the occurrence of the dry coastal belt in Ghana and Togo on the Gulf of Guinea in the central part of a coast which otherwise has a tropical wet climate. Thirdly is the area along the Guinea coast with a high-sun (with the sun being directly overhead) secondary rainfall minimum. A fourth anomaly is the restricted latitudinal spread of wet-and-dry climates to the north of the Equator in contrast to the south because of the unusual southward distribution of the Saharan dry climate (Goudie, 1996).

This environmental diversity, the result of Africa's abundant combinations of climatological, geological and pedological factors, is reflected in the production of Africa's fauna and flora. These communities have evolved over time as a result of this heterogeneity (Meadows, 1996). The flora itself, occurring as diverse vegetation types (rain forest, savanna, grassland and desert) with distinct distribution patterns, is an indication of enormous, often destructive, climatic changes in the past. This destruction explains Africa's relative floristic poverty in tropical regions, spectacular disjunction in vegetation distribution, close similarities among species now widely separated geographically and islands of floristic similarity now distantly isolated (Meadows, 1996).

During the late Cretaceous period (135 to 65 million years ago), Africa was still positioned to the south of its present position across the Equator and tropical conditions prevailed mainly in the northern part of the continent. Rain forest dominated the region up to 15° N and 15° S of the Equator, a tropical humid-climate vegetation formation, indicating that the flowering plants had replaced the gymnospermous *Glossopteris* flora completely (Axelrod and Raven, 1978).

The East African Mountains had still to be formed, so the extension of rain forest from coast to coast is a likely scenario, even though it retained only partial connection with the South American tropical flora after the separation from Africa during the early Cretaceous (Meadows, 1996). Another plant community present on the continent at this time was temperate rain forest, ample indications of which are found in Cretaceous-age fossil-bearing deposits, although, in southern Africa, it appears to have been species-poor and lacking the characteristic *Nothofagus* (southern beech) element (Van Steenis, 1972), characteristic of the rest of Gondwana at the time.

Additional seasonal climates also occurred at this time, which promoted the widespread occurrence of savanna woodland and sclerophyllous scrub, although severe aridity still had to develop in Africa (Meadows, 1996).

Africa was located approximately at its present latitude by the mid-Tertiary period, about 33.7 million years ago. The major changes this brought on relates to the 'migration' of the rain forest flora southwards to inhabit Central Africa, still connecting the west and east coasts (Meadows, 1996). The development of the East African Mountains led to the evolution of montane elements along the mountain spine. For the first time sclerophyll-dominated vegetation is found in the south-western parts of Africa, as verified by the fossil Banke flora at Arnot, in Namaqualand (Scholtz, 1985). Climates that were more seasonal also existed at this time. These supported the general occurrence of savanna woodland and sclerophyllous scrub, even though severe aridity still had not developed in Africa (Meadows, 1996). This is significant, for it documented the arrival of flora that has subsequently diversified to become one of the most species-rich in Africa, if not in the world (Linder et al., 1992).

Aridity was becoming a dominant environmental factor in parts of northern Africa (the proto-Sahara) and in southern Africa by the late Tertiary period (65 – 1.75 million years ago). The arid and semi-arid areas of southern Africa were established because of the isolation of Antarctica (Kennett, 1980), the growth of an Antarctic ice sheet with the resulting global drop in sea-level and the development of the cold Benguela current along the west coast (Siesser, 1980).

The cooler and drier climates reduced the area of rain forest, so much so that savannas became progressively more widely distributed in Africa (Axelrod and Raven, 1978). This change virtually eliminated the temperate rain forest flora from the continent.

Even though Africa is geologically one of the oldest continents and has a fossil biota going back to the origin of life itself, the major modern-day distribution patterns seem largely to have been determined by the dramatic events of the last 65 million years. The contrast between vegetation mapped as major biomes and mean annual precipitation shows prominent similarities and conformities, despite the obvious oversimplification in doing so.

The modern-day biomes of Africa therefore have a character which, with the exception of the Equatorial rain forests, is controlled to a greater or lesser degree by seasonal precipitation (Meadows, 1996).

The five major biomes of Africa can be categorized as tropical rain forest, tropical savannas (with or without an overstorey of trees), semi-arid and arid formations, areas of shrubland with a Mediterranean-type climate and the montane regions (Meadows, 1996).

In contrast to these five major biomes, White (1983) divides Africa into eighteen phytochorological units, based mainly on geography. The phytochoria are classified as Regional Centres of Endemism, Archipelago-like Centres of Endemism, Archipelago-like Centres of Extreme Floristic Impoverishment, Transitional Zones and Mosaics and are characterised in terms of the vegetation and rainfall (Table 2.1; Fig. 2.1). White (1979) defines a Regional Centre of Endemism as a phytochorion which has both more than 50% of its species confined to the area and a total of more than 1000 endemic species. The phytochoria regarded as regional centres of endemism fulfil these criteria with only the Sudanian Region, the status of which is still uncertain, as the exception. The regional centres of endemism are separated by transition zones. The Guinea-Congolia/Sudania, Guinea-Congolia/Zambezia and the Kalahari-Highveld Transition Zones are larger than some regional centres of endemism but have few endemic species and the majority of their species also occur in adjacent phytochoria (White, 1983).

(i) Guineo-Congolian Regional Centre of Endemism

Rain forest on well-drained sites and swamp forest on hydromorphic soils initially covered this area. At present little undisturbed rain forest remains while secondary grassland and various stages of forest regrowth are widespread. Small patches of edaphic grassland on certain hydromorphic and other soils not suited to the growth of trees are also present. Stunted forest and various types of bushland and thicket occur in upland areas, above about 1000 m, especially in rocky places. A number of Afromontane species are found in upland areas but it is only on the highest peaks such as Mount Cameroon that they form distinct Afromontane communities which are excluded from the Guineo-Congolian Region.

Table 2.1. Classification of the main phytochoria of Africa (White, 1983).

Regional Centres of Endemism	Archipelago-like Centre of Endemism	Archipelago-like Centre of Extreme Floristic Impoverishment	Regional Transition Zones	Mosaics
(i) Guineo-Congolian	(viii) Afromontane	(ix) Afroalpine	(x) Guinea-Congolia/Zambezia	(xii) Lake Victoria
(ii) Zambezian			(xi) Guinea-Congolia/Sudania	(xiii) Zanzibar-Inhambane
(iii) Sudanian			(xiv) Kalahari-Highveld	(xv) Tongaland-Pondoland
(iv) Somalia-Masai			(xvi) Sahel	
(v) Cape			(xvii) Sahara	
(vi) Karoo-Namib			(xviii) Mediterranean/Sahara	
(vii) Mediterranean				

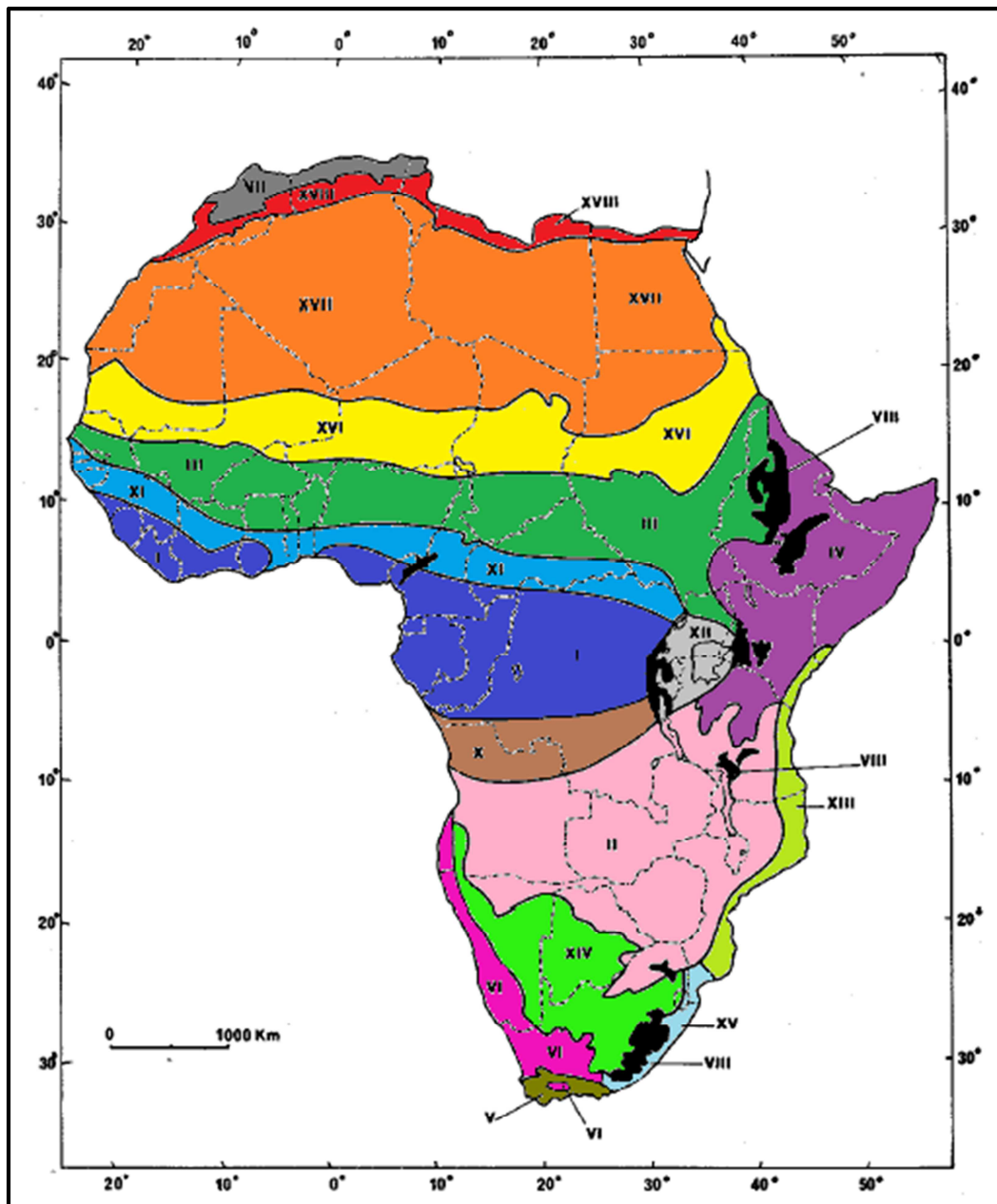


Fig. 2.1. White's (1983) vegetation map of Africa indicating the main phytochoria. (i) Guineo-Congolian Centre, (ii) Zambezian Centre, (iii) Sudanian Centre, (iv) Somalia-Masai Centre, (v) Cape Centre, (vi) Karoo-Namib Centre, (vii) Mediterranean Centre, (viii / ix) Afroalpine Archipelago-like Centre / Afroalpine Archipelago-like Region of Extreme Floristic Impoverishment, (x) Guinea-Congolia/Zambezia Transition Zone, (xi) Guinea-Congolia/Sudania Transition Zone, (xii) Lake Victoria Mosaic, (xiii) Zanzibar-Inhambane Mosaic, (xiv) Kalahari-Highveld transition zone, (xv) Tongaland-Pondoland Mosaic, (xvi) Sahel Transition Zone, (xvii) Sahara Transition Zone, (xviii) Mediterranean/Sahara Transition Zone.

Most of the Guineo-Congolian Region is relatively dry when compared to rain forests in other areas and receives between 1600 and 2000 mm of rainfall per year (White, 1983).

(ii) Zambeian Regional Centre of Endemism

The Zambeian Region is Africa's second largest major phytochorion and shows the widest range of vegetation types and probably the richest and most diversified flora. These vegetation types range from dry forest, swamp and riparian forest, transition woodland, woodland, thicket, scrub woodland to grassland. Almost the whole of the Zambeian Region lies within the tropical summer-rainfall zone, except towards the coast where the climate is continental in character with a noticeably larger seasonal variation in temperature than that of the Guineo-Congolian Region. There is a single rainy season, mainly from November to April, but in some places it may be interrupted by a dry spell lasting for two to three weeks. Rainfall in this region is between 500 and 1400 mm per year, decreasing from north to south, but there are distinct regional variations (White, 1983).

(iii) Sudanian Regional Centre of Endemism

The Sudanian Centre lies inside the northern tropical summer rainfall zone just like the Zambeian Region south of the Equator and the climates of the two are broadly similar especially with regard to rainfall, but temperatures in the Sudanian Region are appreciably higher and because of the harmattan wind, the dry season is more severe than in its southern counterpart. This Centre is bordered in the north by the deserts and semi-deserts of the Saharo-Sindian Region, in to the south it stretches to the tropical forests of the Guineo-Congo Region. The strongly seasonal climate is reflected in the variety of vegetation types from poor thorn scrub and savanna to quite rich deciduous woodland (White, 1983).

(iv) Somalia-Masai Regional Centre of Endemism

The biggest part of this region is covered with deciduous bushland and thicket which grade into and are replaced by semi-evergreen and evergreen bushland and thicket on the lower mountain slopes. There are smaller areas of scrub forest, riparian forest, secondary grassland and wooded grassland, seasonally waterlogged grassland, semi-desert grassland and shrubland and desert.

The climate is arid to semi-arid and rainfall is generally less than 500 mm per year and in some places as low as 20 mm. Throughout the Somalia-Masai Region there are great variations in rainfall from year to year. Most areas have two rainy seasons, separated by periods of drought due to the influence of the SW monsoon in summer and the NE monsoon in winter, but for the most part, these monsoons do not bring rain. Instead, rainfall occurs during the prevailing periods of calm (White, 1983).

(v) Cape Regional Centre of Endemism

Typical Cape vegetation does not inhabit the whole area. Large communities of Karoo and Afromontane vegetation and small patches of bushland of Tongaland-Pondoland affinity are also present. Rainfall ranges from 300 to 2500 mm, but in the mountains it can reach up to 5000 mm per year. The highest winter rainfall is usually during the winter months (White, 1983).

(vi) Karoo-Namib Regional Centre of Endemism

The vegetation of the Namib Desert ranges from Outer Namib fog desert, gravel desert, rocky outcrops, *Welwitschia mirabilis* transition zone to river-bed communities. The semi-desert vegetation of the Karoo ranges from shrubland, dwarf succulents and succulent shrubs, non-succulent bushes, bushy trees and tall shrubs, grasses, geophytes and annuals to riparian scrub forest. Rainfall in the Namib Desert is less than 100 mm per year and elsewhere it hardly ever exceeds 250 mm. The seasonality of rainfall varies greatly throughout this region and considerable variation in the amount and distribution of rainfall occur from year to year, particularly in the more arid parts. Even in the wetter part of the summer-rainfall belt, winter influences dominates this rainfall pattern approximately one in every twelve years (White, 1983).

(vii) Mediterranean Regional Centre of Endemism

A large part of the Maghreb (northern part of Morocco, Algeria and Tunisia) was formerly covered with forest, but on clay soils in the semi-arid scrub forest, which was then dominated by *Olea europaea* L. and various types of bushland or thicket. Where the Maghreb was not covered by forest, non-forest woody vegetation occurred and was confined to shallow soils, wind-swept ridges, coastal habitats and the summits of the higher mountains.

Precipitation occurs mainly in winter and range between 250 and 1000 mm per year. The summer is hot and dry and is more extreme than that of the Cape Region (White, 1983).

(viii and ix) Afromontane Archipelago-like Regional Centre of Endemism and Afroalpine Archipelago-like Region of Extreme Floristic Impoverishment

The Afroalpine Archipelago-like Region of Extreme Floristic Impoverishment is embedded within the Afromontane Region with a variety of vegetation occurring on the mountains. The vegetation types include forest, bamboo, evergreen bushland and thicket, shrubland, grassland and mixed Afroalpine communities. Very few species of the Afroalpine region are not shared with the Afromontane region. The climate is extremely varied. In the forest belt, mean annual rainfall is typically more than 1000 mm, but is less in drier types of transitional- to lowland vegetation. Above the forest belt precipitation lessens and in the Afroalpine belt of some mountains appears to be much less than 1000 mm per year (White, 1983).

(x) Guinea-Congolia/Zambezia Regional Transition Zone

The largest part of this Transition Zone is occupied at present by secondary grassland and wooded grassland dominated almost exclusively by Zambebian species. Other vegetation types include drier peripheral semi-evergreen rain forest, dry evergreen forest, transition woodland and coastal mosaic. In most of this region the climate is similar to that of the Guineo-Congolian and Zambezia Regions.

The dry season is more severe than in the Guineo-Congolian Region but less so than in the Zambebian Region. Rainfall declines rapidly near the Atlantic coast to below 800 mm per year, but relative humidity during the dry-season is high (White, 1983).

(xi) Guinea-Congolia/Sudania Regional Transition Zone

The larger part of this Transition Zone is covered with secondary grassland and secondary wooded grassland. Various types of forest were previously widespread in this area, but have been extensively destroyed by fire and cultivation, peripheral semi-evergreen rain forest being the only survivor. Almost everywhere the climate is intermediate between those of the Guineo-Congolian and Sudanian Regions.

A narrow strip of coastal plain in West Africa, extending from Ghana eastward to the Benin Republic, has an anomalously dry climate. In the driest part, near Accra, rainfall is only 733 mm per year (White, 1983).

(xii) Lake Victoria Regional Mosaic

This Regional Mosaic is the meeting-place of five distinct phytochoria, namely Guineo-Congolian, Sudanian, Zambezan, Somalia-Masai and Afromontane Regions. The vegetation comprises a mosaic of floristically poor variants of the characteristic vegetation of the first four regions which are semi-evergreen Guineo-Congolian rain forest, transitional rain forest, swamp forest and scrub forest and in some cases with an admixture of Afromontane species. Climatic gradients are frequently steep and are related to the complex physiography and the distance from Lake Victoria, which is a significant source of precipitation. Around the lake the rainfall is sufficiently high, 1500 to 2000 mm per year, and well distributed throughout the year to support the rain forest. More distanced from the lake, the rainfall is too low to support rain forest and not adequately seasonal to support woodland (White, 1983).

(xiii) Zanzibar-Inhambane Regional Mosaic

Forest once was the most widespread vegetation, but has largely been replaced by secondary wooded grassland and cultivation. There are also extensive areas of scrub forest, edaphic grassland and smaller areas of transition woodland, bushland and thicket. Rainfall is typically between 800 and 1200 mm per year and there is a well-defined dry season. Appreciably higher rainfall is experienced in a few places such as the East Usambara Mountains at Amani with 1946 mm and on the islands of Zanzibar and Pemba where 1964 mm falls at Wete. In these places the amount and distribution of precipitation are enough to support rain forest (White, 1983).

(xiv) Kalahari-Highveld Regional Transition Zone

This zone separates the Zambezan and Karoo-Namib Regional Centres of Endemism and occurs mainly on the great Interior Plateau of southern Africa. Vegetation is complex and can range from scrub forest, riparian scrub, rupicolous bushland and shrubland, wooded grassland to grassland.

Rainfall is in-between that of the Zambebian and Karoo-Namib Regions, ranging between 250 and 500 mm per year, increasing somewhat in the east towards the Drakensberg. Most of the rainfall occurs in summer (White, 1983).

(xv) Tongaland-Pondoland Regional Mosaic

Vegetation consists of a complex mosaic of forest, scrub forest, evergreen and semi-evergreen bushland and thicket in a matrix of secondary grassland and wooded grassland where it has not been completely destroyed. There are small patches of woodland in the north and edaphic grassland and swamp forest on the coastal plain. Due to the ameliorating effect of the warm Mozambique Current the coastal regions have a moderately high and well-distributed rainfall (White, 1983).

(xvi) Sahel Regional Transition Zone

The Sahel Zone occupies a band which stretches from the Atlantic Ocean on the east to the Red Sea on the west with the Saharan Desert that borders on the north and the Central African rainforests on the south. This band forms a flat or gently undulating landscape below 600 m. Wooded grassland in the south and semi-desert grassland in the north of the Sahel are supported by the extensive sand sheet. Bushland is much more restricted and is mostly confined to rocky outcrops. Numerous types of scrub forest and bushland also occur. Rainfall is unreliable and varies between 150 and 500 mm per year but rises to more than 1000 mm on Jebel Marra. Most rain falls in the three to four summer months, while the dry season is long and severe (White, 1983).

(xvii) Sahara Regional Transition Zone

The vegetation of this Transition Zone ranges from dwarf shrubland, grassland to absolute desert. Three climate zones, northern, central and southern, can be recognized on the basis of rainfall distribution. In the northern zone the rain falls during the cold season with two maxima, in autumn and spring. Even though rain falls every year, there is considerable variation from one year to the next both in distribution and amount. Rainfall declines rapidly toward the south. Rain is episodic in the central region, with the mean annual rainfall being less than 20 mm, except in the high mountains. The southern part receives more rain than the latter two regions and is subject to summer rain (White, 1983).

(xviii) Mediterranean/Sahara Regional Transition Zone

There is considerable change in the vegetation from west to east. In western Morocco the predominant types of vegetation are scrub forest and bushland. A mosaic of grassland dominates the landscape from eastern Morocco to Tunisia. Rainfall is between 100 and 250 mm per year. Precipitation is typically concentrated in the winter months, but in the rain shadow of the Atlas Mountains and on the High Plateau the main peaks are in spring and autumn, but the rainfall may be uneven through the year (White, 1983).

It is hypothesised that climatic stability is an important determinant of a high degree of endemism and that the degree of endemism is not coupled with high rainfall. It is also stated that areas that are consistently arid are just as likely to have endemics as areas that are consistently mesic (Linder, 2001).

When low levels of orbitally forced range dynamics (ORD) (climatic shifts leading to large changes in a species geographical distribution) occur it enables the survival of palaeoendemics as well as the preservation of genetic variation within those persisting populations, whereas high levels of ORD reduces endemism (Jansson, 2003). It is also said that the smaller the climatic shifts in an area, the more likely that neoendemic species will evolve and that it is more probable that new clades will persist and not go extinct (Jansson, 2003).

2.2. The family Apocynaceae

The Apocynaceae was first described by Jussieu in 1789 under the name Apocineae. Robert Brown (1810) separated the Asclepiadeae from Apocineae in his publication "On the Asclepiadeae". The main reason for this separation was the absence of translators (complex pollen carriers formed from hardened secretions of the style-head) and complex pollinia in the Apocineae and the presence thereof in the Asclepiadeae. This proved to be an incongruous character as demonstrated by Endress and Bruyns (2000) when they found that the presence and/or absence of pollinia and translators form a continuum between the two extremes in the modern Apocynaceae (Apocineae).

The relationship between the Apocynaceae and Asclepiadaceae, as defined by Brown (1810) has always been the subject of taxonomical controversy due to their obviously close relationship. Attempts to resolve this problem included combining them as an order separate from the Gentianales, as one family (Judd et al., 1994), or as a suborder within the Gentianales (Endress and Bruyns, 2000). After careful consideration of the distribution of character states of various morphological characteristics between the two families, Endress (1997) concluded that the delimitation between the two families is artificial, because there is a gradation of many character states from one family to the other. Endress and Bruyns (2000) therefore proposed that these two families be united.

Central in modern classification is the establishment of monophyletic taxa. Molecular analyses have indicated that the Apocynaceae *sensu stricto* (as in Brown, 1810) is paraphyletic when excluding the Asclepiadaceae (Judd et al., 1994; Civeyrel, 1996; Endress et al., 1996; Sennblad and Bremer, 1996; Sennblad et al., 1998; Endress and Bruyns, 2000; Sennblad and Bremer, 2000; Endress, 2001; Endress and Stevens, 2001). Combining the Apocynaceae *sensu stricto* and Asclepiadaceae into one family is the most effective way to achieve a monophyletic family. The cladistical results of Livshultz et al. (2007) also support this conclusion.

At present, the Apocynaceae is circumscribed in the sense of Jussieu, therefore including Brown's (1810) Asclepiadaceae and is referred to as the Apocynaceae *sensu lato* (Endress, 2004). For the sake of clarity, Brown's (1810) Apocynaceae is called the Apocynaceae *sensu stricto*.

Robert Brown was unquestionably one of the most significant people regarding the early taxonomy of the Apocynaceae. He described over 40 of the 53 genera comprising the Apocynaceae and Asclepiadaceae of the time, the majority of which are still valid today (Meve, 2002). Thus far, the estimated number of genera in this family has grown to 357 with roughly 5100 species (Nazar et al., 2013), more than seven times the number of genera and 30 times the number of species that was known to Robert Brown.

2.3. The Subfamily Periplocoideae

2.3.1. Circumscription of the Periplocoideae

Brown (1810) proceeded to subdivide his new family, Asclepiadeae (presently the Asclepiadaceae), into three groups or “tribes”, namely Periploceae, Asclepiadeae *verae* and an unnamed group containing only the genus *Secamone* R.Br., based on morphological differences and similarities, primarily the number of pollinia per flower and the type of translator. The ‘Periploceae’ was characterised by pollen clustered in tetrads (rarely in pollinia), each anther containing four pollen sacs and the tetrads (or two pollinia) from the thecae of two adjacent anthers deposited onto a spoon-like translator.

The unnamed group containing *Secamone* differed in that pollen occur in pollinia, each anther is divided into four pollen sacs, producing four pollinia, and two pollinia from each of the thecae of two adjacent anthers are attached onto a clamp-like translator. The ‘Asclepiadeae *verae*’ also has pollen in pollinia, but each anther has only two pollen sacs producing two pollinia. One pollinium from each of the thecae of two adjacent anthers is attached onto a clamp-like translator.

Schlechter (1905) elevated Brown’s Periploceae to family level with the name Periplocaceae. Botanists such as Bullock (1956), Kunze (1990; 1993), Dave and Kuriachen (1991), Liede and Kunze (1993), Nilsson et al. (1993) and Swarupanandan et al. (1996), concurred with the idea that the Periplocaceae is a separate family, closely related to the Asclepiadaceae.

However, after extensive morphological research, Endress and Bruyns (2000) concluded that there is little support for separating the Periplocaceae from the remainder of the ascleps at family level. They proposed that the Periplocaceae be reduced to subfamily status within the Asclepiadaceae. Molecular studies using the *rbcL* gene region did not support the monophyly of the Asclepiadaceae and Periplocaceae as separate families but rather indicated a close relationship between the Apocynaceae *sensu stricto* and Periplocaceae (Kunze, 1990; 1996; Judd et al., 1994; Struwe et al., 1994; Sennblad and Bremer, 1996; Endress, 1997; Sennblad, 1997).

The bulk of the new information on relationships among genera is based on DNA data, especially since it has continually revealed convergences that were not realised as such in earlier classifications based on morphological data alone (Endress, 2004).

Various phylogenetic analyses did support the grouping of the genera of the Apocynaceae *sensu lato* into five subfamilies in accordance with Endress and Bruyns (2000), as well as the monophyly of the Periplocoideae (Sennblad and Bremer, 1996; Civeyrel et al., 1998; Potgieter and Albert, 2001; Ionta and Judd, 2007; Lahaye et al., 2007; Ionta, 2009). However, the studies of Sennblad and Bremer (1996), Potgieter and Albert (2001) and Livshultz et al., 2007 also show that the Periplocoideae are likely nested within the paraphyletic Apocynoideae clade.

Fishbein's study (2001), based on the chloroplast *matK* gene, confirms the monophyly of the subfamilies where he assumes a closer relationship of Periplocoideae to non-asclepiad Apocynaceae than to the remainder of the Asclepiadaceae.

Today, two centuries later, Brown's original three groups within the Asclepiadaceae are thus reinstated and defined using his morphological criteria, supported by molecular data as representing monophyletical groups or subfamilies, these being the Periplocoideae, Secamonoideae and Asclepiadoideae (Endress, 2001; 2004).

Periploca (Linnaeus, 1753) was the first genus described in the Periplocoideae. Brown (1810; 1819) then added *Cryptolepis*, *Gymnanthera*, *Hemidesmus* and *Cryptostegia*, Blume (1825 – 1826; 1828) added *Leposma*, *Phyllanthera* and *Lepistoma*, Wallich (1832) contributed *Finlaysonia*, while Wight and Arnott (Wight, 1834) added *Decalepis* and *Streptocaulon*. With the exception of *Periploca*, all these genera were described from specimens collected in India and Indonesia. The first genera described from Africa were *Ectadium* by E. Meyer (1837), *Raphionacme* by Harvey (1842) and *Aechmolepis*, *Tacazzea* and *Suchellia* by Decaisne (1844). From Madagascar, the third significant centre of diversity for the Periplocoideae, Decaisne (1844) described *Camptocarpus*, *Harpanema* and *Pentopetia*.

To date, 86 generic names by 30 authors have been contributed to the Periplocoideae. The number of recognized genera in this subfamily has changed considerably throughout the years and is still in flux. Venter and Verhoeven (1997) proposed a tribal classification of the Periplocoideae that included 44 genera. Since then two of the smaller genera were placed in synonymy by Klackenberg (1997) and Venter and Verhoeven (2001), reducing the number of genera to 42. The number of species is now approximately 190, so that the number of genera, approximately 42, is proportionally high. Given the small size of the subfamily, an excessively high number of monotypic and ditypic genera have been described. About 117 species belongs to six major genera, while most of the other genera are uni- or bispecific. The largest genera are *Raphionacme* (36 species and 2 subspecies), *Cryptolepis* R.Br. (30 species), *Pentopetia* Decne. (23 species), *Periploca* (13 species), *Camptocarpus* Decne. (9 species) and *Streptocaulon* Wight & Arn. (9 species) (Venter, 2009).

These changes may be attributable to a variety of factors such as the significant morphological diversity of the group, the undercollection of numerous taxa (particularly in Asia) which are therefore poorly known (Venter and Verhoeven, 1997; Meve and Liede, 2004) and the small yet highly complex flowers that are difficult to interpret from herbarium material.

2.3.2. Distribution and habitat of the Periplocoideae

When looking at the distribution of the Periplocoideae, this subfamily is limited to the Old World, which is in Africa, Madagascar, Europe, Asia and Australia, (Venter et al., 1990a; Venter, 1997; Venter and Verhoeven, 1997). Most of the genera and species are woody climbers, some very large (*Mondia* Skeels, *Myriopteron* Griff. and *Tacazzea*), inhabiting tropical rain and swamp forests or, rarely, temperate forests. A small number of species, such as *Raphionacme galpinii* and *R. hirsuta*, inhabit the grasslands of Africa and a few are erect or straggling shrubs and are found in arid semi-desert and desert habitats (e.g., the three *Ectadium* species, *Periploca aphylla* Decne., *P. visciformis* (Vatke) K.Schum., *Raphionacme haeneliae* and *R. namibiana* (Venter and Verhoeven, 1986; 1996; Venter et al., 1990b; Venter, 1997).

Interestingly, no members of the Periplocoideae inhabit the South African Cape Floral Kingdom with its winter rainfall. Nevertheless, a few species, like *Periploca angustifolia* Labill. and *P. gracilis* Boiss., occur in the Mediterranean macchia of North Africa and Europe (Venter, 1997).

Periplocoideae are never dominant in the plant communities where they occur, although lianas like *Mondia* and *Tacazzea* may be prominent because of their size. The taxa are often widely distributed, but large numbers of individual plants seldomly occur (Venter and Verhoeven, 1988).

The exception is *Cryptostegia grandiflora* Roxb. ex R.Br. of Madagascar, which has become common in disturbed locations in Madagascar. In Queensland, Australia, it has become a major noxious weed of grazing lands (Marohasy and Forster, 1991).

2.4. The genus *Raphionacme*

2.4.1. Historical notes on *Raphionacme*

The name *Raphionacme* was established by William Henry Harvey in 1842 when he described a number of new genera from South Africa. He gave no indication as to the origin of the name, but *Raphionacme* could be an inflection of *Raphanus* (radish) due to the similarity between the tubers of the two genera (Venter, 2009).

Variations of this generic name have been proposed, for instance *Rhaphiacme* or *Raphiacme* (Schumann, 1893; 1895) and *Rhaphidonacme* or *Raphidacme*, but the International Code of Botanical Nomenclature (ICBN) (Greuter et al., 2000) confirmed the name *Raphionacme*.

Harvey described two species, *R. zeyheri* and *R. divaricata*, in his new genus *Raphionacme*. In his revisions of *Raphionacme*, N.E. Brown (1902; 1907) regarded an older species, *Brachystelma hirsutum* E.Mey., as synonymous with *R. divaricata* Harv., a dubious conclusion (Venter, 2009). However, Dyer (1942) accepted Brown's synonymy and *B. hirsutum* E.Mey. being the basionym, he changed the species name to *R. hirsuta*. Phillips (1951) then designated *R. hirsuta* as the generic type.

Raphionacme is the largest genus in the Periplocoideae. Various authors have described new *Raphionacme* species with the main contributions by N.E. Brown, F.F.R. Schlechter, H.J.T. Venter and R.L. Verhoeven and to a lesser extent S. Moore, K. Schumann and A.A. Bullock, resulting in about 70 published species names. According to the latest revision by Venter (2009) the genus consists of 36 species and 2 subspecies, with the other names placed in synonymy.

2.4.2. Sectional classification of *Raphionacme*

Raphionacme was divided into four sections by Schumann (1895) based on the ten species known to him. When Venter and Verhoeven (1988) classified the 31 species of *Raphionacme* known at the time, they used the names given by Schumann (1895) and amended the descriptions of the sections (Table 2.2). These sections were based on morphological structure of inflorescences, flowers and growth habit.

2.4.3. Distribution and habitat of *Raphionacme*

Raphionacme is virtually endemic to Africa, with only one species, *R. arabica*, occurring on the Arabian Peninsula. *Raphionacme* is absent from the Sahara Desert, the winter rainfall region of the western/southwestern Cape Region of South Africa, the very wet tropical forests of western Africa and virtually excluded from the Mediterranean winter rainfall region of North Africa (except two collections of *R. splendens*). The highest concentration of *Raphionacme* species is found between 15 – 20° S and 25 – 35° E, comprising northern Botswana, Zimbabwe, southwestern Zambia and northwestern Mozambique (Venter, 2009).

In his biogeographical analysis of the flora of Oman, Ghazanfar (1992) uses White's (1983) classification and Leonard's (1988) extension of the phytochoria in Asia. The Dhofar Mountains in the southeast of Yemen form a steep, arid escarpment rising above the coastal plain bordering the Arabian Sea.

Table 2.2. *Raphionacme* species within Venter and Verhoeven's (1988) four sections.

<i>Raphionacme</i> sect. <i>Raphionacme</i>	<i>Raphionacme</i> sect. <i>Cephalacme</i> K.Schum.	<i>Raphionacme</i> sect. <i>Speiracme</i> K.Schum.	<i>Raphionacme</i> sect. <i>Pseudochironia</i> K.Schum.
<i>R. dyeri</i>	<i>R. angolensis</i>	<i>R. flanagani</i>	<i>R. bingeri</i>
<i>R. hirsuta</i>	<i>R. galpinii</i>	<i>R. keayii</i>	<i>R. brownii</i>
<i>R. lanceolata</i>	<i>R. globosa</i>	<i>R. longifolia</i>	<i>R. caerulea</i>
<i>R. longituba</i>	<i>R. utilis</i>	<i>R. monteiroae</i>	<i>R. chimanimaniana</i>
<i>R. madiensis</i>	<i>R. vignei</i>	<i>R. procumbens</i>	<i>R. excisa</i>
<i>R. palustris</i>		<i>R. sylvicola</i>	<i>R. grandiflora</i>
<i>R. velutina</i>		<i>R. welwitschii</i>	<i>R. linearis</i>
<i>R. zeyheri</i>			<i>R. michelii</i>
			<i>R. namibiana</i>
			<i>R. splendens</i>
			<i>R. sp. nov. = R. borenensis</i>

The climate and floristic composition correspond to the Somalia-Masai Regional Centre of Endemism of Africa (White, 1983) and is also influenced by the southwestern Monsoon (Ghazanfar, 1992). It would seem as if *Raphionacme arabica* forms part of the concentration of endemic species found in the escarpment woodlands of Dhofar.

Raphionacme plants are usually found in savanna and grassland where the climate ranges from arid to seasonally wet (Venter and Verhoeven, 1988; Venter, 2009). A few exceptional species can be found in seasonally dry swamps (*R. linearis*, *R. palustris* and *R. splendens*) (Venter, 2009) or moist and dense escarpment woodland but with a marked dry season (*R. sylvicola*) (Pers. com. Dr David Goyder, Herbarium Royal Botanic Gardens, Kew, Richmond, United Kingdom). The members of the genus are always lesser components of the communities in which they occur (Venter and Verhoeven, 1988; Venter, 2009).

2.4.4. Morphology of *Raphionacme*

The genus consists of latex bearing geophytes, with most species being prostrate or erect herbs and a small number being climbers (Venter, 2009). A single subterranean taproot tuber is usually turnip-shaped, with one to a few subterranean, erect, perennial stems occurring at the crown of the tuber.

Annual (rarely perennial) aerial stems develop from these subterranean stems and can be twining, erect or procumbent, with dichotomous or lateral branching. Interpetiolar ridges with reddish turbinate colleters occur on the stems.

Opposite leaves are sessile to petiolate, with blades broadly to narrowly ovate, elliptic, obovate or linear. Leaf texture varies from herbaceous to leathery, the surface can be glabrous or hairy, the main veins are prominently visible abaxially and the secondary veins are arching, divaricate or patent.

The cymose inflorescences occur terminally or pseudo-axillary and can be raceme-like, plume-like, corymbose-like or globose in appearance, either few or many flowered, lax or compact.

The species of *Raphionacme* are distinguishable by their floral morphology. Flowers are typically pentamerous and actinomorphic, bisexual and semi-epigynous. The free sepals may or may not have paired colleters at inner bases with the sepal shapes ranging from ovate to triangular. In most species the corolla is glabrous inside but can be either glabrous or hairy outside. The corolla is divided into a distinct upper tube (that is the portion above the circle of nectaries) and a very short lower tube that is annular around the upper half of the ovaries. The shape of the upper tube varies from campanulate to cylindrical with the inner face vertically fluted due to coronal ridges. The corona lobes, which can be spreading to reflexed, are ovate, obovate or triangular. The corona arises from the corolla mouth with the lobes opposite the sepals. The corona consists of five lobes, borne in the corolla mouth, attached to coronal feet, fused to the corolla just below the corolla lobe sinuses. The simple or variously incised corona can be glabrous or hairy. Five stamens, that dehisce laterotrorsely with full or terminal half-length slits, arise from the coronal feet and are glabrous with free filaments. A gynostegium is formed by ovate, oblong-ovate, triangular or hastate shaped anthers which are fused to the style-head. The gynostegium is found in the corolla mouth or could be elevated above it. Pollen is usually in tetrads with single grains being 8 – 16-porate. Pocket-like, deep green nectaries are found at the base of the upper corolla tube and are fused to the coronal feet via coronal ridges. The two semi-inferior ovaries are unilocular and many ovuled. The two styles are basally free, but fuse into one compound style that is terete or rarely fluted, glabrous or hairy and becomes terminally enlarged to form a pentagonal style-head which is broadly ovoid, oblong-ovoid or deltoid.

Five translators, each consisting of a receptacle (spoon-like structure) with a stalk and viscous disc, are embedded into the upper surface of the style-head. The follicles are erect or divaricate, narrowly ovoid to cylindrical and can be single or in pairs. A coma or a ring of hairs occur on the obliquely ovate seeds. (Venter, 2009)

2.5. Molecular analysis

2.5.1. Ribosomal DNA (rDNA)

Multiple copies of ribosomes are important for the functioning of organisms. The ribosome genes exist in tandem arrays composed of thousands of copies per array. The ribosomes translate mRNA to build polypeptide chains, thus, making the ribosomes important structures in a cell (Baldwin et al., 1995; Wendel et al., 1995; Cronn et al., 1996; Soltis et al., 1997; Kuzoff et al., 1998; Soltis and Soltis, 1998; Small et al., 2004; Poczai and Hyvönen, 2010).

The rDNA locus have virtually the same structure within a wide variety of taxa, but due to functional constraints, it is commonly assumed that all ribosomal copies present in the genome have fairly identical sequences (Small et al., 2004). Coding regions, like the small and large subunit genes of the ribosomes, signify some of the most conserved sequences in eukaryotes as a result of a strong selection for the regions to prevent against any loss of function (Hillis and Dixon, 1991; Poczai and Hyvönen, 2010).

Since the specific regions of the rDNA loci are conserved differently, the sequence information from the different rDNA locus can be utilized at different taxonomic levels. The internal spacer regions can be beneficial from generic to species level and are now commonly used in phylogenetic studies (Poczai and Hyvönen, 2010).

2.5.2. Characterising the Internal Transcribed Spacer (ITS)

The ITS region is located in the 18S-5.8S-26S region and consists of three components: two spacer regions (ITS-1 and ITS-2) and a highly conserved 5.8S rDNA exon (Fig. 2.2) (Baldwin et al., 1995; Li et al., 2011). The spacer sequences of ITS-1 and ITS-2 are both incorporated into the mature ribosome. The ribosomal RNA undergoes a specific splicing process during maturation, before mRNA translation (Poczai and Hyvönen, 2010).

The 5.8S subunit is almost constant in length (mostly 163 or 164 bp) in reported angiosperms. The whole ITS region in angiosperms seems to be between 500 – 750 bp in length. In other seed plants, it can be up to 1500 – 3500 bp.

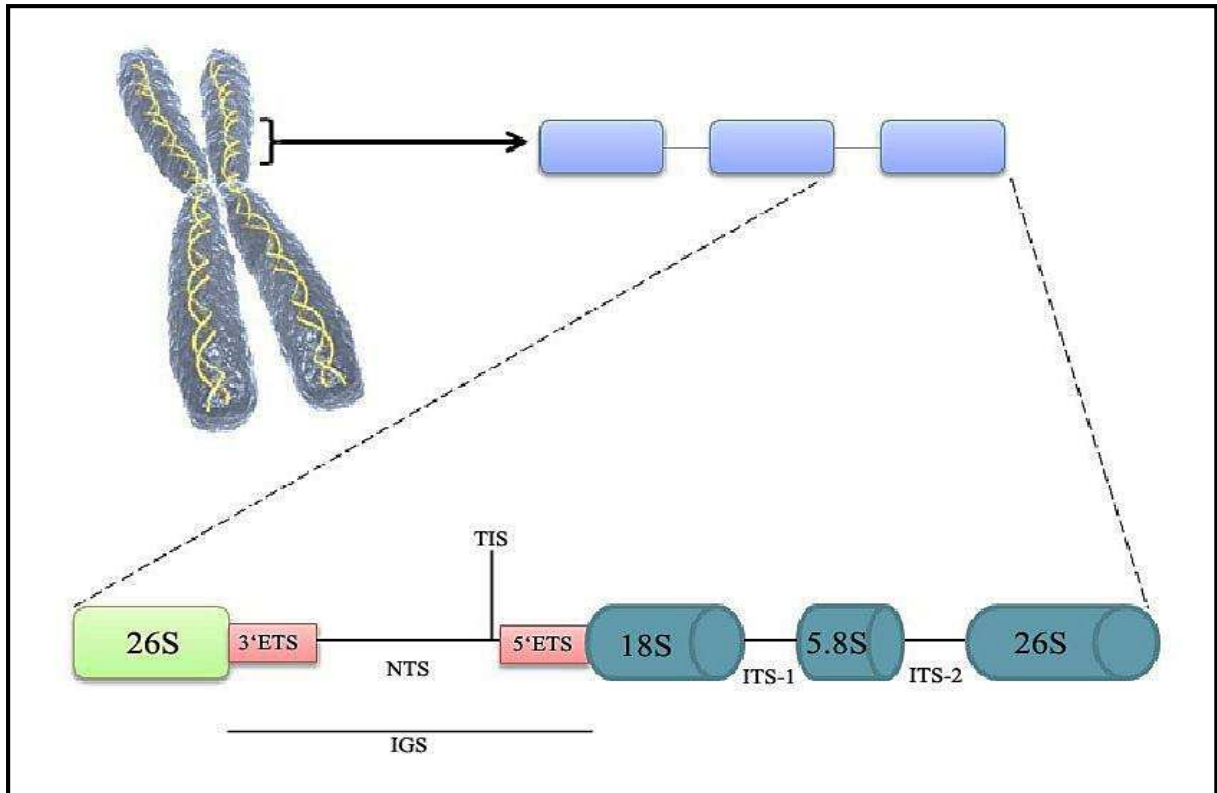


Fig. 2.2. Schematic representation of the universal structure of the rDNA region in plants as adopted from Poczai and Hyvönen (2010). The small subunit (18S) and large subunit genes (5.8S and 26S) are separated by the internal transcribed spacer 1 (ITS-1) and internal transcribed spacer 2 (ITS-2).

The lengths of ITS-1 and ITS-2 are between 187 – 298 bp and 187 – 252 bp respectively in all flowering plants reported to date. Much longer spacers are found in various eukaryotes (Baldwin et al., 1995; Poczai and Hyvönen, 2010). The relative sizes of ITS-1 and ITS-2 can differ between and within families, with little or no indication of a broad-scale phylogenetic pattern. It is also found that ITS-1 is consistently longer than ITS-2 (Baldwin et al., 1995).

According to Poczai and Hyvönen (2010), the ITS region evolve at a faster rate compared to coding regions like the small- and large subunit genes. Thus, ITS can be widely used as a marker for phylogenetic reconstruction at different levels. Baldwin et al. (1995) stated that the ITS sequences have evolved at a slower rate in some ancient woody groups than in herbaceous, mostly annual groups of fairly recent origin. Compared to the 5.8S gene, ITS-1 and ITS-2 are significantly more variable in primary sequence. Furthermore, ITS-2 is slightly more conserved than ITS-1 and can allow for alignments in ranks above the genus level (HersHKovitz and Lewis, 1996; Calonje et al., 2009).

It is recognized that ITS-1 is more variable than ITS-2 (Poczai and Hyvönen, 2010). This is due to the two spacers having different evolution rates for nucleotide substitution and the rates appear to vary from species to species. Thus, a universal rate cannot be applied to plants, it must be modified and determined for each genus. The variation between the two spacers is due to their function and role in the ribosome biogenesis. ITS-2 is more significant in the development of the mature ribosome and its sequence is therefore more conserved than that of ITS-1.

Although a universal rate of substitution cannot be determined, a mean substitution rate for ITS was determined to be about 2.86×10^{-9} substitutions/site/year, which is already among the highest known in plants (Kay et al., 2006; Calonje et al., 2009).

Liu and Schardl (1994) identified a 20 bp region in the inferred secondary structure of ITS-1; which is highly conserved amongst angiosperms. This was confirmed by Goertzen et al. (2003). Currently there are no reports of other conserved motifs within the structure of ITS-1. The small number of nucleotide positions available for analysis in both spacers is compensated for by the high levels of variation found in ITS-1 and ITS-2 (Baldwin et al., 1995).

The high interspecific divergence and low intraspecific variation of the ITS-2 region are key advantages for the distinction between closely related species, and some of these variations were potentially informative for resolving relationships among subspecies (Baldwin et al., 1995; Li et al., 2011).

Complementarity of ITS-1 and ITS-2 data sets was further indicated by more comprehensive and robust resolution in trees based on joint data than in trees on either ITS-1 or ITS-2 alone (Baldwin et al., 1995). No discordance between ITS-1 and ITS-2 data sets in angiosperms has been observed. These two spacers appear to be particularly good examples of “homogeneous” sets of characters that have evolved similarly and are best analysed together for maximal phylogenetic resolution and support (Bull et al., 1993; Poczai and Hyvönen, 2010).

2.5.3. Complications with ITS

Limitations of the ITS region in particular taxa are well-known. These include species-level variability, cloning of numerous copies due to divergent paralogues and poor quality of data due to secondary structure problems (Baldwin et al., 1995; Álvarez and Wendel, 2003; Kress et al., 2005). Recurrent DNA, like ITS, might experience non-Mendelian transference and cause this region to undergo concerted evolution and/or be transportable within a genome (Small et al., 2004).

The diverse sequences acquired from both parents could be standardized by concerted evolution of the nuclear ribosomal DNA (nrDNA) gene family. Nuclear ribosomal DNA is not a reliable marker even though it is biparentally inherited (Wendel et al., 1995; Sang, 2002).

For phylogenetic reconstruction, ITS or any rapidly evolving non-coding region, can involve intricate sequence alignment for homology assessments (Kress et al., 2005).

Sequence alignment in the ITS region can be promoted by the combination of conserved and variable positions through both spacers. It was found that in both ITS-1 and ITS-2 at least one region appears to have experienced relaxed evolutionary limitation on length in most groups (Baldwin et al., 1995).

Because of independent and matching length mutations (indels) that have come about in different taxa, definite alignment of sequences in these segments may be impossible.

According to Baldwin et al. (1995), insertions and deletions (indels) complicate alignment of the ITS region. Gaps are inserted in the alignment to conserve positional homologies of nucleotides. The location of gaps in most indel sections is shown clearly due to the flanking conserved site. The greatest amount of deviation between angiosperm ITS sequences is accredited to point mutations instead of length mutations. The huge amount of site-substitutions compared to indel mutations and the interspersed nature of conserved and variable sites, support ITS sequence alignment and the use of ITS indels as characters in phylogenetic analysis (Baldwin et al., 1995; Poczai and Hyvönen, 2010). Because of the limited number of indels found in plants and fungi, alignments are trivial to optimize for 5.8S (Hershkovitz and Lewis, 1996).

Another problem using the ITS region is the amplification of fungal DNA when using universal primers. The fungal contamination can be as a result of fungi growing on the plant before or after collection (during the drying process in the herbarium) or endophytes within the leaves. Phylogenetic analyses based on the 5.8S sequences indicated that the fungi were most likely basidiomycetes (Zhang et al., 1997). This problem can be overcome by generating plant-specific primers (Cullings and Vogler, 1998; Kress et al., 2005; Li et al., 2011).

2.5.4. The value of the ITS region

ITS was first used by Porter and Collins (1991) to reconstruct phylogenies and hence became widely used thereafter. The two most widely used non-coding DNA marker systems are the nuclear ribosomal encoded ITS and the plastid *trnL-F* region (Calonje et al., 2009).

Baldwin et al. (1995) found that the ITS region present distinct benefits for evolutionary studies. ITS is a particular substantial resource of angiosperm systematics due to its immeasurable properties for phylogenetic studies of closely related species and the absence of other identified nuclear DNA regions.

This is verified by the fact that the ITS region can provide valuable characters for use in lower level phylogenetic questions in flowering plants.

The ITS nrDNA is present in virtually all organisms as part of a transcriptional unit (Calonje et al., 2009). Refinement on species-level and practical ease have been confirmed in a large number of phylogenetic studies that made use of ITS. Sequence data already exists for this nrDNA region (>172 000 unfiltered angiosperm sequences were obtainable from GenBank in April 2013 but due to the sequences not being filtered for taxa, it is unclear how many species are represented) (Kress et al., 2005).

Of the multitude nuclear regions available ITS is being used extensively in systematic and phylogenetic treatments (Stoeckle, 2003; Chase et al., 2005; Kress et al., 2005), often discerning successfully between species (Newmaster et al., 2006).

At lower taxonomic levels, internal and external intergenic spacers are more frequently used (Baldwin et al., 1995; Álvarez and Wendel, 2003; Bailey et al., 2003; Small et al., 2004). A particularly affirmative role should be played by ITS data in angiosperm studies by offering independent evaluation of lower-level phylogenetic hypotheses built on morphology or chloroplast DNA proof (Baldwin et al., 1995). The ITS region can be amplified in two smaller fragments (ITS-1 and ITS-2), which was proven particularly useful for degraded samples, such as old herbarium material (Baldwin et al., 1995; Poczai and Hyvönen, 2010).

For order- and division discrimination, the moderately conserved 5.8S region has sufficient phylogenetic signal (Cullings and Vogler, 1998; Kress et al., 2005). In angiosperms the ITS sequences are phylogenetically valuable at numerous intrafamilial levels (Baldwin et al., 1995).

Depending on the lineage, ITS is not likely to preserve adequate evolutionary signal or alignability for examining relationships between species in different plant families. The 5.8S locus aids as an acute alignment-free anchor point for search algorithms.

The practicality of conserved regions to generate a pool of nearest neighbours for refined comparisons will be critical for effective database searches, especially when comparing a sequence that has no identical matches in a sequence library (Kress et al., 2005). ITS sequences demonstrate certain promise for investigations of relationships between allopatric or disjunct populations within species. For example, up to 3.7% ITS sequence divergence was found between individuals from conspecific, allopatric populations in *Calycadenia* DC. (Baldwin, 1993).

2.6. Morphological analysis

2.6.1. The value and application of morphological data

Most of the present knowledge about phylogeny was derived primarily from morphological analyses as interpreted by many generations of taxonomists. These taxonomists developed the concepts of groups or taxa, which they acknowledged in classifications, due to the coincidence of some morphological similarities being synapomorphies (Scotland et al., 2003; Pisani et al., 2007).

One of the greatest advantages of morphological over molecular approaches to systematics, is the much greater applicability of the former approach to the extensive collections of preserved specimens in museums and herbaria. For many groups of poorly known organisms, the only known specimens of species are denoted by the holotype specimen. Further collection of such species can be prohibitive due to scarcity of the species, remoteness of the habitat, destruction of identified collection localities and legal protection of the habitat or species (Hillis, 1987).

Most taxonomic studies based on morphology include large samples of each species throughout its distribution range and the examination of all known specimens so as to define the extent of intraspecific variation of morphological characters. Even though general geographical analyses exist for some molecular characters, sample sizes in molecular studies are considerably smaller than in morphological studies, often as small as a single individual (Hillis, 1987). Taxonomists thus have a collection of complex morphological characters upon which to base their conclusions, rather than relying on part of a single gene.

Although morphological data sets usually comprise relatively small numbers of characters in contrast to the total amount of included molecular data, Baker et al. (1998) and Meier and Baker (2002) found that morphology may add more support and stability to the combined analysis when considered per character than molecular data alone. Through a comprehensive range of different taxa they also determined morphology to be up to twice as informative per included character in a combined analysis as molecules.

Morphological evidence may appear to be somewhat incongruent with molecular data when analysed separately, but when combining morphology and molecular data, the total data interact to produce a new and well-supported phylogeny. Morphology may contribute to the total evidence analyses in the form of hidden clade support, only becoming apparent when data sets are combined (Jenner, 2003). Results of Pisani et al. (2007) suggest that both molecular and morphological trees are valuable estimates of a common underlying phylogeny. Thus, when molecular and morphological data are incongruent, molecular phylogenies should not be considered more reliable.

Serious errors are seen in the reasoning, methodology and interpretation of results excluding morphological studies in favour of a narrow and exclusive molecular identification system. If a program was instigated at the expense of morphological systematics, as recommended by Hebert et al. (2003), there would be severe negative consequences for accurate biodiversity assessments (Kipling and Rubinoff, 2004).

Establishing monophyletic groups is one of the main aims of systematics, but finding morphological characters supporting those groups is just as significant. Even though no uncertainty exist that molecular data obscures morphological data when it comes to the construction of a robust phylogeny, a phylogenetic hypothesis founded entirely on molecular data is too narrow (Schols et al., 2004).

Our taxonomic concepts are, or should be, built on a constellation of data, based on not only molecular analyses, but also morphology which in part is often gathered by non-systematists during their investigations. Reciprocity between morphological and molecular data may slow the progress, but the ultimate products are of greater value (Kipling and Rubinoff, 2004).

2.6.1. Problems with and restrictions of morphological data

A serious restriction with morphological data within the phylogenomic context is that only a few morphological character sites exist, compared to an increasingly number of molecular character sites used to construct phylogenies (Dunn et al., 2008).

Another problem is that numerous aspects of morphological character analysis are debatable. Smith (1994) explains that morphological characters are perceived and defined in different ways by different taxonomists. This difficulty of character definition and coding of morphological features leads to variances in character concepts which present a further level of ambiguity regarding morphological data analyses and phylogenetic reconstruction (Scotland et al., 2003).

Most morphological characters are quantitative data which are frequently divided into arbitrary states (e.g. leaves longer than 5 cm and leaves shorter than 5 cm) or described with a vocabulary that hides the quantitative nature of the data (e.g. leaf surface reticulate) so as to avoid coding difficulties. Other problems may include the way in which characters are constructed, whether intraspecifically variable characters can be included, how within-species variation is coded and how the character states are ordered (Slowinski, 1993; Wiens, 1995; Rae, 1998; Lee and Bryant, 1999; Strong and Lipscomb, 1999; Smith and Gutberlet, 2001). These coding methods are somewhat challenging to reproduce and they disregard a lot of information contained within the data (Scotland et al., 2003; Schols et al., 2004). Different choices and assumptions are significant since they can lead to completely contradictory trees (Wiens, 2001).

Morphological character analysis involves substantial effort, involving countless methodological decisions and implicit assumptions during the process. This is in contrast to the analyses of molecular data, in which the designation of characters and character states is nearly automatic (Wiens, 2001; Schols et al., 2004).

Even though related problems of homology assessment occurs for molecular data relative to problems such as alignment (Goldman, 1998; Simmons and Ochoterena, 2000), the critical issue for morphology is that the already small quantity of morphological characters is further compromised by ambiguous homology assessment (Scotland et al., 2003).

Our present inability to combine models of morphological evolution into phylogeny reconstruction methods confines the range of techniques available for analyses of matrices comprising only morphological data (Scotland et al., 2003).

2.7. Phylogenetics

Sennblad and Bremer (2000) did a comprehensive molecular survey that included at least one taxon from all recognized tribes of the combined Apocynaceae *sensu stricto*-Asclepiadaceae, but support values for many of the clades were low and relationships between the clades were mainly unresolved.

Potgieter and Albert (2001) contended that the monophyly of the combined Apocynaceae *sensu stricto*-Asclepiadaceae had not been adequately tested as many relevant taxa were not considered by the earlier cladistical analyses.

The molecular phylogenies of the Apocynaceae *sensu stricto*-Asclepiadaceae combination have been based on chloroplast DNA (*rbcL*, *matK*, *trnL*), either singly, combined or in conjunction with morphological data (Potgieter and Albert, 2001; Sennblad and Bremer, 2002; Endress, 2004). However, it would seem that these gene regions are evolving too slowly to be used alone for determining relationships. The next logical step is to sequence nuclear genes and combine that with the chloroplast DNA data (Endress, 2004).

The most comprehensive phylogenetic study of Apocynaceae *sensu lato* was carried out by Potgieter and Albert (2001). The study was based on the *trnL* intron, *trnL*-F spacer gene regions and propagule characters of 147 species. The Apocynaceae was found to be a monophyletic group as well as the subfamily Periplocoideae represented by six species (five genera).

The findings of this study supported that of Civeyrel et al. (1998) based on *matK* data and was then further supported by the results obtained by Sennblad and Bremer (2002) which used *rbcL* and *ndhF* data.

The most comprehensive phylogenetic study of Periplocoideae was carried out by Ionta (2009). This study was based on the analyses of *trnT-F*, *trnD-T*, *ycf1* and morphological characters of 32 taxa. Periplocoideae was again found to be a monophyletic group. The Raphionacme-clade included *Raphionacme* (represented by 16 of the 36 species) and *Schlechterella* (represented by two species). These results support the findings of Meve and Liede (2004) who used ITS, *trnT-L*, *trnL-F* spacers and the *trnL* intron and by Ionta and Judd (2007) where *trnD-T*, *trnT-F* and ITS gene regions were used.

A close-up photograph of a green plant stem with several yellow-green buds and one partially open yellow flower. The flower has a dark red center. The background is dark, making the plant stand out.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Specimens

Leaf material for DNA extraction was sampled from herbarium specimens (with permission of relevant Herbaria) or freshly collected plant material dried in silica gel. The 87 herbarium specimens, representing 30 *Raphionacme* species, two subspecies and two forms, used in this study are listed in Appendix 1. The herbarium acronyms are used according to Holmgren et al. (1990). Four *Raphionacme* species, *R. borenensis*, *R. caerulea*, *R. inconspicua* and *R. linearis* are also listed but no amplification of the ITS region could be achieved. Two species, *R. arabica* and *R. sylvicola*, were not included because only type material are available and destructive sampling is not allowed. As outgroups *Chlorocyathus lobulata* (Venter & R.L.Verh.) Venter, *Schlechterella abyssinica* (Chiov.) Venter & R.L.Verh. and *Stomatostemma monteiroae* N.E.Br. were chosen. These three outgroups as well as *Raphionacme* form part of the grooved translator clade of Ionta and Judd's (2007) and Ionta's (2009) phylogenetic analyses of the Periplocoideae.

3.1.2. Morphological characters and character states

Data used in the morphological matrix were obtained from the revisions of *Raphionacme* (Venter, 2009), *Chlorocyathus* (Venter, 2008), *Schlechterella* (Venter and Verhoeven, 1998), *Stomatostemma* (Venter and Verhoeven, 1993) and herbarium specimens. Characters and character states are listed in Table 3.1.

3.1.3. Morphological terminology

Terminology is used in accordance with Beentje (2010). Due to the complexity of the Periplocoideae flower, a unique terminology was created to describe these flowers (Venter and Verhoeven, 2001; Venter, 2009). This terminology is explained using a generalized *Raphionacme* flower (Fig. 3.1). Some vegetative and reproductive terms are also defined as it was interpreted in this study.

Table 3.1. List of morphological characters with character states as used in the data-matrix.

Characters	Character States		
	0	1	2
1. Plant growth form	Hemicryptophyte	Phanerophyte	
2. Habit	Herb	Herbaceous climber	Woody climber
3. Position of tuber on plant	Modified tap root	On lateral roots	
4. Number of tubers per plant	One	More than one	
5. Tuber shape	Globose to sub-globose	Cylindrical	
6. Stem texture	Herbaceous	Woody	
7. Stem habit	Erect	Spreading to decumbent	
8. Stem branching	Lateral	Dichotomous	
9. Stem indumentum	Puberulous/ tomentose/velutinous	Hirsute/hispid/scabrous	Glabrous
10. Leaf indumentum	Puberulous/ tomentose/velutinous	Hirsute/hispid/scabrous	Glabrous
11. Secondary venation	Arching	Patent	Divaricate
12. Leaf apex	Acuminate/acute/attenuate/cuspidate	Obtuse/Truncate	Mucronate
13. Leaf base	Cuneate/tapering	Obtuse	
14. Inflorescence density	Compactly flowered	Laxly flowered	
15. Bract shape	Acicular/subulate/triangular	Ovate	Oblong
16. Sepal shape	Triangular	Ovate	Subulate
17. Sepal indumentum	Puberulous/pubescent/velutinous	Hirsute/hispid/scabrous	Glabrous
18. Corolla tube shape	Campanulate	Cylindrical	Pentagular-campanulate
19. Corolla tube length	Shorter than or equal to 5 mm	Longer than 5 mm	

Table 3.1. Continued...

Characters	Character States		
	0	1	2
20. Corolla lobes spreading/reflexed	Spreading	Reflexed	
21. Corolla lobe apex shape	Obtuse	Acute	
22. Upper surface colour of corolla lobes	Brown/green/white/yellow	Blue/pink/purple	Green with brown/purple base
23. Presence or absence of coronal tube	Absent	Present	
24. Coronal lobe shape	Simple	Basally divided	Apically divided
25. Coronal feet absent/present	Absent	Present	
26. Coronal feet shape	Inconspicuous	Conical	Collumnar
27. Coronal feet free or fused to each other	Free	Fused	
28. Stamen insertion	Corolla tube	Coronal foot	
29. Stamen insertion on corolla tube	Base of corolla tube	Apex of corolla tube	
30. Stamen insertion when fused to coronal foot	From inner base of coronal foot	From inner apex of coronal foot	From inner face of coronal foot
31. Filament length	Longer than or equal to 1 mm	Shorter than 1 mm	
32. Filament base shape	Filiform	Linear	Columnar/deltoid/dilated/ovoid/spherical
33. Filament apex shape	Filiform	Linear	Dilated

Table 3.1. Continued...

Characters	Character States		
	0	1	2
34. Anther basal callosities absent or present	Absent	Present	
35. Anther dehiscens	Full length slits	Slits restricted to terminal half of anther	
36. Pollen in tetrads or in pollinia	Tetrads	Pollinia	
37. Style-head shape	Ovoid	Oblong	
38. Translator spoon shape	Elliptic	Ovate	Triangular
39. Gynostegium position	In mouth of corolla tube	Elevated above mouth of corolla tube	Included in corolla tube
40. Follicle shape	Ovoid	Keel-shaped	
41. Follicles paired/solitary	Paired	Solitary	
42. Follicle orientation	Erect	Pendulous	
43. Follicle divergence	Narrow	Wide	
44. Habitat	Swamp	Terrestrial	
45. Terrestrial habitat	Grassland/open savannah	Closed savanna/dry forest	Desert/semi-desert

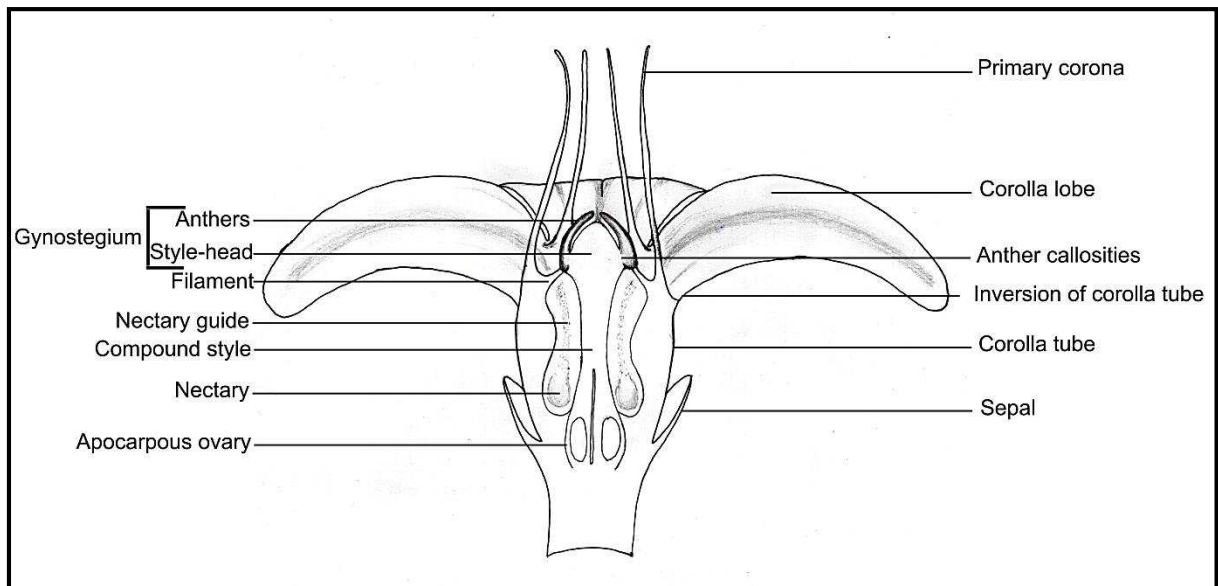


Figure 3.1. Schematic representation of a generalized *Raphionacme* flower.

Definitions

Anther callosities: a pair of spongy bodies at the anther base where the anther is fused to the style-head.

Corolla: fused into a tube with free lobes.

Corolla lobes: can be spreading (horizontally orientated) or reflexed (curved downwards).

Corolla tube pentangular-campanulate: tube distinctly five-angular in a cross section and bowl-shaped.

Corona: only a single or primary corona is present, divided into an upper segment or lobe and a foot at the base.

Follicle divergence: paired follicles may have a divergence that is: (i) narrow – the angle between the two follicles are smaller than 90° ; (ii) wide – the angle between the two follicles are equal to or larger than 90° .

Gynostegium: a compound structure comprising the anthers fused to the style-head.

Hemicryptophyte: a plant with an apical meristem that survives adverse seasons as a dormant bud at or near the soil surface.

Indumentum: covering of hairs on epidermal surface of any plant part. For the purpose of this study, the indumentum was divided into three groups: (i) hairs that are coarse or stiff to the touch (hirsute/hispid/scabrous); (ii) hairs that are soft to the touch (puberulous/pubescent/velutinous); (iii) hairless (glabrous).

Inflorescence density: the cyme can be laxly or loosely flowered due to the lengthened internodes or flowers can be clustered and compact when the internodes are contracted.

Phanerophyte: a plant with an apical meristem that survives adverse seasons as a dormant bud well above the soil surface.

Secondary venation: the orientation of secondary veins is regarded as: (i) arched when the angle between lateral veins and main vein is less than 45° ; (ii) divaricate when the angle is between 45° and 85° ; (iii) patent when the angle is approximately 90° .

3.1.4. Biogeography

Distribution data and coordinates were obtained from an unpublished database of Prof H.J.T. Venter of the University of the Free State (UFS).

3.2. Methods

3.2.1. DNA extraction from dried leaf tissue

DNA extraction from fresh and herbarium leaf material was based on the modified cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1987). Leaf tissue was ground to a fine powder with a Qiagen® TissueLyzer, after which 750 µL CTAB extraction buffer [2% (w/v) CTAB, 100 mM Tris(hydroxymethyl)aminomethane-hydrochloride (Tris-HCl) pH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 0.2% (v/v) 2-mercapto-ethanol] was added. The material was incubated at 65 °C for 1 h. After incubation, 500 µL chloroform (CHCl₃)/isoamylalcohol (IAA) (24:1 v/v) was added, the suspension was thoroughly mixed and centrifuged at 12 000 g for 10 min at 4 °C. DNA was precipitated from the aqueous phase by adding 500 µL isopropanol and the tubes incubated at room temperature for 20 min. After centrifugation at 12 000 g for 10 min at 4 °C, the supernatant was discarded and the pellet washed with 500 µL ice-cold 70% (v/v) ethanol. After an overnight incubation step at 4 °C followed by 40 min at room temperature, the extracts were again centrifuged and the pellet washed with 70% (v/v) ethanol for 20 min at room temperature. After centrifugation for 5 min, the pellet was air-dried for 1 h at room temperature. The pellet was finally resuspended overnight at 4 °C in 200 µL TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Two microliter ribonuclease A (RNase A) (10 mg/mL) was added to the extract and incubated at 37 °C for 2 h. The DNA was extracted once with 20 µL 7.5 M ammonium acetate and 200 µL CHCl₃/IAA (24:1 v/v) and then centrifuged at 12 000 g for 10 min at 4 °C. The DNA was precipitated from the aqueous phase with 500 µL ice-cold 100% (v/v) ethanol for 3 days at -20 °C. After centrifugation at 12 000 g for 15 min at 4 °C, the pellet was washed twice with 500 µL ice-cold 70% (v/v) ethanol, dried and finally resuspended in 50 µL TE buffer.

The DNA concentration was determined with the NanoDrop™ 2000 spectrophotometer (Thermo Scientific) and expressed as ng/μL. The DNA samples were diluted to a final concentration of 10 ng/μL.

3.2.2. Sequence analysis of plant specimens

3.2.2.1. Amplification of the ITS region

The entire ITS region was amplified from purified genomic DNA in a single PCR reaction using primers ITS-A and ITS-B (Fig. 3.2; Table 3.2).

When the template DNA was degraded or no amplification was achieved, ITS-1 and ITS-2 were amplified separately using the primer combinations ITS-A, ITS-C and ITS-B, ITS-D respectively. This resulted in a 72 bp overlap at the beginning of the 5.8S rDNA corresponding to 5.8S rDNA alignment positions 6 – 78 (Blattner, 1999).

To obtain the optimal annealing temperature for the four primers, a PCR gradient was run with the following regime: 96 °C for 2 min followed by 30 cycles of 94 °C for 30 sec, 51 – 57 °C for 30 sec and 72 °C for 2 min with a final step of 72 °C for 5 min. The optimal annealing temperature was determined by examining the amplified DNA quality on a 1% (w/v) agarose gel prepared in 0.5 x TAE buffer [20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 0.01% (v/v) acetic acid containing 10 mg/mL ethidium bromide (Sambrook et al., 1989)].

The running buffer used was 0.5 x TAE. The DNA was visualized under UV light illumination and the image captured with the Gel Doc XR™ system (BioRad). Subsequently an annealing temperature of 57 °C was used for all the primer pairs.

Each amplification reaction contained 10 ng genomic DNA, 10 pmol of each primer and 1 x KAPATaq ReadyMix (KAPA Biosystems). A G-Storm™ G1 Thermal Cycler was used and the amplification regime was as follows: 94 °C for 2 min followed by 40 cycles of 94 °C for 30 sec, 57 °C for 30 sec and 72 °C for 2 min, with a final extension step at 72 °C for 5 min. To confirm amplification of the PCR product, 10 μL of the products were mixed with DNA loading buffer [0.015% (w/v) Orange G, 2.5% (w/v) ficoll] and separated on a 1% (w/v) agarose gel and visualized under UV light illumination as described above.

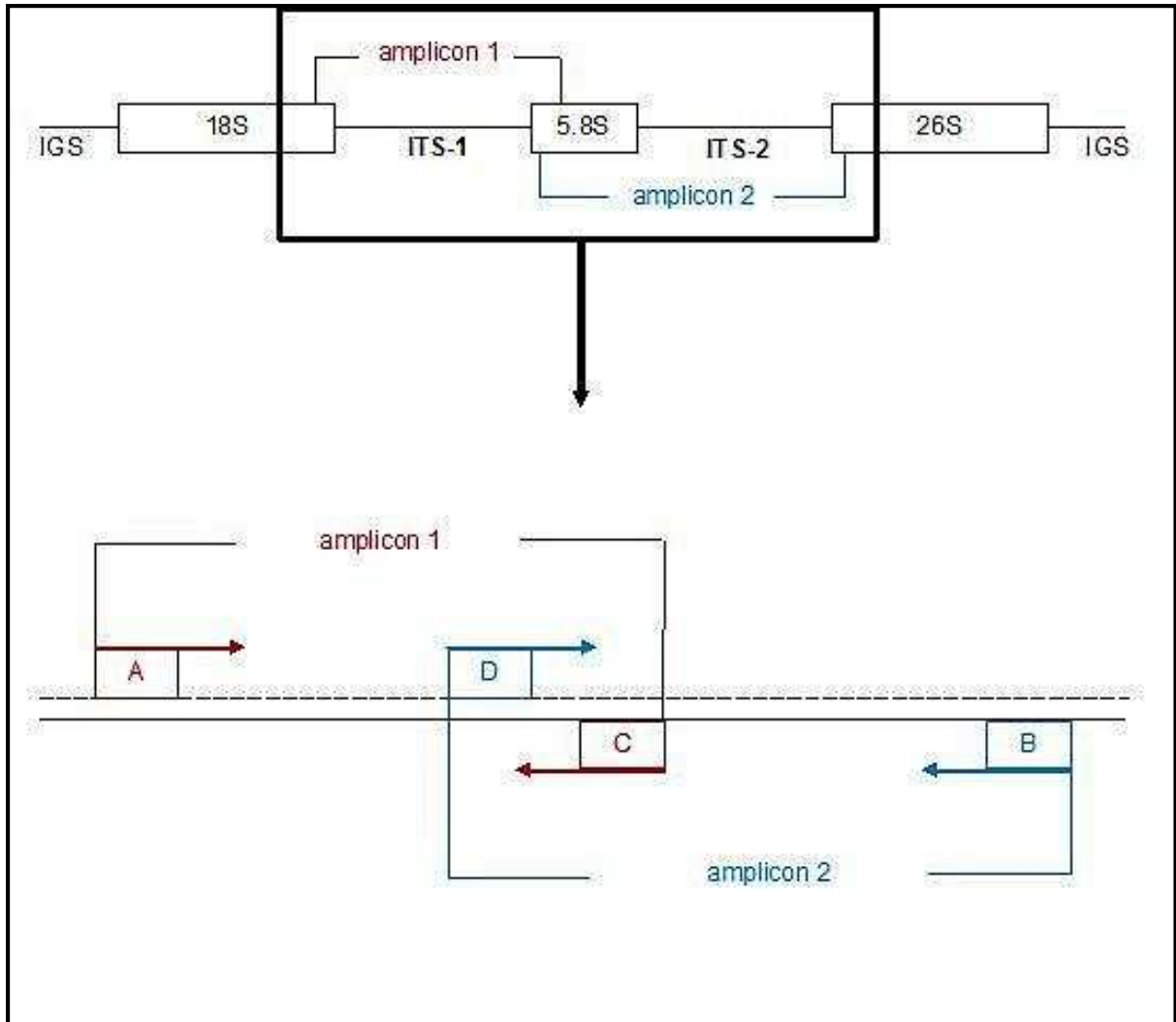


Fig. 3.2. Schematic representation of ITS regions 1 and 2 as adopted from Blattner (1999). Primer positions are indicated with A (ITS-A), B (ITS-B), C (ITS-C) and D (ITS-D). The arrows indicate the direction of amplification.

Table 3.2. Nucleotide sequences of ITS primers used in this study.

Gene Region	Primer	Primer sequence	Amplification direction
Ribosomal ITS	ITS - A	5'-GGAAGGAGAAGTCGTAACAAGG-3'	Forward
	ITS - B	5'-CTTTTCCTCCGCTTATTGATATG-3'	Reverse
	ITS - C	5'-GCAATTCACACCAAGTATCGC-3'	Reverse
	ITS - D	5'-CTCTCGGCAACGGATATCTCG-3'	Forward

When problems persisted with the amplification of the gene region, EmeraldAmp® MAX HS PCR Master Mix (Takara Biotechnology (Dalian) Co., Ltd.) was used according to manufacturer's instructions. The regime was as follows: 30 cycles of 98 °C for 10 sec, 57 °C for 30 sec and 72 °C for 1 min.

The amplified DNA fragments were purified from either the gel or the PCR reaction with the Favorgen® Gel/PCR Purification Kit (Favorgen® Biotech Corp.) according to the manufacturer's instructions.

3.2.2.2. DNA sequencing of ITS

Gradient PCR sequencing reactions were run for each of the primers used, to achieve optimal sequencing of the ITS region (Table 3.2). Approximately 40 ng of the purified PCR product was sequenced in a 10 µL reaction containing 3.2 pmol of each respective primer, using the BigDye™ Ready reaction mix (Applied Biosystems) according to the manufacturer's specifications. The cycling regime was 96 °C for 1 min followed by 25 cycles of 96 °C for 10 sec, 51 – 56 °C for 5 sec and 60 °C for 4 min. The optimal annealing temperature was determined by examining the quality of the resulting electropherograms. Subsequently an annealing temperature of 55 °C was used for all the primers.

Purification of the sequenced products were done by transferring each 10 µL sequence reaction to a 1.5 mL Eppendorf tube and adding 10 µL water, 5 µL of a 125 mM EDTA solution and 60 µL 100% (v/v) ethanol. Each reaction was vortexed and incubated at room temperature for 15 min and centrifuged at 12 000 *g* for 15 min at 4 °C. The supernatant was removed and the pellet washed with 60 µL of 70% (v/v) ethanol. After centrifugation for 5 min at 12 000 *g* at 4 °C, the pellet was air-dried overnight in the dark. The pellet was then sent to the Department of Microbial, Biochemical and Food Biotechnology (UFS) for separation.

3.2.2.3. Sequence editing and alignment

Editing of the obtained sequences was carried out in Chromas Lite v2.01 (Technelysium Pty Ltd). Sequences were manually adjusted and consensus sequences created in BioEdit Sequence Alignment Editor v7.0.9 (Tom Hall, Ibis Biosciences). Consensus sequences were created using ITS-A and ITS-B (to create an entire ITS 1 and ITS 2 continuum sequence per specimen), or using ITS-C and ITS-D (to also create an entire ITS 1 and ITS 2 continuum sequence per specimen).

Sequences were then automatically aligned in Mafft online version 6 (mafft.cbrc.jp/alignment/server/index.html) and further manually aligned using GeneDoc v2.7.0.0 (<http://www.psc.edu/biomed/genedoc>).

The ITS sequences for the three outgroups as well as five *Raphionacme* species were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>). The accession numbers of these species are listed in Table 3.3.

3.2.3. Phylogenetic analyses

3.2.3.1. Data

The data-matrix comprised two submatrices consisting of the ITS gene region and the morphological data respectively. The morphological submatrix (Appendix 2) comprised 45 floral, fruit and vegetative characters.

3.2.3.2. Parsimony

Analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Heuristic analyses were performed with all characters given unit weight (Fitch parsimony; Fitch, 1971). Each submatrix was analysed separately before analysing the combined ITS and morphological data.

All of the analyses were done with the following settings: 10 000 replicates of random taxon entry, the Tree-Bisection-Reconnection (TBR) branch swapping algorithm and MULTREES on (saving multiple equally parsimonious trees) but holding only 10 trees per replicate.

Table 3.3. *Raphionacme* and outgroup species with their respective accession numbers for which ITS sequences were obtained from GenBank.

Species name	Accession number
<i>R. dyeri</i>	AJ581686.1
<i>R. elata</i> [= <i>R. galpinii</i>]	AJ581687.1
<i>R. flanagani</i>	AJ581688.1
<i>R. hirsuta</i>	AJ581689.1
<i>R. madiensis</i>	AJ581690.1
<i>R. lobulata</i> [= <i>Chlorocyathus lobulata</i>]	DQ916862.1
<i>Schlechterella abyssinica</i>	AJ581691.1
<i>Stomatostemma monteiroae</i>	AJ581694.1

Bootstrap (BS) values (Felsenstein, 1985) were calculated with 100 replicates on each of the submatrices as well as on the combined matrix. Scale for BS-MP values is as follows: low – 30% to 50%; moderate – 51% to 80%; well – 81% to 100%. Congruence of nuclear and morphological data were assessed using a partition homogeneity test, as implemented in PAUP* 4.0b10 (Swofford, 2002).

3.2.3.3. Maximum likelihood

Maximum likelihood (ML) analysis was performed on the ITS data only. An appropriate model of nucleotide substitution was determined using Modeltest (Posada and Crandall, 1998), using the Akaike information criterion (AIC) (Akaike, 1974). The F81+I+G model (Felsenstein, 1981) was subsequently chosen and used in the ML analysis. Heuristic searches were performed with 100 random addition replicates, each with 10 random additions and TBR branch swapping. Scale for BS-ML values is as follows: low – 30% to 50%; moderate – 51% to 80%; well – 81% to 100%.

3.2.3.4. Bayesian analysis

Bayesian analyses (BI) were performed on both ITS and morphological data in the software application MrBayes 3.1 (Heuleisenbeck and Ronquist, 2001; Ronquist and Heuleisenbeck, 2003). Each analysis started from random trees in which the Markov Chain Monte Carlo (MCMC) included four chains for 2×10^6 generations. Default settings for priors were used. Scale for BI values is as follows: low – 0.3 to 0.5; moderate – 0.51 to 0.75; well – 0.76 to 1.00.

3.2.4. Biogeography

Species distribution data were read into the Botanical Research And Herbarium Management System (BRAHMS), v. 7.1.1. and then exported to DIVA-GIS (Hijmans et al., 2012), where distribution and species diversity maps were generated.

A close-up photograph of a plant stem. The stem is brown and covered in fine, white, hair-like structures. Several green, pointed, fleshy leaves are clustered around the stem. In the center, a small, purple, star-shaped flower is in bloom. The background is dark and out of focus.

CHAPTER 4

RESULTS

4.1. Biogeography

4.1.1. Distribution of *Raphionacme*

Raphionacme species are widely distributed throughout Africa. *Raphionacme arabica* was collected in Oman and is the only *Raphionacme* species found outside Africa. The fact that Arabia and Southwest Asia is adjacent to Africa means that much of the flora of southern Oman is African in origin (Ghazanfar, 2007). According to White (1983), the region in which *R. arabica* occurs also falls with the Somalia-Masai Region and therefore shares the same vegetation type where the climate is influenced by the SW monsoon in the summer (Miller and Biagi, 1988) and the NE monsoon in the winter. The genus is absent from the Cape Region, a large part of the wet tropical Guineo-Congolian Region as well as 20° N of the Equator which forms the Sahara Regional Transition Zone. According to the known distribution of *Raphionacme*, ten of the 36 species are widely distributed throughout sub-Saharan Africa occurring in six of White's (1983) regions of endemism. These species are *R. brownii*, *R. dyeri*, *R. globosa*, *R. hirsuta*, *R. lanceolata*, *R. madiensis*, *R. michelii*, *R. procumbens*, *R. utilis* and *R. welwitschii* (Fig. 4.1).

4.1.2. *Raphionacme* species richness

Species richness of *Raphionacme* is high, from five to eight species are concentrated at four localities: (i) in the northern part of KwaZulu-Natal (South Africa) which falls within the Tongaland-Pondoland Regional Mosaic; (ii) northeastern part of Zimbabwe which falls within the Zambezi Region of Endemism; (iii) southwestern corner of Angola which falls within the Karroo-Namib Region and Kalahari-Highveld Regional Transition Zone; (iv) and the northern part of Zambia which also falls within the Zambezi Region of Endemism (Fig. 4.2).

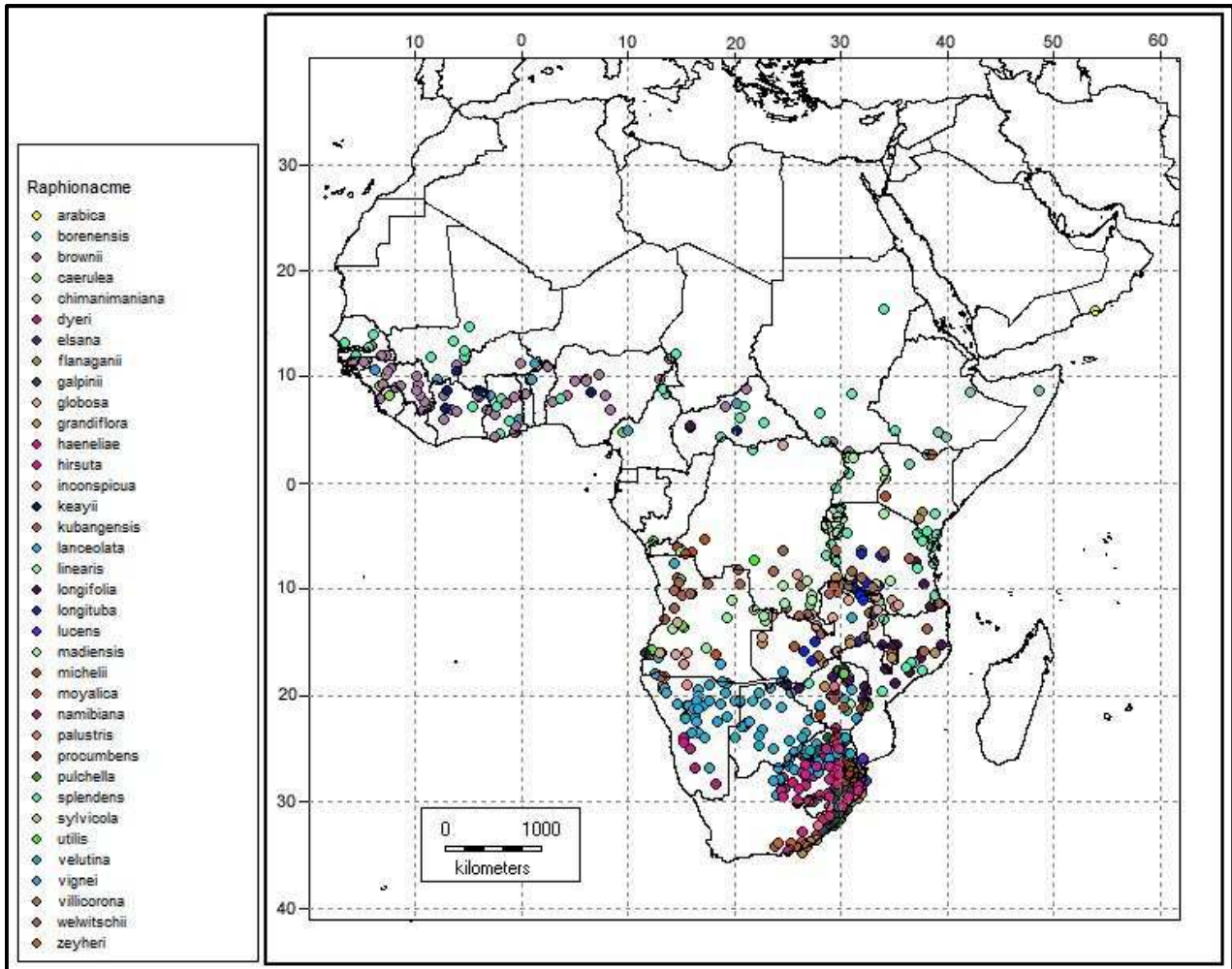


Fig. 4.1. Known distribution of *Raphionacme* species in Africa.

Raphionacme arabica from Oman is included.

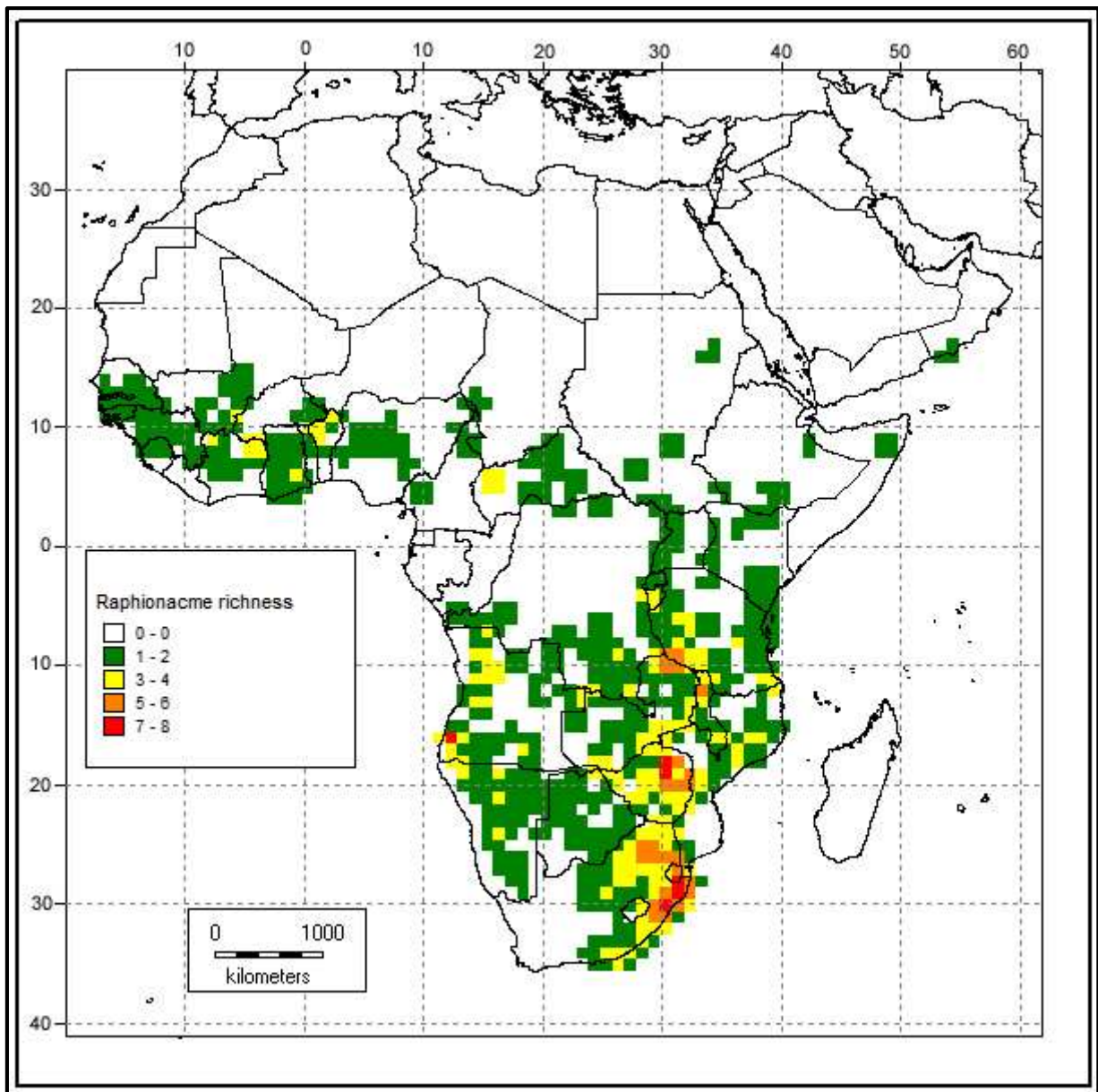


Fig. 4.2. Map indicating *Raphionacme* species richness in Africa. *Raphionacme arabica* collected in Oman is also included. Scale: each cell represents 1° square.

4.1.3. Endemism in *Raphionacme*

Fourteen of the 36 *Raphionacme* species are endemic to five of the 18 African centres of endemism (White, 1983). These species are *R. borenensis*, *R. chimanimaniana*, *R. elsana*, *R. flanaganii*, *R. haeneliae*, *R. linearis*, *R. longituba*, *R. lucens*, *R. moyolica*, *R. namibiana*, *R. pulchella*, *R. sylvicola*, *R. villicorona* and *R. zeyheri*. The centres of endemism in which these species occur are Somalia-Masai Region, Zambezian Region, Tongaland-Pondoland Regional Mosaic, Karoo-Namib Region and Kalahari-Highveld Regional Transition Zone (Table 4.1). *Raphionacme arabica* is also endemic to Somalia-Masai Region in the Dhofar Mountains of southeastern Oman.

4.2. Cladistical analyses

4.2.1. Morphological data

The first analysis of the morphological dataset consisted of all 36 species and two subspecies. The resulting cladogram was then compared to a cladogram which only included the 33 species of which molecular data were available. No significant differences were found between these two cladograms. The parsimony analysis of the morphological dataset consisted of 33 species which included two subspecies, two forms and three outgroup species. The dataset consisted of 45 characters of which 41 characters were parsimony-informative. The heuristic search analysis resulted in a tree of 214 steps with a consistency index (CI) of 0.280, homoplasy index (HI) of 0.720 and a retention index (RI) of 0.488. One of the 10 000 equally most parsimonious trees shows that the position of several of the species is unresolved as indicated by the very low bootstrap (BS) and posterior probability (PP) values (Fig. 4.3). Bootstrap values lower than 30% and PP values lower than 0.50 were not indicated, as it was found to be insignificant.

According to the cladogram constructed with morphological data the outgroup species *Stomatostemma monteiroae* and *Chlorocyathus lobulata* are sister to the ingroup. *Schlechterella abyssinica* is morphologically more closely related to *Raphionacme* than it is to the other two outgroup species (BS 90%, PP 0.97).

Table 4.1. *Raphionacme* species endemic to White's (1983) phytochoria.

Somalia-Masai Regional Centre of Endemism	Zambeziian Regional Centre of Endemism	Tongaland-Pondoland Regional Mosaic	Karoo-Namib Regional Centre of Endemism	Kalahari-Highveld Regional Transition Zone
<i>R. borenensis</i>	<i>R. chimanimaniana</i>	<i>R. elsana</i>	<i>R. haeneliae</i>	<i>R. villicorona</i>
<i>R. moyalica</i>	<i>R. linearis</i>	<i>R. flanagani</i>	<i>R. namibiana</i>	
	<i>R. longituba</i>	<i>R. lucens</i>		
	<i>R. pulchella</i>	<i>R. zeyheri</i>		
	<i>R. sylvicola</i>			

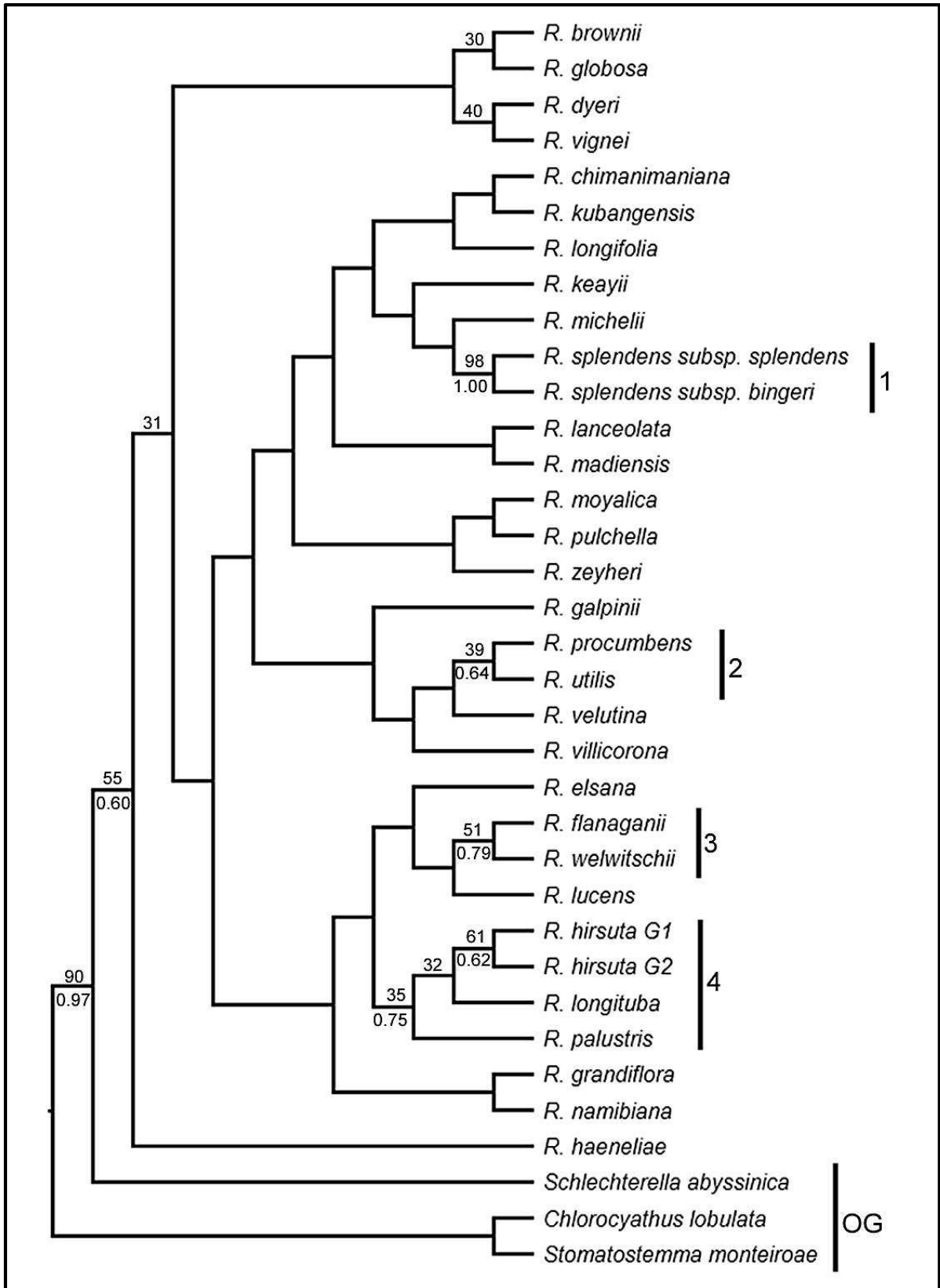


Fig. 4.3. Morphological data cladogram of *Raphionacme* species. Values above the lines indicate MP BS-values and below the lines indicate BI PP-values. Clades are indicated by numbers 1 to 4 and OG represent the outgroup.

Clade 1 consisted of the two *R. splendens* subspecies and is very well supported (BS 98%, PP 1.00), while clade 3 consisted of *R. flanagani* and *R. welwitschii* and is also moderately to well supported with BS and PP values of 51% and 0.79 respectively. Clades 2 and 4 have low to moderate support, where clade 2 consisted of *R. procumbens* and *R. utilis* (BS 39%, PP 0.64) and clade 4 of *R. hirsuta* G1, *R. hirsuta* G2, *R. palustris* and *R. longituba* (BS 35%, PP 0.73).

4.2.2. Molecular data

The aligned ITS data matrix contained 705 characters, whereof 129 characters were parsimony informative. The parsimony analysis of the ITS data matrix consisted of 30 *Raphionacme* species which included two subspecies, two forms and three outgroup species. The heuristic analysis resulted in a tree of 415 steps with a CI of 0.737, HI of 0.263 and a RI of 0.776. One of 10 000 equally most parsimonious trees is illustrated in Fig. 4.4.

The outgroup species, *Chlorocyathus lobulata* and *Stomatostemma monteiroae* are sister to the ingroup, but *Schlechterella abyssinica* is nested in the ingroup. The ITS tree is divided into four clades, namely clades A, B, C and D. Clade A is then further subdivided into sub-clades A1, A2, A3 and A4, while clade C is subdivided into sub-clade C1 (Fig. 4.4).

Clade A is supported by moderate to high values (BS-MP 58%, BS-ML 52%, PP 0.90) and comprises 20 species. Moderate to high values support sub-clade A1 (BS-MP 42%, BS-ML 50%, PP 0.92) which consists of *R. brownii*, *R. vignei*, *R. michelii* and *R. zeyheri*, while sub-clade A2 is supported with high values (BS-MP 93%, BS-ML 96%, PP 1.00) and consists of *R. chimanimaniana* and *R. villicorona*. Support values for sub-clade A3 is low to moderate (BS-MP 35%, BS-ML 30%, PP 0.85), with the clade consisting of *R. dyeri*, *R. velutina* and *R. elsana*, while sub-clade A4 is supported by moderate to high values (BS-MP 62%, BS-ML 54%, PP 0.93) and consists of *R. keayii* and *R. kubangensis*.

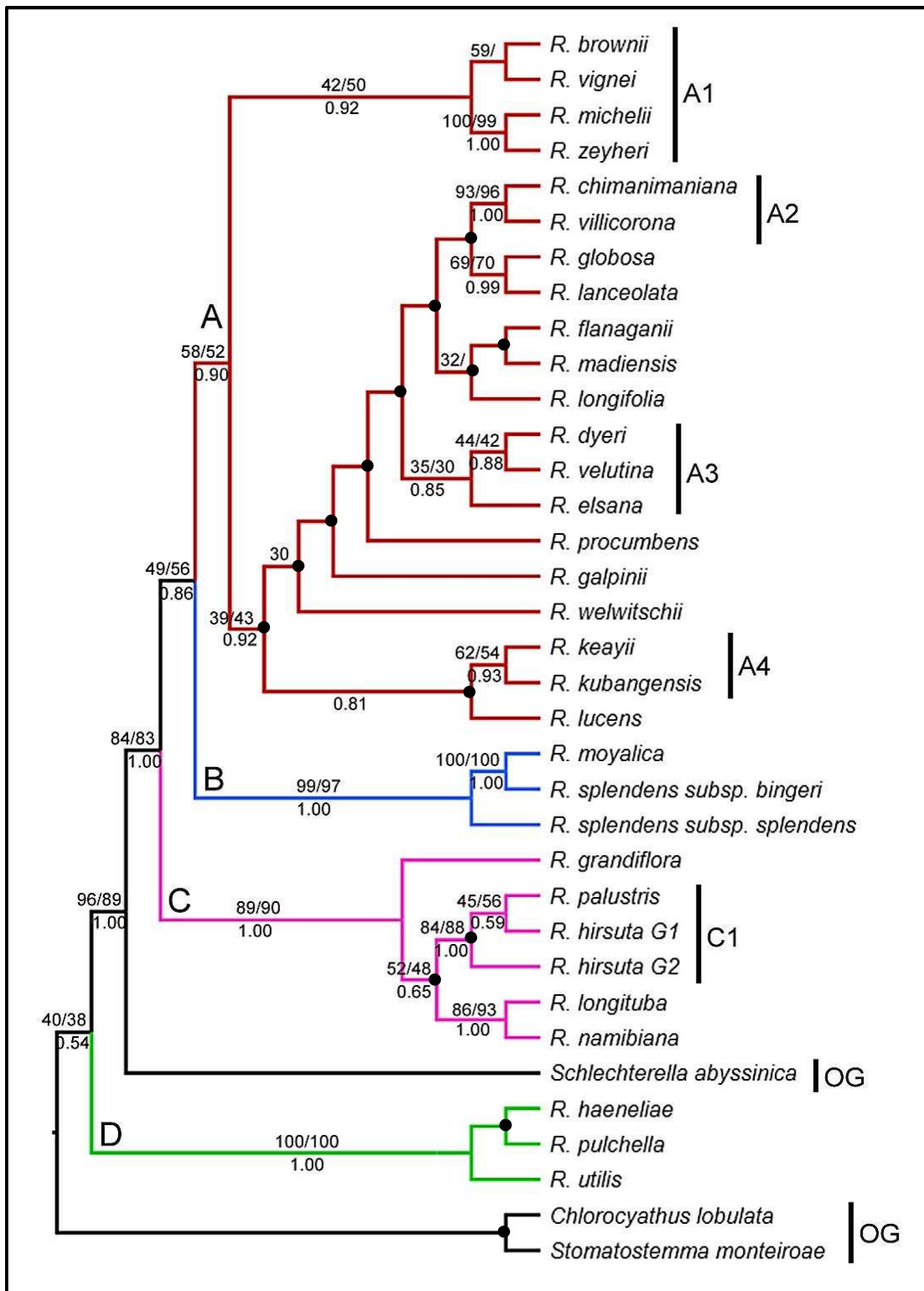


Fig. 4.4. ITS cladogram of *Raphionacme* species. Values above the lines indicate MP and ML BS-values respectively and below the lines indicate BI PP-values. Clades are indicated by A, B, C, and D while sub-clades are indicated by numbers A1 to A4 and C1. OG indicates the outgroup. Black bullets indicate branches that collapsed in the consensus tree.

Clade B is sister to clade A and this grouping is supported with high values (BS-MP 99%, BS-ML 97%, PP 1.00) and contains *R. moyalica*, *R. splendens* subsp. *bingeri* and *R. splendens* subsp. *splendens*.

Clade C is sister to clade A and B, with high values for the grouping of this clade (BS-MP 89%, BS-ML 90%, PP 1.00). The five species contained in this clade are *R. grandiflora*, *R. palustris*, the two *R. hirsuta* forms, *R. longituba* and *R. namibiana*. Sub-clade C1 is supported by high values (BS-MP 84%, BS-ML 88%, PP 1.00) and consists of *R. palustris* and the two forms of *R. hirsuta*.

Clade D is sister to clades A, B, C and *Schlechterella abyssinica*. Clade D is very well supported by high values (BS-MP 100%, BS-ML 100%, PP 1.00) and consists of *R. haeneliae*, *R. pulchella* and *R. utilis* (Fig. 4.4).

4.2.3. Combined data

In order to combine the morphological and molecular data, the species with missing molecular data were left out of the morphological analysis. Visual inspection of the separate analyses of the ITS and morphological datasets revealed contradictory clades. A partition homogeneity test indicated that the ITS and morphological datasets were incongruent ($P = 0.01$).

Even though the data sets were incongruent, they were still combined to give an indication of changes that may have occurred in the major clades A, B, C and D. The combined data matrix included 750 characters of which 136 characters were parsimony-informative. The heuristic analysis resulted in a tree of 693 steps. The tree had a CI of 0.553, HI of 0.447 and a RI of 0.610. One of 374 equally most parsimonious trees is illustrated in Fig. 4.5.

The topography of the combined tree has not change drastically when compared to that of the ITS tree. The species that formed the major clades A, B, C and D and the sub-clades A1, A2, A3, A4 and C1 in the ITS tree, remained the same in the combined tree. Only minor topographical changes are found in the major clades, when compared to the ITS tree. Support values of the major and sub-clades either increase or decrease only slightly (Fig. 4.4; Fig. 4.5).

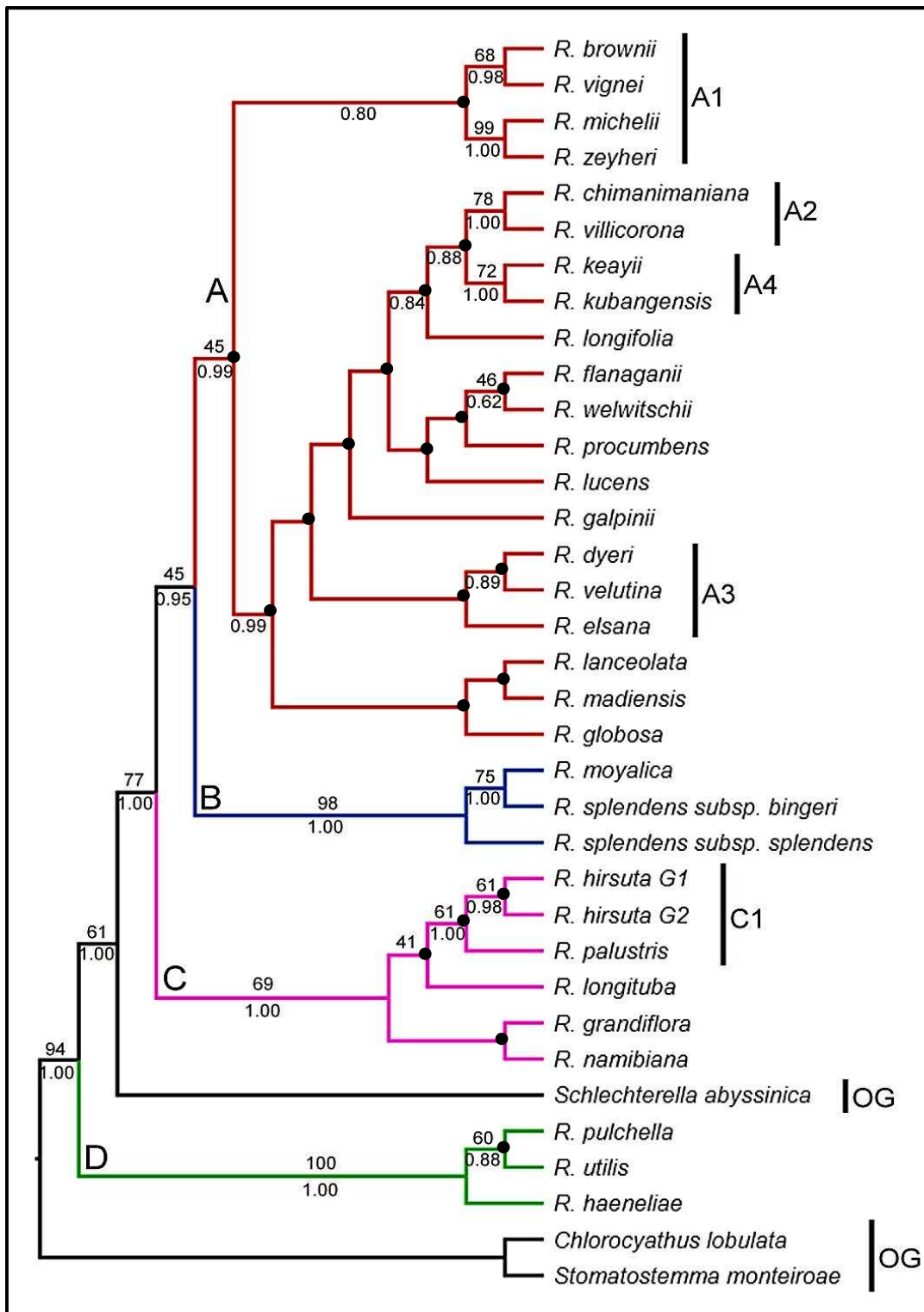


Fig. 4.5. Cladogram based on combined data of ITS and morphology of *Raphionacme* species. Values above the lines indicate MP and ML BS-values respectively and below the lines indicate BI PP-values. Clades are indicated by A, B, C and D while sub-clades are indicated by numbers A1 to A4 and C1. OG indicates the outgroup. Black bullets indicate branches that collapsed in the consensus tree.



CHAPTER 5

DISCUSSION AND CONCLUSION

5.1. Incongruence between the morphological and nuclear dataset

Conflict between the nuclear ITS and morphological datasets of *Raphionacme* lies in the relative positions of several *Raphionacme* species as well as the placement of *Schlechterella abyssinica* (Fig. 4.3; Fig. 4.4). This conflict may result from factors such as weak resolution of the morphologically base topologies and insufficiency of discrete characters suitable for parsimony analysis. It was impossible to partition characters into discrete states across the entire genus because of the high level of diversity among *Raphionacme* species.

Combining different datasets often increases the resolution of the ingroup and the bootstrap support of the internal nodes of the phylogenetic trees (Olmstead and Sweere, 1994; Chase et al., 1995; Yukawa et al., 1996; Soltis et al., 1998; Meerow et al., 1999; Meerow and Snijman, 2001). Kluge (1989) and Nixon and Carpenter (1996) argued that simultaneous analysis of multiple datasets better maximizes parsimony and allow secondary signals to appear from the combined data. Dolphin et al. (2000), Barker and Lutzoni (2002) and Norup et al. (2006) found the Incongruence Length Difference test (ILD) to be unreliable and thus assessed congruence by comparing topology and support values of the separate consensus trees.

The same assessment for congruence between morphological and molecular datasets was followed in this study. Incongruence between clades was considered conflicting phylogenetic signal when each node had BS and PP support values larger than 95% and 0.85 respectively. In this study no such conflicts occurred and the nuclear ITS and morphological trees were thus combined and used in the phylogenetic description of *Raphionacme*.

5.2. Phylogeny, morphology and biogeography of *Raphionacme*

5.2.1 Phylogenetic relationships and biogeographical affinities in *Raphionacme*

Phylogenetic analyses of the combined nuclear ITS region and morphology of *Raphionacme* species have generated a well-supported cladistical hypothesis for the genus (Fig. 4.5).

Previous molecular studies where a small sample of *Raphionacme* species were included as part of the Periplocoideae, were done by Meve and Liede (2004) using seven *Raphionacme* species, Ionta and Judd (2007) with three species and Ionta (2009) included up to 16 species in various analyses. In all these, the representative *Raphionacme* species formed a distinct clade supported by BS values ranging from 61% to 100%. However, in the present study that included 30 of the 36 *Raphionacme* species, monophyly of the genus could only be achieved by including the one outgroup species, *Schlechterella abyssinica*. The resultant clade was very well supported with a BS value of 94% and a PP value of 1.00. Four consistent clades were identified in the ingroup containing *Raphionacme* and *Schlechterella*. Clades A, B, C and D were supported with moderate to high BS and PP values. Although relationships could be established between most of the species, the positions of the remaining species were unresolved or poorly supported.

Clade A, the largest within *Raphionacme*, consisted of 20 species and was supported with moderate to high values (BS 45%, PP 0.99). According to the phylogeny, the most recently diverging *Raphionacme* species were found in this clade. Morphological characters typical for all these species are corolla tube length of 5 mm or shorter and coronal feet free from one another. As could be expected in a clade of this size, the species were spread throughout Africa (Fig. 5.1). This clade was found in all the phytochoria of White (1983) except the Cape Regional Centre, Sahel Regional Transition Zone and further north. It was also distributed in a diversity of vegetation types ranging from swamp to desert, grassland to dry forest. Clade A is further sub-divided into sub-clades A1, A2, A3 and A4 which were well to indifferently supported.

The oldest diverging sub-clade within clade A was A1, supported by a moderately high PP value of 0.80, but with no BS support value. The four species forming this sub-clade, *R. brownii*, *R. vignei*, *R. michelii* and *R. zeyheri*, are all herbs, have spreading corolla lobes and stamens fused to the coronal foot. Members of this sub-clade are distributed throughout six of White's (1983) phytochoria, namely Guineo-Congolian Region, Zambebian Region, Sudanian Region, Guinea-Congolia/Zambia Transition Zone, Guinea-Congolia/Sudania Transition Zone and Tongaland-Pondoland Mosaic, in vegetation types ranging from short grassland to savanna.

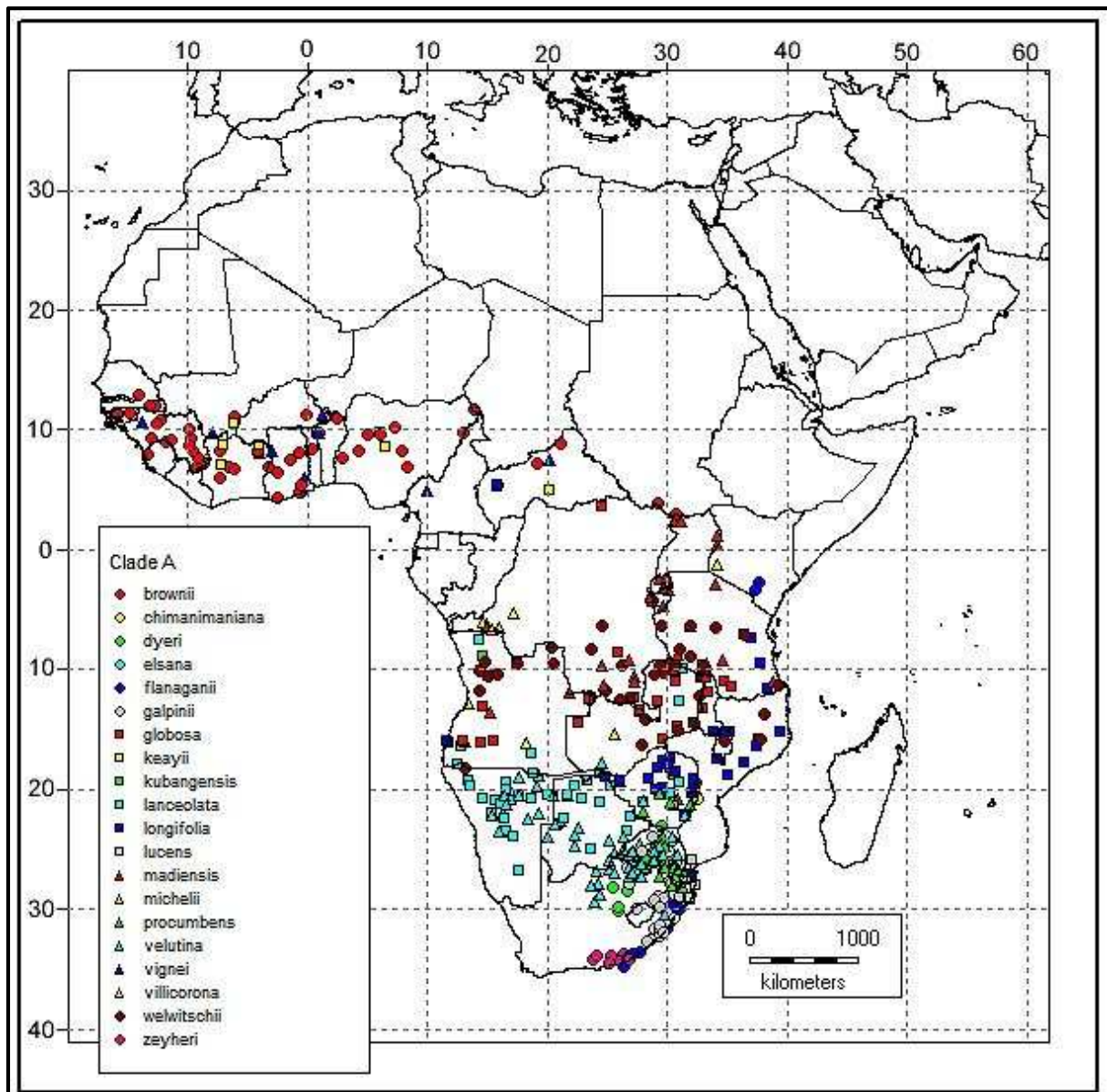


Fig. 5.1. Distribution of *Raphionacme* species within clade A.

Raphionacme brownii and *R. vignei* appeared to be closely related, supported by moderate to high values (BS 68%, PP 0.98). Both species occur between 0° N and 20° N, with a similar distribution pattern mainly in West Africa, as well as the same type of habitat such as rocky pans or sandy to rocky outcrops, grassland and dry open savanna.

Raphionacme michelii is found between 0° S and 20° S while *R. zeyheri* has the furthest southern distribution of all the *Raphionacme* species, occurring below 30° S in the Eastern Cape Province of South Africa (Fig. 5.2). A close relationship between *R. michelii* and *R. zeyheri* was indicated by very high support values (BS 99%, PP 1.00). Even though these two species do not share the same distribution pattern, they are found in similar vegetation types ranging from grassland to savanna, all of which are fairly dry.

The five most recently diverging *Raphionacme* species in clade A were *R. chimanimaniana*, *R. villicorona*, *R. keayii*, *R. kubangensis* and *R. longifolia*. This clade, supported by a moderately high value (no BS value, PP 0.84), was divided into two sub-clades, A2 and A4. Moderate to high values supported sub-clade A2 containing *R. chimanimaniana* and *R. villicorona* (BS 78%, PP 1.00) and sub-clade A4 consisting of *R. keayii* and *R. kubangensis* (BS 72%, PP 1.00).

The two species of sub-clade A2 share lax inflorescences, whereas the majority of species in clade A have compact inflorescences. A unique feature shared by these two species and found in only one other *Raphionacme* species (*R. splendens*), is the gynostegium that is prominently exerted from the corolla tube mouth. Both species were collected in mountainous areas in the Zambezian region (Fig. 5.3).

Raphionacme chimanimaniana was collected above the level of *Brachystegia tamarindoides* woodland in Zimbabwe and *R. villicorona* was collected in mountain bushveld of the Limpopo Province of South Africa. As only a limited number of specimens are available for both these species, description of the vegetation type is probably incomplete.

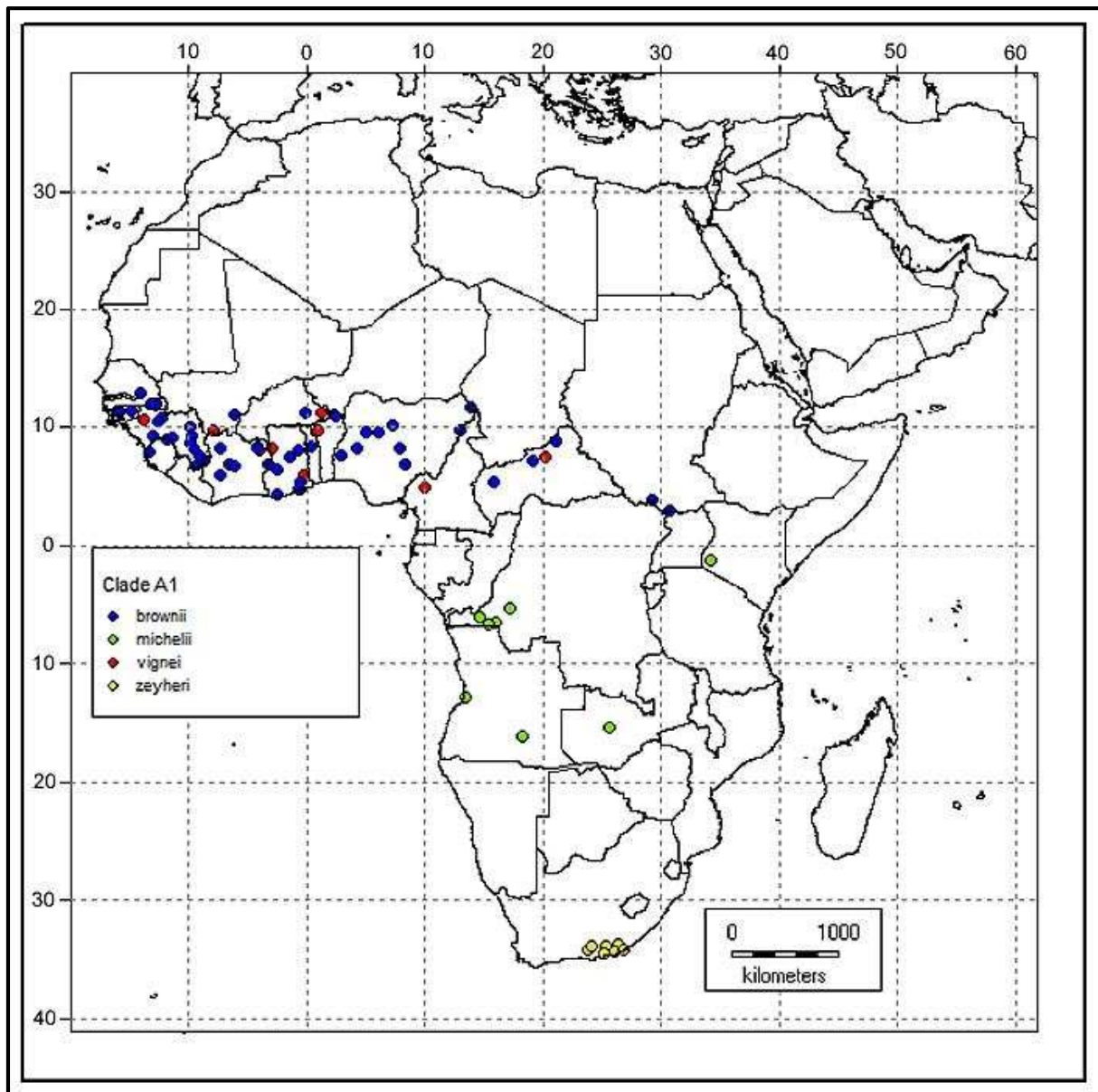


Fig. 5.2. Distribution of *Raphionacme* species within sub-clade A1.

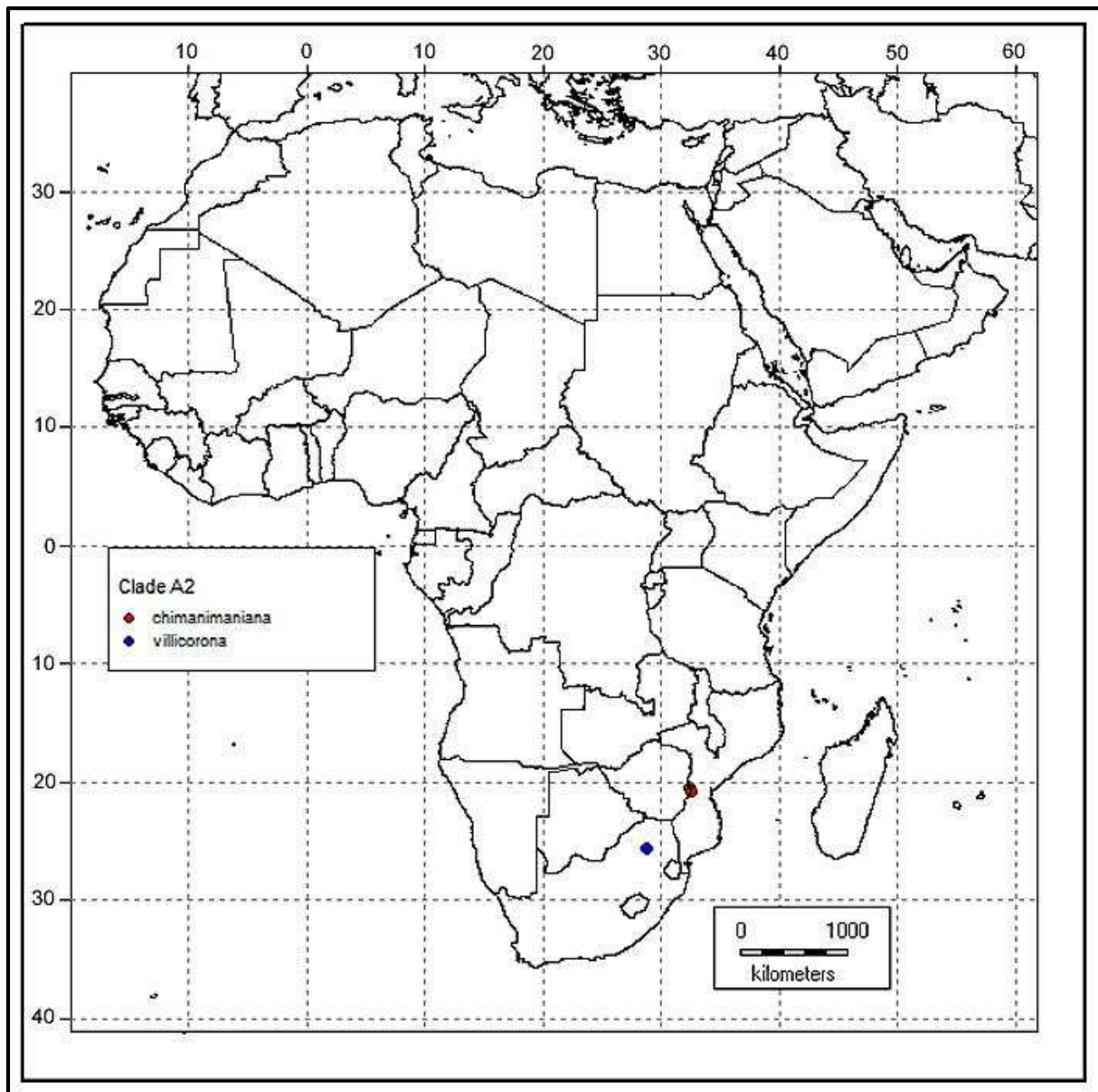


Fig. 5.3. Distribution of *Raphionacme* species within sub-clade A2.

Sub-clade A3 consisting of *R. dyeri*, *R. velutina* and *R. elsana* had very low support in the combined tree (BS 18%, with no PP value), although moderate to high support was evident in the nuclear ITS tree with a PP value of 0.85, but no BS value. These three species are distributed throughout four phytochoria, these being Zambezian Region, Karoo-Namib Region, Kalahari-Highveld Transition Zone and Tongaland-Pondoland Mosaic, in vegetation types ranging from grassland to open savanna (Fig. 5.4). All three species have spreading to decumbent stems with glabrous leaves. The coronal lobes are basally divided with the upper corolla lobe surfaces being green with purple/brown bases.

Raphionacme keayii and *R. kubangensis* of sub-clade A4, like the majority of species in clade A, have compact inflorescences. Morphologically the two species differ in that the upper corolla lobe surface of *R. keayii* flowers is green with a purple/brown base and the coronal lobes are basally divided, whereas uniformly pale green corolla lobes are found in *R. kubangensis* and the coronal lobes are simple. These two species are distributed throughout four phytochoria, namely Sudanian Region, Karoo-Namib Region, Guinea-Congolia/Zambezia Transition Zone and Guinea-Congolia/Sudania Transition Zone. *Raphionacme keayii* occur between 0° N and 15° N in open savanna in West and Central African countries, while *R. kubangensis* occur in Angola between 5° S and 20° S in mixed deciduous woodland (Fig. 5.5).

Clade B was sister to clade A and this relationship was moderately to well supported (BS 45%, PP 0.95). *Raphionacme moyalica*, *R. splendens* subsp. *bingeri* and *R. splendens* subsp. *splendens* were grouped together in the very well supported clade B (BS 98%, PP 1.00). The species of this clade are characterised by herbaceous plants with erect, laterally branching stems, lax inflorescences and adaxially purple corolla lobes, campanulate corolla tubes 5 mm or shorter, while the stamens are attached at the inner apices of the conspicuous coronal feet.

All material was collected from localities between 20° S and 20° N (Fig.5.6). The only available *R. moyalica* specimen was collected in the Somalia-Masai Region in Kenya.

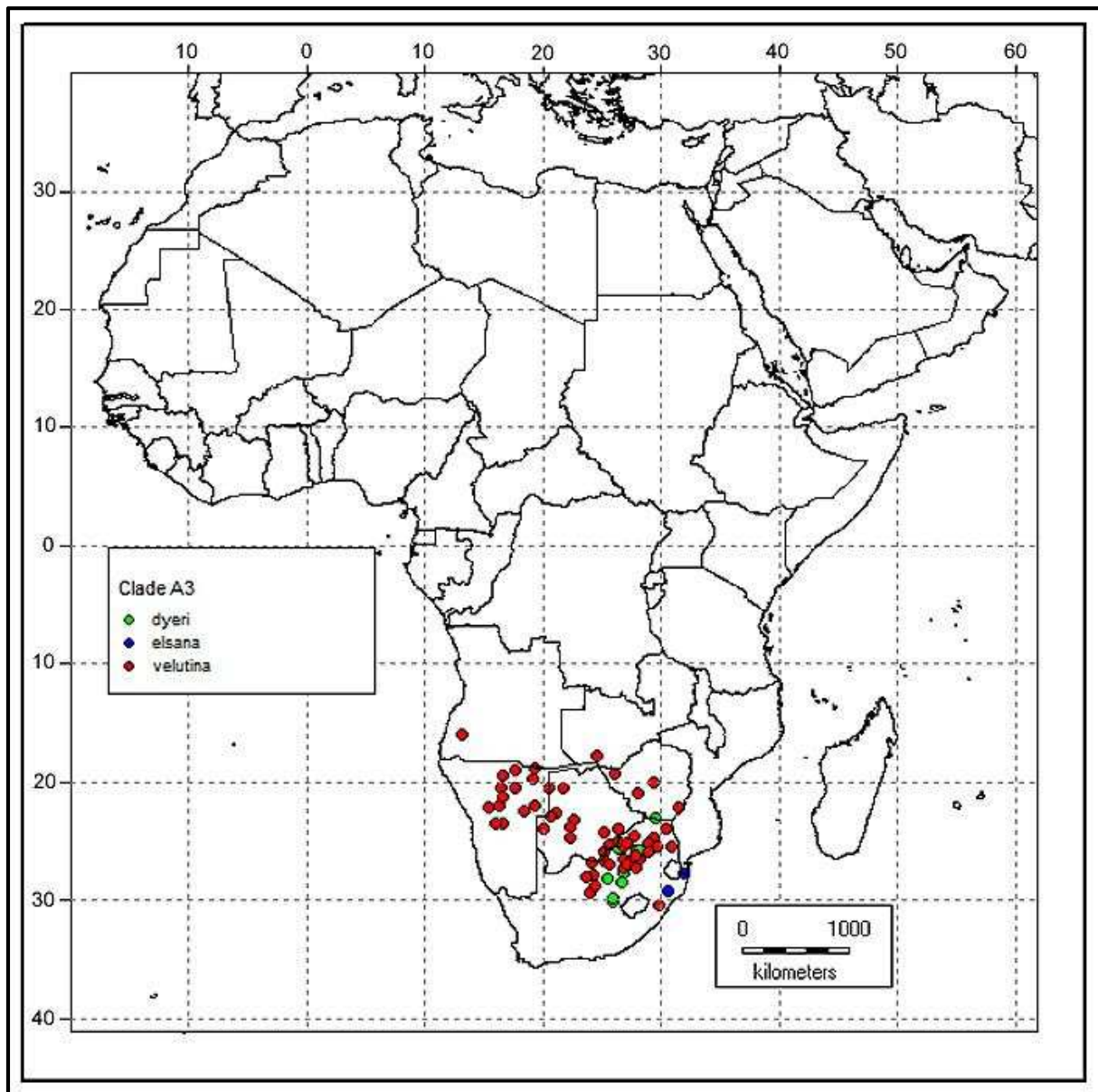


Fig. 5.4. Distribution of *Raphionacme* species within sub-clade A3.

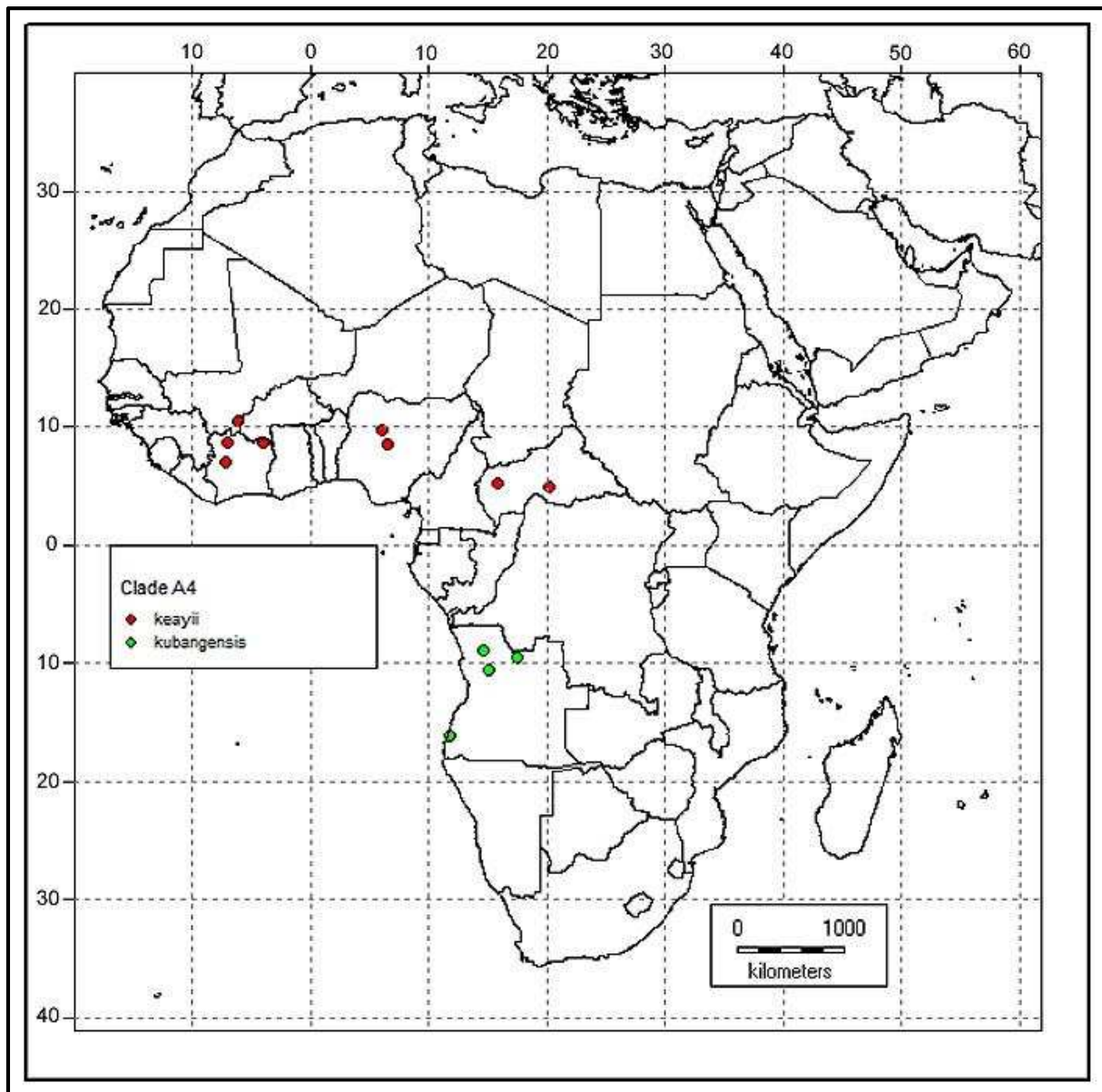


Fig. 5.5. Distribution of *Raphionacme* species within sub-clade A4.

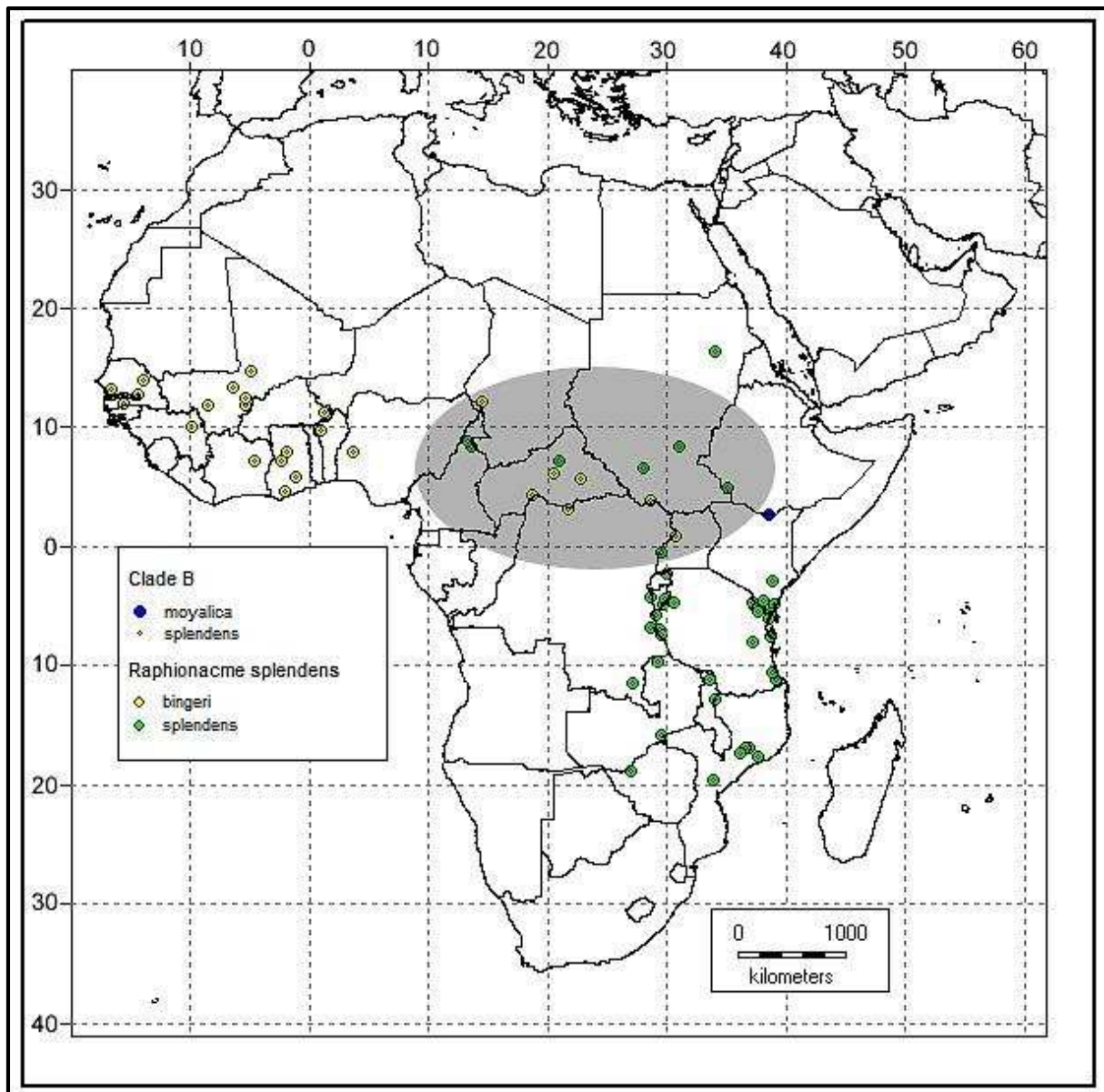


Fig. 5.6. Distribution of *Raphionacme* species within clade B. The grey circle in the middle of the map indicates the distribution overlap of *R. splendens* subsp. *bingeri* and *R. splendens* subsp. *splendens*.

The two subspecies of *R. splendens* are distributed throughout seven of the phytochoria, namely Zambebian Region, Sudanian Region, Somalia-Masai Region, Guinea- Congolia/Sudania Transition Zone, Lake Victoria Mosaic, Zanzibar-Inhambane Mosaic and Sahel Transition Zone.

Raphionacme moyolica was collected in mountain scrub while the two *R. splendens* subspecies occur in a wide array of vegetation types, ranging from scrub forest to coastal and inland open or dense savanna and grassland. Some collections of *R. splendens* were made in seasonal swamps. Even though both *R. splendens* subspecies occur in the same type of vegetation, *R. splendens* subsp. *bingeri* is found in more humid habitats compared to *R. splendens* subsp. *splendens*. *Raphionacme splendens* subsp. *bingeri* has a more western distribution, while *R. splendens* subsp. *splendens* were found to be distributed in eastern Africa.

The second largest clade in *Raphionacme* was clade C, sister to clades A and B and this branch was supported by moderate to high values (BS 77%, PP 1.00), as was the support of clade C itself (BS 69%, PP 1.00). Sub-clade C1, with moderate to high support values (BS 61%, PP 1.00), included *R. palustris* and two forms of *R. hirsuta*. Placement of the rest of the species in this clade was unresolved with no support.

The five species in this clade are herbaceous, corolla lobes adaxially blue/pink/purple and spreading and the coronal feet free from one another. These species occur in the southern hemisphere between 0° S and 30° S (Fig. 5.7) and are distributed throughout six phytochoria, namely the Zambebian Region, Karoo-Namib Region, Afromontane Archipelago-like Region, Kalahari-Highveld Transition Zone, Tongaland-Pondoland Mosaic and Zanzibar-Inhambane Mosaic. The vegetation types, in which these plants occur, range from woodland and savanna to heathland and grassland. Some of the *R. longituba* material was collected in temporary marshes while all *R. palustris* specimens came from seasonally wet swamps and moist grassland. In contrast, *R. namibiana* occurs in the arid semi-desert karroid-succulent Nama-Karoo Biome.

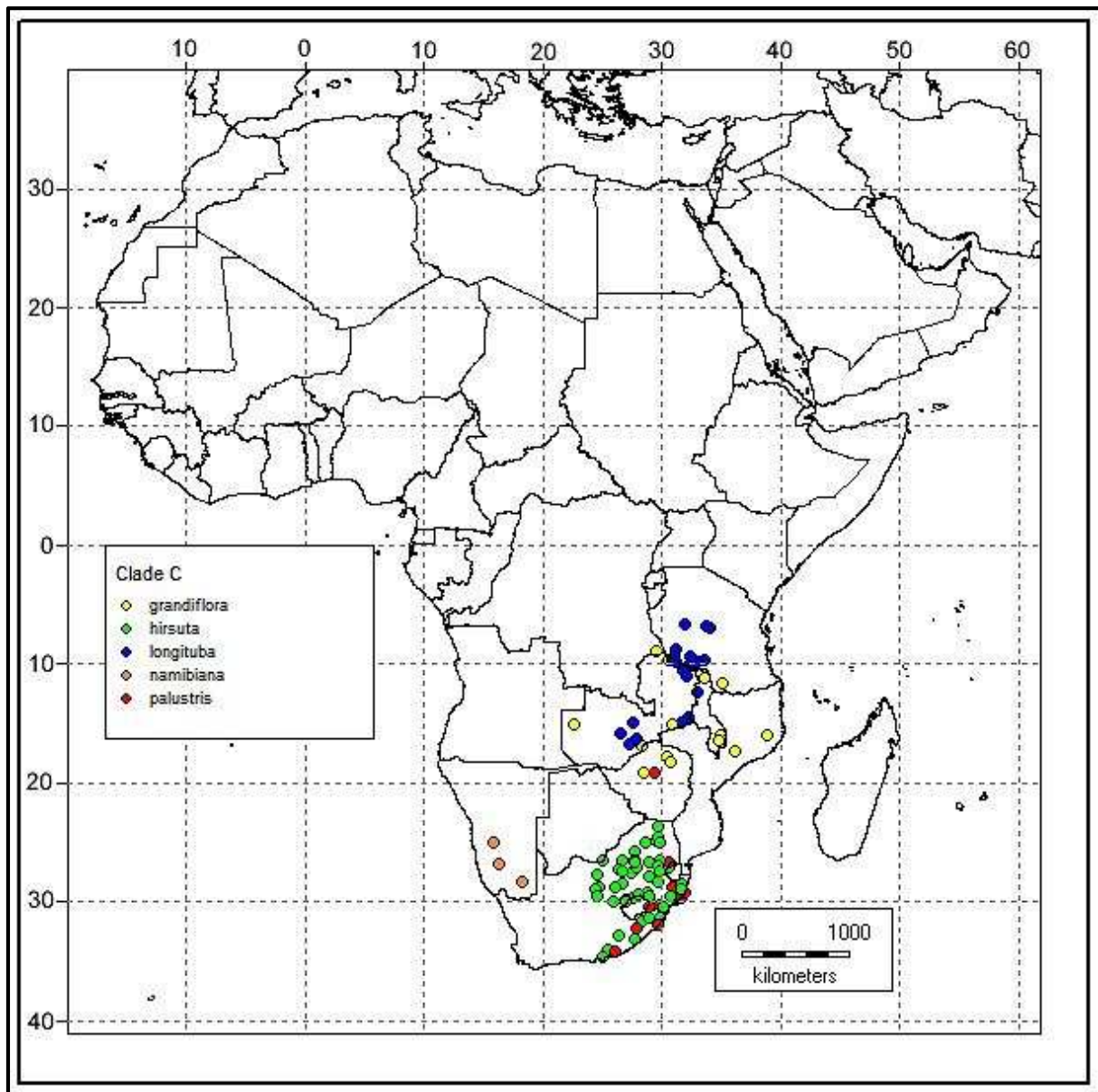


Fig. 5.7. Distribution of *Raphionacme* species within clade C.

In clade C, sub-clade C1 was identified consisting of the two forms of *R. hirsuta* and *R. palustris*. Both species have typically dichotomously branching stems (a characteristic considered to be primitive), campanulate corolla tubes equal to 5 mm or shorter and apically divided coronal lobes. The two species of sub-clade C1 are distributed through four phytochoria namely the Zambezi Region, Afromontane Archipelago-like Region, Kalahari-Highveld Transition Zone and Tongaland-Pondoland Mosaic (Fig. 5.8) and are usually associated with savanna and grassland.

Morphologically *R. longituba* shares the typical dichotomously branched stems and apically divided coronal lobes of the species in sub-clade C1 but support for inclusion of *R. longituba* as part of this sub-clade was weak (BS 41%, no support for PP). However, *R. longituba* is the only other species to have a corolla tube longer than 5 mm as in the other species of clade C, *R. grandiflora* and *R. namibiana*. This morphological affinity of *R. longituba* to both sub-clade C1 and the other two species would explain the weak support values within this sub-clade rendering the placement of the species in this clade as unresolved. The presence of anther callosities in only *R. grandiflora* and *R. namibiana* (also in *R. caerulea*, not included in this study) should be of significant value as these structures occur only rarely in the Periplocoideae. In the African members of the Periplocoideae, only one other species, *Chlorocyathus lobulata*, has callosities (Venter, 2008), while only one Asian genus, *Finlaysonia*, has been positively characterised as having anther callosities (Nwigwe, 2012).

Clade D was the earliest diverging clade in *Raphionacme* and was sister to the rest of the *Raphionacme* species including the outgroup species *Schlechterella abyssinica* (BS 94%, PP 1.00). The very well supported clade D (BS 94%, PP 1.00) consisted of three species, *R. haeneliae*, *R. pulchella* and *R. utilis*. According to the phylogenetic results, this clade is probably closest to the ancestral *Raphionacme* species. These three species are herbs with lateral branching stems and campanulate corolla tubes equal to or shorter than 5 mm. Distribution of this clade's species are throughout four phytochoria, namely Zambezi Region, Karoo-Namib Region, Guinea-Congolia/Zambezi Transition Zone and Guinea-Congolia/Sudania Transition Zone (Fig. 5.9). *Raphionacme pulchella* and *R. utilis* are found in vegetation types varying from savanna to grassland, but *R. haeneliae* is found in true desert habitat.

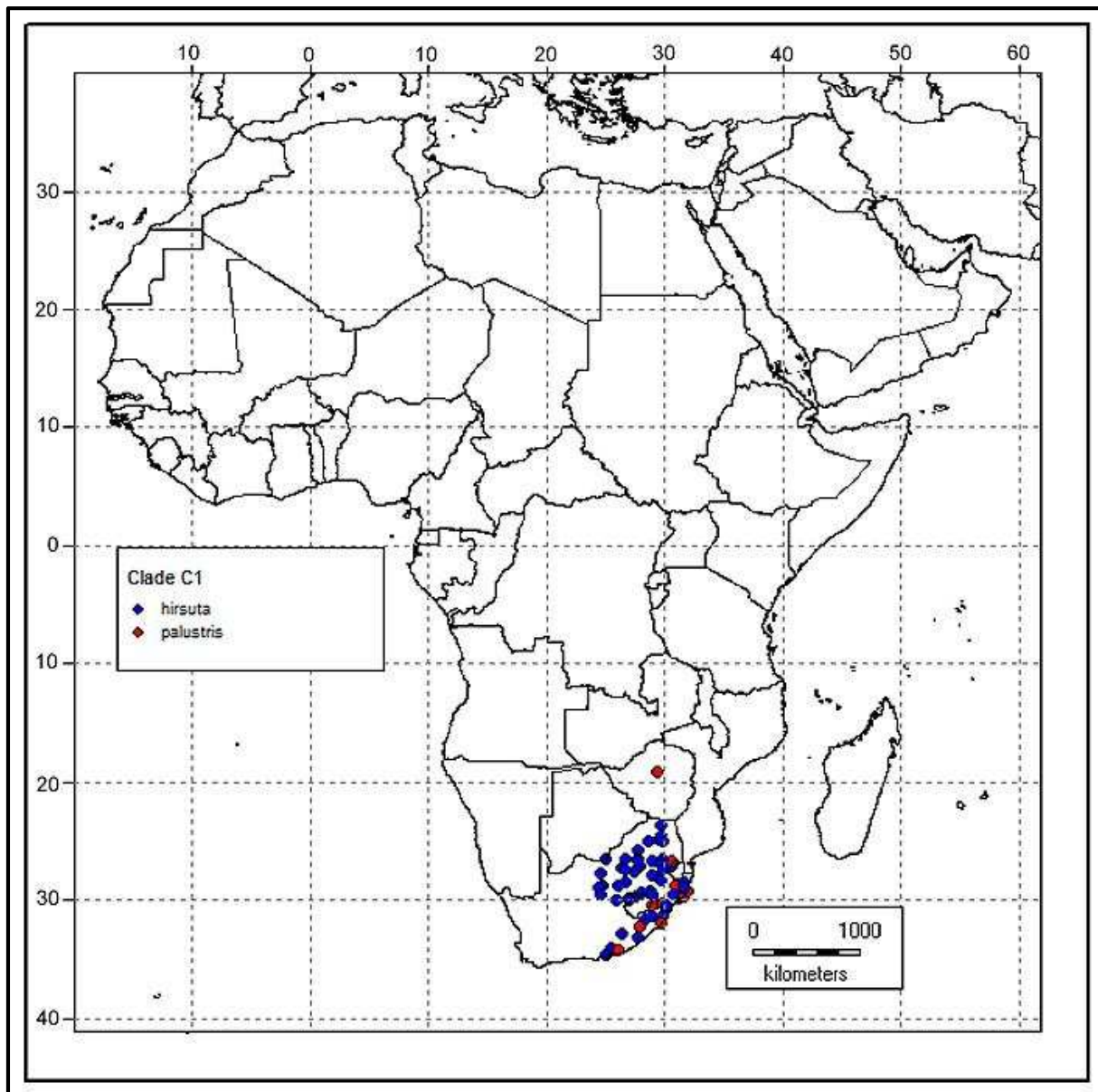


Fig. 5.8. Distribution of *Raphionacme* species within sub-clade C1.

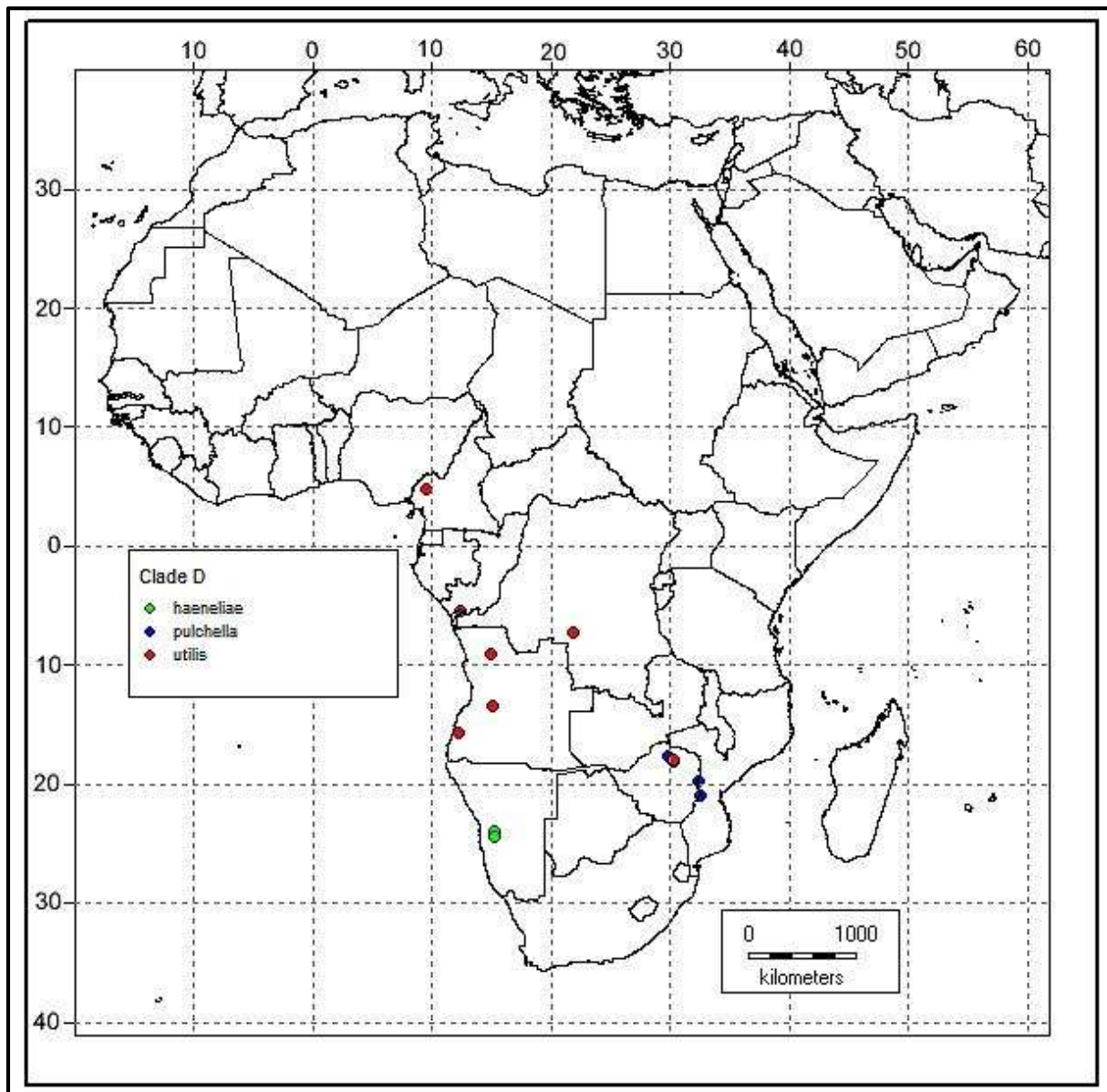


Fig. 5.9. Distribution of *Raphionacme* species within clade D.

5.3. Cladistical indications for taxonomic questions

5.3.1. Forms of *Raphionacme hirsuta*

Venter (2009) distinguished two forms of *R. hirsuta* based on morphological differences. In the first form, leaves are nearly as wide as they are long and elliptic to broadly elliptic to nearly discoid, the lamina apex is truncate-mucronate and indumentum on the plants ranges from glabrous to somewhat hirsute.

The second form has elliptic to narrowly elliptic or obovate to narrowly obovate leaves, acuminate, rarely obtuse-mucronate, apices and indumentum ranges from glabrous to densely hirsute (Fig. 5.10). The distribution of these two forms indicates that they grade into each other, although they are somewhat isolated from each other. The phylogenetic analysis indicates that the two forms of *R. hirsuta* are closely related and comparison of the nucleotide sequences shows only eight differences in the alignment of the nuclear ITS. These nucleotide differences are as follows: one possible transition (C/Y), one A/G transition, five C/T transitions and one deletion/insertion (Fig. 5.11).

The first form is distributed in areas west of the Drakensberg, South Africa, while the second form is distributed from Lydenburg in Mpumalanga, through Swaziland and KwaZulu-Natal to the Transkei in the Eastern Cape Province (Venter, 2009). The slight molecular variation and the geographical overlap of the two *R. hirsuta* forms prevent any formal taxonomic status, such as variety or subspecies rank (Du Rietz, 1930). A similar conclusion was reached by Venter (2009) who suggested that *R. hirsuta* be regarded as a morphologically heteromorphic species which has dispersed over a large area of varied environments.

5.3.2. *Raphionacme splendens* – unite or divide

Meve et al. (2002) combined three *Raphionacme* species, namely *R. excisa*, *R. splendens* and *R. bingeri*, under *R. splendens* giving as motivation the grading of growth form where these three species come into contact. Although the three species have some morphological differences, they exhibit the same unique floral morphology, even though the flowers differ in size (Fig. 5.12).



Fig. 5. 10. Voucher specimens of the two *Raphionacme hirsuta* forms. (i) *R. hirsuta* form 1. (ii) *R. hirsuta* form 2.

R. hirs G1	CGGAAGGATCATATGTCGAATCCTAC-AAAAGC-AAATGA-CTAGCGAAAGTATGTTTTTC
R. hirs G2	CGGAAGGATCATATGTCGAATCCTAC-AAAAGC-AAATGA-CTAGCGAAAGTATGTTTTTC
R. hirs G1	AAT---TAGGGAGGT-AGGCAG-TTGGGCT-CAAACCCCTTTGC--AATCTCTCCTCTCG
R. hirs G2	AAT---TAGGGAGGT-AGGCAG-TTGGGCT-CAAACCCCTTTGC--AATCTCTCCTCTCG
R. hirs G1	GTCGA-TCGGTGCCTTTGCACGGTTCTC-GGTTGTGCCGTATAACAAATTAATAAATTCGG
R. hirs G2	GTCGA-TCGGTGCCTTTGCACGGTTCTC-GGTTGTGCCGTATAACAAATTAATAAATTCGG
R. hirs G1	CGTGGGAA--GCGCCAAGG-ACTATACTAAAGGAATGCCTCTTGGCGATCTGGTTGTTCTG
R. hirs G2	CGTGGGAA--GCGCCAAGG-ACTATACTAAAGGAATGCCTCTTGGCGATCTGGTTGTTCTG
R. hirs G1	CAACCATGAGTCGTCGGGGTTAAGGCTCCATTT-AAATCTAAATGACTCTCGGCAACGG
R. hirs G2	CAACCATGAGTCGTCGGGGTTAAGGCTCCATTT-AAATCTAAATGACTCTCGGCAACGG
R. hirs G1	ATATCTAGGCTCTCGCATCGATGAAGAAGCTAGCAAAGTGGGACTTGGTGTGAATTGC
R. hirs G2	ATATCTAGGCTCTCGCATCGATGAAGAAGCTAGCAAAGTGGGACTTGGTGTGAATTGC
R. hirs G1	AGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCC-TGAAGCCATTAGGCTGA
R. hirs G2	AGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCC-TGAAGCCATTAGGCTGA
R. hirs G1	GGGCACGTCTGCCTGGGGCGTCACACAATGCG-TCGCTCCCTCACAGCTCG-CCT----TG
R. hirs G2	GGGCACGTCTGCCTGGGGCGTCACACAATGCG-TCGCTCCCTCACAGCTCG-CCT----TG
R. hirs G1	CATG-GGAGGAGTGTTC-TAA-TGGGGGC-GGAGAATGGCCTCCCCTGCATAGTTGCGG
R. hirs G2	CATG-GGAGGAGTGTTC-TAA-TGGGGGC-GGAGAATGGCCTCCCCTGCATAGTTGCGG
R. hirs G1	CTGGCTGAAATTGAAGTC--CCTCATCGC-GGGGGTCGCAACAAGTGGTGGTTGAAAAGC
R. hirs G2	CTGGCTGAAATTGAAGTC--CCTCATCGC-GGGGGTCGCAACAAGTGGTGGTTGAAAAGC
R. hirs G1	TCGAACGAGTTGTGCGCACCATGCGATTGA-GGTGACATTTTAGACCCTAAGGTGATCTT
R. hirs G2	TCGAACGAGTTGTGCGCACCATGCGATTGA-GGTGACATTTTAGACCCTAAGGTGATCTT
R. hirs G1	CC-TATAGAGGAGCGTTTGTACGACTG-CGACCCCAAGGT-CAGG
R. hirs G2	CC-TATAGAGGAGCGTTTGTACGACTG-CGACCCCAAGGT-CAGG

Fig. 5.11. ITS sequence alignment of the two *Raphionacme hirsuta* forms. Differences in nucleotides are indicated in red.



Fig. 5.12. Specimens of (i) *Raphionacme splendens* subsp. *bingeri* and (ii) *R. splendens* subsp. *splendens*.

The phylogenetic results of this study necessitated a re-evaluation of the relationship between these taxa. *Raphionacme splendens* together with *R. moyalica* forms clade B. According to both the nuclear ITS and combined phylogenetic trees *R. splendens* subsp. *bingeri* is more closely related to *R. moyalica* than to *R. splendens* subsp. *splendens* (Fig. 4.4; Fig. 4.5). This result is an indication that the combination of the three species may be incorrect and that at least two separate entities can be identified.

Morphologically both *R. splendens* and *R. excisa* are characterised by erect stems which produce linear to narrowly lanceolate leaves only after flowering with the flowers being relatively large in the former species and smaller in *R. excisa*. *Raphionacme splendens* is distributed through Uganda, the Democratic Republic of the Congo, Rwanda, Burundi and Tanzania while the distribution of *R. excisa* stretches from the Sudan southwards to Mozambique. However, the third species, *R. bingeri*, is morphologically distinct from the previous two in having zigzag-shaped stems, distinctly broader leaves ranging from ovate to elliptic and flowers that are conspicuously smaller (Fig. 5.12). *Raphionacme bingeri* is distributed in the western tropical countries of Africa, from Senegal to Nigeria, with some specimens collected in Chad, southern Sudan, the Central African Republic and Uganda (Venter, 2009). Venter (2009) concurs with the combination of *R. excisa* and *R. splendens* but regards *R. bingeri* morphologically as sufficiently unique to be given subspecies status.

According to Du Rietz (1930), a subspecies is regarded as a population of several biotypes forming more or less distinct regional forms of a species. Morphologically distinct but interfertile populations of a species growing in different geographical regions are maintained as distinct subspecies due to the geographical isolation of the species. However, the distribution ranges of the two *R. splendens* subspecies overlap in the Central African Republic, southern Sudan and Uganda (Fig. 5.6).

Whereas molecular evidence generated in this study and morphological differences support Venter's (2009) division of *R. splendens*, the distribution overlap is contrary to the definition of subspecies. It might be appropriate to elevate the two subspecies to species rank once more.

5.3.3. Sectional classification of *Raphionacme*

Venter and Verhoeven (1988), recognising Schumann's (1895) sectioning of *Raphionacme* according to morphological characteristics, divided the 31 *Raphionacme* species known to them then, into four sections, namely *Raphionacme* sect. *Raphionacme*, *Raphionacme* sect. *Cephalacme*, *Raphionacme* sect. *Speiracme* and *Raphionacme* sect. *Pseudochironia* (Table 2.2). The eight species in *Raphionacme* sect. *Raphionacme* are all erect or decumbent herbs, while the five species in *Raphionacme* sect. *Cephalacme* are erect herbs with flowers borne in compact terminal inflorescences. *Raphionacme* sect. *Speiracme* contains seven species of which five (*R. flanaganii*, *R. longifolia*, *R. monteiroae*, *R. sylvicola* and *R. welwitschii*) are climbers and *Raphionacme* sect. *Pseudochironia* with 11 species has the most conspicuous flowers and is also the most tropical (Venter and Verhoeven, 1988).

Comparing these morphologically based sections with the cladistical results in the combined tree, it is clear that the clades do not correspond to the sections proposed by Venter and Verhoeven (1988) (Fig. 5.13). The species in *Raphionacme* sect. *Raphionacme* are spread out across clades A and C, while the species in *Raphionacme* sect. *Cephalacme* occur in both clades A and D.

Raphionacme sect. *Speiracme* seems to be a more natural grouping, consisting of species more closely related and are found in clade A only, while *Raphionacme* sect. *Pseudochironia* are found in all the clades except in clade D. Sub-clade A3 and C1 are the only sub-clades containing species which occurs in the same sections.

5.3.4. Position of *Schlechterella abyssinica*

Schlechterella was first described by Karl Schumann in 1899 as a monotypic genus to accommodate one African species, *S. africana* (Schltr.) K.Schum. In 1998, Verhoeven and Venter discovered the presence of pollinia in *Raphionacme abyssinica* Chiov., a unique characteristic in the African Periplocoideae at the time, thus also in *Raphionacme*. However, when pollinia were discovered in *S. africana*, *R. abyssinica* was transferred to *Schlechterella* as *S. abyssinica* (Chiov.) Venter & R.L.Verh.

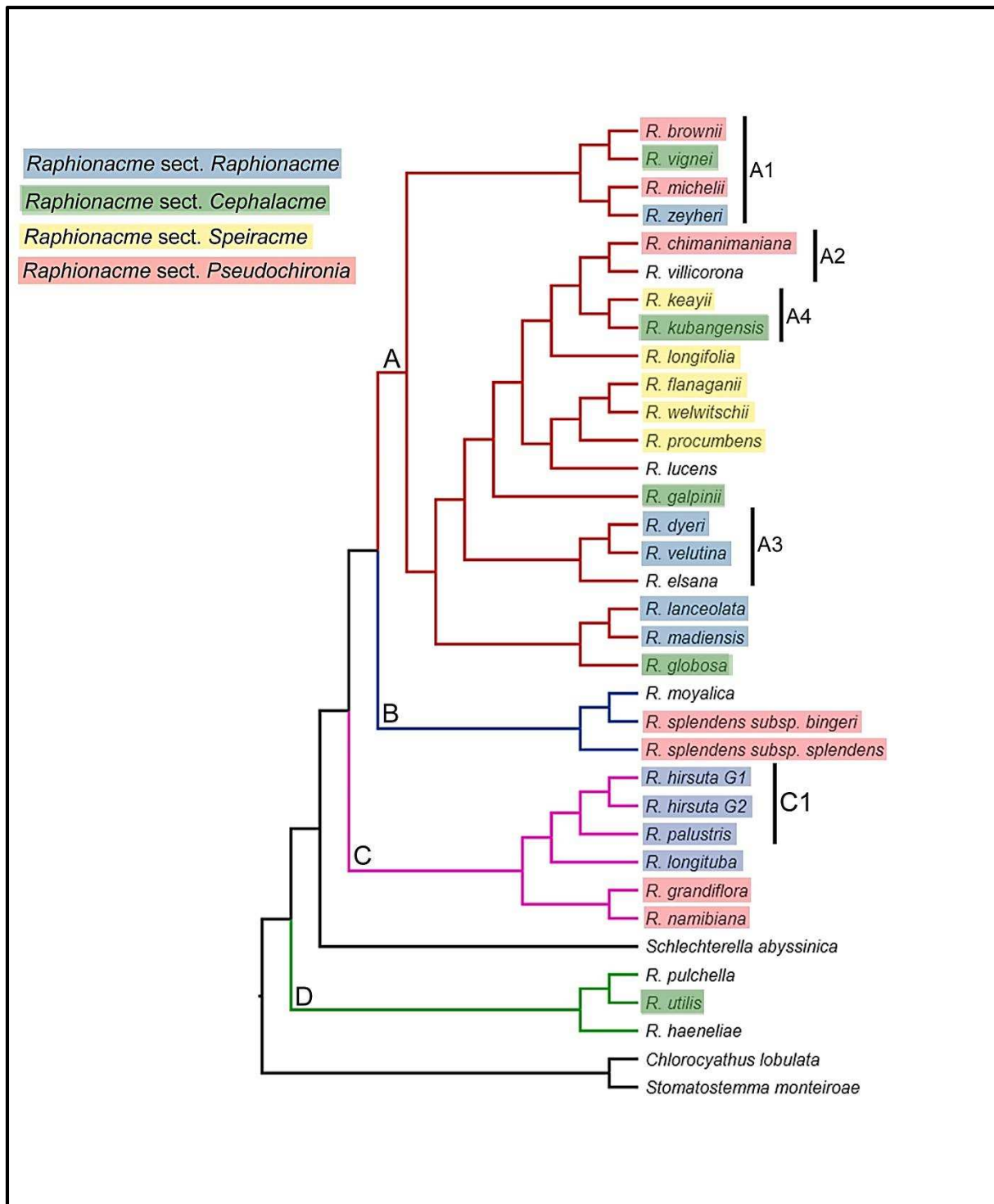


Fig. 5.13. Sections of *Raphionacme* plotted onto the combined data cladogram.

The morphological phylogenetic results of the current study indicates, with moderate support, that *Raphionacme* is monophyletic (BS 55%, PP 0.60), but when the outgroup species, *Schlechterella abyssinica*, is included in the ingroup, the support increases dramatically (BS 90%, PP 0.97) (Fig. 4.3).

A morphological study by Venter and Verhoeven (2001) that included all the *Raphionacme* and *Schlechterella* species indicated a strong morphological relationship between these two genera (BS 97%). This result is supported by the findings of Ionta (2009) in which the majority rule tree from maximum parsimony, based on morphological data, indicated that both *Schlechterella* species are nested within *Raphionacme* with a very high support value of 100%.

The nuclear ITS phylogenetic tree (Fig. 4.4) indicated that *Schlechterella abyssinica* is nested within the *Raphionacme* clade, but with low to moderate support (BS-MP 40%, BS-ML 38%, PP 0.54). However, the results for the combined molecular and morphological data supported *Schlechterella abyssinica* as part of the *Raphionacme* clade with high values (BS 94%, PP 1.00) (Fig. 4.5). These results indicate that to obtain a monophyletic *Raphionacme* genus, *Schlechterella abyssinica* has to be included. It would, therefore, seem that the transfer of *R. abyssinica* to *Schlechterella* was incorrect, and that there is indeed one *Raphionacme* species with pollinia. It is unfortunate that the only other species in *Schlechterella*, *S. africana* (Schltr.) K.Schum., was not included in this analysis, but as this species identity was not in doubt initially, it was excluded.

5.4. Distribution of endemism and species richness

The term 'endemic' refers to a taxon restricted in its range to a specified geographical region. This limitation in range can be due to historical, ecological or physiological reasons. Endemic species can be classified according to their presumed origins. Palaeoendemic species are remnants of species that previously had a wider distribution.

This is due to the shrinkage of widespread geographic ranges as a result of climatic or habitat changes which became fragmented, are well-differentiated and taxonomically isolated (clearly delimited species) (palaeoendemism).

Neoendemic species are new or evolutionary young species, typically with existing relatives in close proximity (neoendemism) (Van Wyk and Smith, 2001). The establishment of neoendemics include allopatric divergence, ecological speciation, peripheral isolate formation and hybridization (Harrison, 2013).

Species that are habitat specialists where special climatic conditions prevail and are confined to a small area because of the restricted range are termed holoendemics (Richardson, 1978; Van Wyk and Smith, 2001). When a taxon is marginally existent elsewhere (in the form of distant satellite populations), it is called a near-endemic. Consideration of the probable evolutionary history of a species and its habitat is vital for the understanding of plant geographical patterns, since the geographical range of a species alters as it evolves (Van Wyk and Smith, 2001).

A geographical region is termed a 'centre of endemism' when this region can be distinguished by a certain combination of endemic plant taxa, with the endemic taxa signifying the geographical element which most naturally depicts the floristic uniqueness of that region (Van Wyk and Smith, 2001). The 'Regional Centre of Endemism' of White (1983) should not be confused with the 'centre of endemism' of Van Wyk and Smith (2001).

5.4.1. Distribution of *Raphionacme* species within Regions of Endemism

The phytochoria of White (1983), including his 'Centres of Endemism', indicate floristic regions of several ranks and indicate a complex hierarchy in which smaller regions are nested within successively larger regions. Due to this fact, it is advisable to also focus on the distribution of endemic species in the localized 'Centres of Endemism' that were identified by Van Wyk and Smith (2001).

According to the phylogenetic results, 19 of the 30 *Raphionacme* species included in this study are present in ten of the Centres of Endemism as identified by Van Wyk and Smith (2001), while three of the four species richness areas of *Raphionacme* also fall within seven of these Centres (Fig. 4.2). The ten Endemic Centres in which *Raphionacme* occur are the Maputaland Centre, Pondoland Centre, Albany Centre, Barberton Centre, Wolkberg Centre, Sekhukhuneland Centre, Soutpansberg Centre, Chimanimani-Nyanga Centre, Great Dyke Centre and Kaokoveld Centre.

Although six *Raphionacme* species, *R. hirsuta*, *R. palustris*, *R. flanagani*, *R. galpinii*, *R. elsana* and *R. lucens*, occur in the Maputaland Centre, a nearly flat low-level coastal plain with a maximum elevation of 150 m, only *R. elsana* and *R. lucens* can be classified as near endemics (Van Wyk and Smith, 2001). The latter two species are endemic to White's (1983) Tongaland-Pondoland Regional Mosaic where a tropical/subtropical climate prevails with relatively high humidity on the plains, even away from the coast. Even with the tropical/subtropical climate, there is a prominent dry season which occurs in winter (White, 1983). Many of the endemics in the Maputaland Centre seems to be of recent diversification, supported by the fact that some are distinguished at infraspecific level only, with the nearest relatives still present. This Centre is well known for its biological evolution, which includes speciation that appears to be very active amongst plants (Van Wyk and Smith, 2001). Both *R. elsana* and *R. lucens* occur in clade A (Fig. 4.4; Fig. 4.5; Fig. 5.1), the youngest and most recently diversified of the four clades which is in accordance with the information given by Van Wyk and Smith (2001). One of these evolutionary events includes that of the development of a geoxylic suffruticose habit (plant producing annual shoots from a woody subterranean base), which signifies a long evolutionary history for this particular vegetation type in this region (Matthews et al., 1999).

The rugged plateaux and subtropical climate characterises the Pondoland Centre, which is deeply dissected by narrow river gorges (Van Wyk and Smith, 2001). The vegetation of this Centre consists mainly of grassland, considered to be the densest in southern Africa and particularly vigorous (Acocks, 1953; Shackleton et al., 1991), with a few isolated forest patches limited mainly to protected riverine gorges. Even though *R. galpinii* and *R. palustris* are distributed through this Centre, they were found not to be endemic to this region.

The Albany Centre consists of a mosaic of diverse floristic and vegetation elements. Five of White's (1983) main phytochoria, Cape Region, Karoo-Namib Region, Tongaland-Pondoland Regional Mosaic, Afromontane Region and the Kalahari-Highveld Regional Transition Zone, meet at this Centre (Van Wyk and Smith, 2001). This results in a wide diversity of vegetation ranging from forest, thicket, savanna, Nama-Karoo, Fynbos and grassland.

The grassland and forest are associated with the inland mountains and the coastal zone, while savanna is locally present in some of the transitional areas between succulent thicket and Karoo (Van Wyk and Smith, 2001). The four *Raphionacme* species occurring in this Centre are *R. hirsuta*, *R. palustris*, *R. flanaganii* and *R. zeyheri*. *Raphionacme zeyheri* appears to be endemic to this Centre while both *R. flanaganii* and *R. zeyheri* are endemic to the Tongaland-Pondoland Regional Mosaic of White (1983) which includes the Albany Centre.

The Barberton Centre, known for being rugged and mountainous with some of the peaks rising to over 1800 m, falls within the summer-rainfall region. The vegetation is generally classified as North-Eastern Mountain Grassland which is intermingled with Sour Lowveld Bushveld and Afromontane Forest. The grassland (North-Eastern Mountain Grassland) predominates and is intermingled with pockets of Sour Lowveld Bushveld and Afromontane Forest which is restricted to incised valley heads, sheltered ravines and moist valleys (Van Wyk and Smith, 2001). This Centre contains three *Raphionacme* species, *R. hirsuta*, *R. galpinii* and *R. procumbens*, none of which are endemic.

The northern and eastern parts of the Wolkberg Centre are very mountainous with steep slopes and vertical cliffs, but to the west the relief becomes less rugged with more rolling hills (Van Wyk and Smith, 2001). This Centre is climatically characterised by hot, wet summers and dry, cool winters. Rainfall declines rapidly away from the crests on both the windward and leeward slopes. Most of the northern area falls within the rain and mist shadow of the main escarpmental crest and is therefore somewhat arid compared to the associated Black Reef Formation and Wolkberg Group (Van Wyk and Smith, 2001).

The vegetation is primarily montane grassland, with pockets of Afromontane Forest which is limited to the slopes of the main Escarpment. The Wolkberg Centre, especially the more arid regions, shares many species with the adjacent Sekhukhuneland Centre (Van Wyk and Smith, 2001). The four species found in the Wolkberg Centre are *R. galpinii*, *R. procumbens* and *R. velutina* was found in the Sekhukhuneland Centre.

The Sekhukhuneland Centre is known for its parallel belts of rocky ridges and mountains with undulating valleys. This Centre falls within the rain shadow of the Drakensberg Escarpment and is therefore fairly arid when compared to the adjacent areas. Some exceptions are the subtropical climate in the bordering Lowveld, temperate climate with frost and much wetter conditions to the north, east and south with precipitation mainly between December and April. Almost pure grassland covers some of the mountain slopes in the region with small patches of Afromontane Forest and associated Fynbos-type vegetation on mountain summits (Van Wyk and Smith, 2001). *Raphionacme villicorona* was found to be endemic to the Sekhukhuneland Centre and this species is endemic to White's (1983) Zambebian Region as well. Only one other species *R. galpinii*, a non-endemic, occurs in this Centre.

The Soutpansberg Centre is a relatively narrow mountain range varying in altitude. In the west it is separated by a narrow gap from the Blouberg, which rises to 2051 m (Van Wyk and Smith, 2001). This Centre mainly receives summer rain, with the southern slopes undergoing orographic rain, contributing to a much wetter habitat than the northern slopes which fall in the rain shadow area. This sharp north-south gradient in rainfall is found to greatly contribute towards the species richness of the mountain range. The vegetation consists mostly of different types of bushveld and thicket, with pockets of well-developed Afromontane Forest on various areas of the wet south- and east-facing slopes. Grassland occurs at higher altitudes, predominantly on the southern slopes. The relatively high proportion of endemic succulents in this Centre indicates a long history of arid climatic conditions, especially for the northern foothills (Van Wyk and Smith, 2001). Although four *Raphionacme* species occur in this Centre, neither *R. hirsuta*, *R. galpinii*, *R. procumbens* or *R. velutina* are endemic to this area.

The Chimanimani Mountains are an exceedingly steep and rugged range with deep gorges and high peaks, with the Nyanga Mountains being less rugged with rolling grassland slopes, steep cliffs, deep gorges and rocky hills. These two mountain ranges constitute the Chimanimani-Nyanga Centre (Van Wyk and Smith, 2001). Fog and orographic rains are common because of the moisture-laden winds blowing across the lowlands from the Indian Ocean, with rain falling throughout the year, peaking in spring and summer (Van Wyk and Smith, 2001). Rainfall generally increases from west to east (Clarke, 1991).

Afromontane grassland predominates at medium and high altitudes with savanna occurring at lower altitudes in warmer and drier sites and *Brachystegia* (miombo) woodland on some of the rain-shadow slopes (Van Wyk and Smith, 2001). Endemism has been encouraged by the Chimanimani Mountains which forms an edaphic ecological island (Wild, 1964). For at least millions of years the Chimanimani-Nyanga Centre must have been isolated from other Afromontane 'islands' by the hot and dry Zambezi Valley in the north and the correspondingly harsh conditions in the Limpopo Valley in the south (Van Wyk and Smith, 2001). Many endemics found in this Centre are very well speciated and are inferred as palaeoendemics (Wild, 1964). Four *Raphionacme* species occur in this Centre, these being *R. pulchella*, *R. chimanimaniana*, *R. lanceolata* and *R. longifolia*, but only *R. chimanimaniana* is endemic to this Centre.

The Great Dyke Centre is associated with rocky hills, whereas in the central sectors ridge topographies are more subdued (Wild, 1965; Proctor and Cole, 1992). This Centre mainly receives rain between December and April and the dominant vegetation is *Brachystegia-Julbernardia* (miombo) woodland, but savanna and grassland are also present. Riverine forest communities are present along the larger streams and rivers, predominantly in the northern part of the Great Dyke. Most of the Great Dyke Centre flora is unmistakably derived from the prevailing surrounding flora which is of Zambezian affinity (Van Wyk and Smith, 2001). Six *Raphionacme* species occur in and around the Great Dyke Centre, these species being *R. pulchella*, *R. utilis*, *R. grandiflora*, *R. lanceolata*, *R. longifolia* and *R. madiensis*. *Raphionacme pulchella* was found to be endemic to White's (1983) Zambezian Region, but not to the Chimanimani-Nyanga Centre. Both *R. pulchella* and *R. utilis* occur in the oldest diverging clade D (Fig. 5.10).

The Kaokoveld Centre consists of rugged mountains divided by broad plains and deeply incised valleys (Hall-Martin et al., 1988). This region includes the Namib Desert and the interior highlands, with this two regions being divided by a deeply dissected escarpment (Van Wyk and Smith, 2001). The vegetation ranges from desert to arid savanna. The coastal plateau and pro-Namib are covered by sparse grassland, while the Escarpment Zone is mountainous and rocky with larger woody plants and trees. Over a long period of time this Centre has seemingly served as a sanctuary for taxa that can survive under arid conditions (Van Wyk and Smith, 2001).

The three *Raphionacme* species occurring in this Centre are *R. kubangensis*, *R. lanceolata* and *R. utilis* but none of them are endemic.

Van Wyk and Smith (2001) list *R. namibiana* as endemic to the Gariiep Centre. According to the present study the distribution of *R. namibiana* is further north of the Orange River in central Namibia, in the Nama-Karoo Biome, where it seems to be endemic (Fig. 4.1).

5.4.2. Species richness

The four phytochoria in which the highest concentration of *Raphionacme* species occur are Tongaland-Pondoland Mosaic, Zambezi Region, Karoo-Namib Region and Kalahari-Highveld transition zone (Fig. 4.2). This finding is in accordance with that of Lebrun (1976), Brenan (1978) and Goldblatt (1978), where they revealed that southern Africa is much richer in species than east Africa. Linder (2001) also found that the southern hemisphere has a much higher grid-diversity than the northern hemisphere for equivalent latitudes. It was suggested by Linder (2001) that the proximity to the Sahara plays a debilitating effect on species richness, therefore there is an occurrence of species poor areas in northern Africa. It was also suggested that rainfall does not adequately explain the differences between the northern and southern hemispheres.

The Tongaland-Pondoland Mosaic contains the highest concentration of *Raphionacme* species and is found at the northeastern side of South Africa. Within this area falls Van Wyk and Smith's (2001) Maputaland Centre, which is well known for its high levels of endemism. This area of species richness contains ten *Raphionacme* species.

The vegetation is very variable and subject to moderately high and well-distributed rainfall along the coast. The ten species are *R. dyeri*, *R. elsana*, *R. flanagani*, *R. galpinii*, *R. hirsuta*, *R. lucens*, *R. palustris*, *R. procumbens*, *R. velutina* and *R. villicorona*.

The Zambezian region contains two areas where there is a high occurrence of *Raphionacme* species. The one region is in the northeastern part of Zimbabwe, which includes the Chimanimani Mountains. Included in this phytochoria of White (1983) are the Chimanimani-Nyanga Centre as well as the Great Dyke Centre of Van Wyk and Smith (2001). This region encompasses ten *Raphionacme* species. These species are *R. chimanimaniana*, *R. grandiflora*, *R. lanceolata*, *R. longifolia*, *R. madiensis*, *R. palustris*, *R. procumbens*, *R. pulchella*, *R. utilis* and *R. welwitschii*. The second region is in the northern part of Zambia, which includes the Zambezi-Congo watershed (Linder, 2001) that was found to be a centre of endemism and had a particular striking richness when compared to equivalent latitudes to the north of the Equator. This region contains seven *Raphionacme* species with these species being *R. globosa*, *R. grandiflora*, *R. lanceolata*, *R. longituba*, *R. madiensis*, *R. sylvicola* and *R. welwitschii*.

The smallest area of *Raphionacme* species richness occurs in the south-western part of Angola and falls within the Karoo-Namib region and Kalahari-Highveld transition zone. Both these phytochoria consist mainly of dry vegetation types and also includes the Kaokoveld Centre of Van Wyk and Smith (2001) which were found to be a centre of endemism. This area of species richness is comprised of seven *Raphionacme* species, including *R. inconspicua*, *R. lanceolata*, *R. utilis*, *R. longifolia*, *R. kubangensis*, *R. globosa* and *R. velutina*.

5.5. Possible origin and distribution of *Raphionacme*

Lahaye et al. (2005) found that the ancestral Secamonoideae had a lianoid growth form and that shrub-like growth forms evolved from these lianescent ancestors. It is clear that the shrub-like Secamonoideae evolved independently in different clades. It is also associated with semi-arid to arid climates of the southwest and central Madagascar, indicating that the driving force behind habit evolution is probably climatic factors.

The phylogenetic results obtained by Livshultz et al. (2007) indicate that the Periplocoideae is one of the early diverging subfamilies in Apocynaceae. The growth forms in the Periplocoideae range from woody climbers in the majority of species, erect or straggling shrubs and rarely herbaceous geophytes and epiphytes (Ionta, 2009). Drawing a parallel between the findings of Lahaye et al. (2005) in the Secamonoideae, the ancestral form in the Periplocoideae seems to be lianescent with herbaceous geophytes and epiphytes the derived habit.

The most recent Periplocoideae phylogenetic study of Ionta (2009) indicates that *Raphionacme* is a recently diverging clade within the Periplocoideae. The fact that all the *Raphionacme* species, except the lianescent *R. sylvicola* that is probably not a *Raphionacme* (Venter and Verhoeven, 2001), are geophytic herbs with taproot tubers (Venter, 2009), therefore exhibiting the derived habit, concurring with Ionta's (2009) phylogenetic results.

According to the phylogenetic results obtained in the current study, the species of clade D were the first to diverge. As all three of the species are found in southern Africa, this could be an indication that *Raphionacme* had its origin south of the Equator, diversified and then radiated northwards (Fig. 4.5; Fig. 5.9).

Raphionacme haeneliae is specifically adapted to the extremely arid habitat of the true desert in the Karoo-Namib Region, the only *Raphionacme* species with succulent stems and leaves (Venter, 2009). This specific adaptation to specialised environmental conditions and the confinement to the small area (Fig. 5.9), suggests that this species is a habitat specialist and can therefore be regarded as a holoendemic. With *R. haeneliae* being one of the early diverging species (Fig. 4.5), this species had time to adapt to the specific environment, as succulence is a specific specialized adaptation to very dry environments.

As described by Stebbins (1952), there are several reasons why plant evolution would be relatively rapid in semi-arid to arid regions. The reasons are that soil and limited precipitation have a greater effect on flora and vegetation than regions with adequate moisture. Semi-arid climates with regional diversity promotes the division of medium to large sized populations into smaller units, which are isolated but can still exchange genes through migration.

Thereafter new populations may be established that can give rise to new species. In dry regions, specialized vegetative structures can evolve which enable the plants to withstand long periods of droughts.

These adaptations include root tubers, deep root systems, succulence, specialized leaf cover as well as reduction in leaf size. Species with low vagility (low dispersal ability and propensity) and high specialization could evolve and these species are more likely to have small distribution ranges (Dynesius and Jansson, 2000; Jansson, 2003), a possible explanation of *R. haeneliae*'s limited distribution.

The five *Raphionacme* species of clade C was the next to diversify and adapt to habitats along the eastern parts of Africa south of the Equator, with *R. namibiana* being the exception, occurring in semi-dry scrub of Namibia. The four eastern species are distributed through savanna and grassland except for *R. palustris* which occur in seasonal swamps. Even though some parts of eastern Africa are humid, rocky habitats which are preferred by *Raphionacme* (Venter, 2009) maintains the presence of arid conditions due to the formation of edaphic sites (Jürgens, 1997).

A factor which could have assisted in the diversification of clade B, consisting of *R. moyolica* and two subspecies of *R. splendens*, into the western parts of Africa north of the Equator, was the continuous belt of arid habitats. This was due to the glacially induced arid phases, which formed a bow east and north around the tropical forest (Jürgens, 1997; Burgoyne et al., 2005). This could have occurred after the movement of the African plate to the north, subjecting the then large forest area to a drier environment, leading to the desiccation of large parts of the forest (Meadows, 1996), but making it a suitable environment for *Raphionacme* to occupy.

The remainder of *Raphionacme* species, comprising clade A, diversified to become widespread throughout an increasingly arid Africa. The majority of these twenty species exhibit a herbaceous geophytic habit, but also include the herbaceous climbers, *R. longifolia* and *R. welwitschii* and a woody climber, *R. flanaganii*. Rowe and Speck (1996) state that clades that have basally evolved a specialized lianoid habit could have developed constraints that limit evolution back to a fully self-supporting habit. Therefore it is suggested that the climbers which occur within *Raphionacme* must not be seen as reversals from their geophytic habit, but as a habit adaptation to their environment.

5.6. Conclusion

This is the first substantial study to include virtually all *Raphionacme* species using molecular analyses with only six of the 36 *Raphionacme* species excluded due to limited or problematic material. Even though the morphological and molecular datasets were found to be incongruent, the combination thereof increased the resolution of the ingroup and maximized parsimony. Overall, the phylogenetic study presented here, has contributed to our understanding of the relationships amongst *Raphionacme* species.

The early systematics of the Apocynaceae *sensu lato* was characterised by major categories based mainly on one or two, easy-to-determine morphological characters – usually orientation of the pollinia and type of corona (Schumann, 1895; Woodson, 1941), even when other characters suggested that this might not be the best choice (Endress, 2004). Especially in the Asclepiadoideae, the two most important characters traditionally used to define tribes are the pollinia orientation and type of corona (Endress, 2004). However, many characters have evolved in parallel at various hierarchical levels, the extent of which has only become apparent with the widespread use of phylogenetic analyses, mainly of molecular data (Endress, 2004).

The results of this study indicate that *Schlechterella abyssinica*, characterized by the presence of pollinia, should be included in *Raphionacme*, a genus previously characterized by the absence of pollinia.

This result supports Venter and Verhoeven's (2001) prediction and the findings of Endress (2004) of multiple origins of pollinia in the Periplocoideae. Pollinia in Periplocoideae evolved polyphyletically (Venter and Verhoeven, 1997) and Venter and Verhoeven (2001) predicted an independent origin of pollinia in the Asian and African Periplocoideae. They concluded that "pollinia of Periplocoideae are not reconstructed as homologous with those of other Asclepiads..., it appears that pollinia may have evolved independently on several occasions in Periplocoideae, based on the distribution of pollinia among putatively isolated genera in each of the three recognized tribes." The position of *S. africana*, the only remaining species in *Schlechterella*, should be investigated to determine whether *Schlechterella* remains 'n monotypic genus or should be transferred to *Raphionacme*.

The transfer of *S. abyssinica*/*Schlechterella* would necessitate an adaptation of the present generic description of *Raphionacme*.

The first diverging *Raphionacme* species originated in southern Africa, indicating an origin south of the Equator. Thereafter the species diversified and radiated northwards, not stretching further than the Saharan desert.

Today, *Raphionacme* are distributed throughout Africa, excluding the Western Cape in South Africa, the wettest parts of Central Africa and the very dry Sahara. The only *Raphionacme* species found outside of Africa is *R. arabica*, which occurs on the Dhofar escarpment in southeastern Oman, Asia. The *Raphionacme* species with the widest distribution are *R. splendens*, *R. brownii*, *R. madiensis*, *R. utilis*, *R. vignei* and *R. welwitschii*. The endemic *Raphionacme* species are *R. arabica*, *R. chimanimaniana*, *R. elsana*, *R. haeneliae*, *R. moyalica*, *R. namibiana*, *R. sylvicola*, *R. villicorona* and *R. zeyheri*.

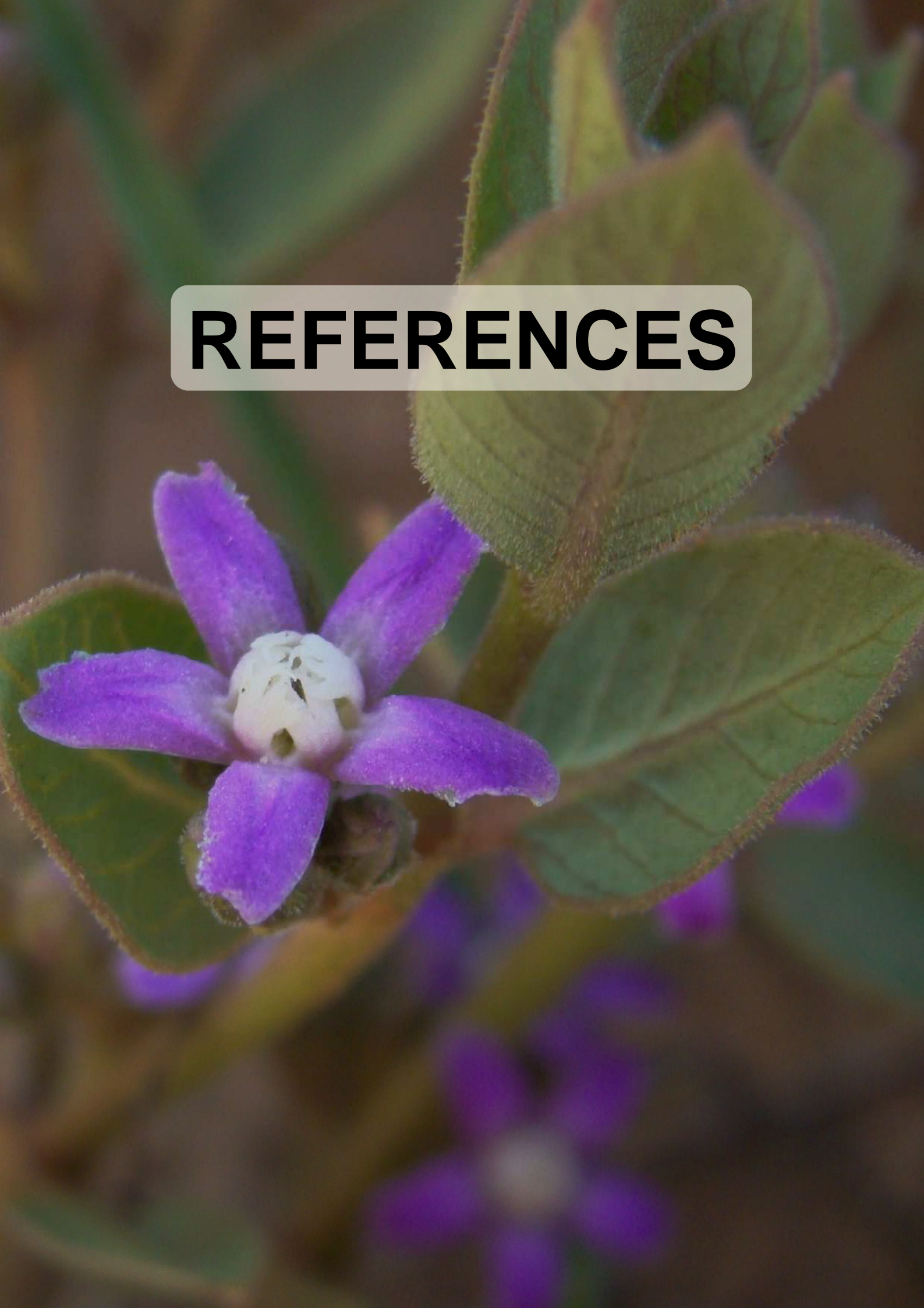
Progress on determining relationships in *Raphionacme* will depend on increasing the number of characters via sequencing additional readily accessible loci that can be combined in simultaneous analyses to increase resolution, accuracy (Hillis, 1996, 1998) and support (Bremer et al., 1999) for critical nodes (Wortley et al., 2005; Hughes et al., 2006). Only then would a classification of *Raphionacme* species be possible.

Species-level phylogenies for detailed evolutionary studies often demand not just complete or near-complete taxon sampling, but ideally multiple accessions within species, as well as complete or near-complete resolution and high statistical support (Hughes et al., 2006). With this being stated, it is also important to emphasize that in many cases only herbarium material are available due to habitats disturbances and eradication of entire habitat, which could lead to the extinction of endemic species.

It is also difficult to obtain material of a species when only type material of that species is available or the voucher specimen is in such a way that it is not possible to obtain a sample without damaging the specimen. All of these reasons mentioned above limits the availability of material for molecular or other analyses.

Even though based on molecular data from one gene region and morphology as additional data source, this study creates a baseline for future phylogenetic work in this large and important genus of the Periplocoideae.

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A close-up photograph of a plant's inflorescence. The central part is a dark brown, fuzzy spike. Surrounding it are numerous small, yellowish-green flowers and buds. Some flowers are fully open, showing delicate white stamens. The overall appearance is that of a dense, developing flower cluster.

APPENDIX

Appendix

Appendix 1: The 87 herbarium specimens, representing 30 *Raphionacme* species, two subspecies and two forms, used in this study.

RAPHIONACME SPECIES	VOUCHER (HERBARIUM)	LOCALITY (YEAR COLLECTED)
<i>R. brownii</i>	Akoegninou, A. 4917 (WAG)	Benin (2001)
<i>R. brownii</i>	Jongkind, C.C.H. 2644 (WAG)	Ghana (1996)
<i>R. brownii</i>	Elliot, F.F. 2921 (LISC)	Guinea (1951)
<i>R. chimanimaniana</i>	Mavi, S. 634 (BLFU)	Zimbabwe (1967)
<i>R. chimanimaniana</i>	Phipps, J.B. 839 (LISC)	Zimbabwe (1957)
<i>R. chimanimaniana</i>	Torre, A.R. & Correia, M.F. 16107 (LISC)	Mozambique (1967)
<i>R. dyeri</i>	Motsamei, J. & Gemmel, J.M. 2092 (BLFU)	South Africa (1929)
<i>R. dyeri</i>	Venter, A.M. 757 (BLFU)	South Africa (2008)
<i>R. dyeri</i>	Pienaar, M. 13 (BLFU)	South Africa (2008)
<i>R. elsana</i>	Venter, H.J.T. 9085a (BLFU)	South Africa (1985)
<i>R. elsana</i>	Venter, H.J.T. 9085b (BLFU)	South Africa (1985)
<i>R. elsana</i>	Venter, H.J.T. 9085d (BLFU)	South Africa (1985)
<i>R. flanaganii</i>	Joubert, L. 65 (BLFU)	South Africa (2009)
<i>R. flanaganii</i>	Nichols, G.R. 1901 (BLFU)	South Africa (1999)
<i>R. flanaganii</i>	Torre, A.R. 1921 (LISC)	Mozambique (1940)
<i>R. galpinii</i>	Venter, F. 13088 (BLFU)	South Africa (1988)
<i>R. galpinii</i>	Pole-Evans, I.B. V-233745 (UPS)	South Africa (1952)
<i>R. galpinii</i>	Rogers, Y.A. 19347 (PRE)	South Africa (1918)
<i>R. galpinii</i>	Pienaar, M. 25 (BLFU)	South Africa (2009)
<i>R. galpinii</i>	Pienaar, M. 11 (BLFU)	South Africa (2008)
<i>R. globosa</i>	Detilleux, E. 206 (BR)	Democratic Republic of the Congo (1956)
<i>R. globosa</i>	Pawek, J. 13527 (WAG)	Malawi (1978)
<i>R. globosa</i>	Eanshawe, D.B. 2693 (BR)	Zambia (1957)

Appendix 1: Continued...

<i>R. globosa</i>	Torre, A.R. 6937 (LISC)	Mozambique (1944)
<i>R. grandiflora</i>	Torre, A.R. & Correia, M.F. 13961 (LISC)	Mozambique (1965)
<i>R. grandiflora</i>	Pawek, J. 13646 (MO)	Malawi (1978)
<i>R. grandiflora</i>	Milne-Readhead, E. & Taylor, P. 8394 (BR)	Tanzania (1956)
<i>R. haeneliae</i>	Robinson, E.R. S-R10 (BLFU)	Namibia (1972)
<i>R. haeneliae</i>	Venter, H.J.T. 9349 (K)	Namibia (?)
<i>R. haeneliae</i>	Duplicate' Venter, H.J.T. 9349 (PRE)	Namibia (?)
<i>R. hirsuta (Group 1)</i>	Du Preez, P.J. 797 (BLFU)	South Africa (1987)
<i>R. hirsuta (Group 1)</i>	Du Preez, C. 230 (BLFU)	South Africa (1977)
<i>R. hirsuta (Group 1)</i>	Pont, J.W. 7721 (BLFU)	South Africa (1928)
<i>R. hirsuta (Group 2)</i>	Pienaar, M. 3 (BLFU)	South Africa (2008)
<i>R. hirsuta (Group 2)</i>	Balkwill, K. & Cadman, M-J. 3609 (BLFU)	South Africa (1986)
<i>R. hirsuta (Group 2)</i>	Du Preez, P.J. 3001 (BLFU)	South Africa (1995)
<i>R. keayii</i>	César, J. 364 (BLFU)	Ivory Coast (1976)
<i>R. keayii</i>	Pobequin, H. 351 (BLFU)	? (?)
<i>R. kubangensis</i>	Menezes, A. 1367 (LISC)	Angola (1964)
<i>R. kubangensis</i>	Leach 14546 (BLFU)	Angola (1970)
<i>R. lanceolata</i>	Menezes, A. 3707 (LISC)	Angola (1970)
<i>R. lanceolata</i>	Giess, W. 209 (BR)	Namibia (1963)
<i>R. lanceolata</i>	Smith, P.A. 3761 (MO)	Botswana (1981)
<i>R. longifolia</i>	Bruyns, P. 6962 (BOL)	Botswana (1996)
<i>R. longifolia</i>	Wild, H. & Drummond, R.B. 6678 (LISC)	Zimbabwe (1964)
<i>R. longifolia</i>	Faulkner, H. KEW203 (BR)	Mozambique (1948)
<i>R. longituba</i>	Greenway, P.J. & Polhill, R.M. 11677 (BR)	Tanzania (1964)
<i>R. longituba</i>	Richards, H.M. 19373 (UPS)	Zambia (1964)

Appendix 1: Continued...

<i>R. lucens</i>	Grandvaux Barbosa, L.A. & De Lemos, F. 7543 (BLFU)	Mozambique (1957)
<i>R. lucens</i>	Pienaar, M. 20 (BLFU)	South Africa (2009)
<i>R. lucens</i>	Venter, H.J.T. 4980 (BLFU)	South Africa (1968)
<i>R. madiensis</i>	Wilson, J. 2067 (LISC)	Uganda (1971)
<i>R. madiensis</i>	Wild, H. 3916 (BLFU)	Zimbabwe (?)
<i>R. michelii</i>	Pennels, Z. 407 (BR)	Democratic Republic of the Congo (1958)
<i>R. michelii</i>	Troupin, G. 2119 (BLFU)	Democratic Republic of the Congo (1952)
<i>R. michelii</i>	Sinnpoon, C.D. 16/19 (BLFU)	Zimbabwe (1962)
<i>R. moyalica</i>	Luke et. al. 7093 (K)	Kenya (2000)
<i>R. namibiana</i>	Bruyns, P. 5803 (WIND)	Namibia (1993)
<i>R. namibiana</i>	Bruyns, P. 5758 (WIND)	Namibia (1993)
<i>R. namibiana</i>	Bruyns, P. 5651 (BOL)	Namibia (1993)
<i>R. palustris</i>	Goldblatt, P. & Manning, J. 8366 (MO)	South Africa (1987)
<i>R. palustris</i>	Pienaar, M. 22 (BLFU)	South Africa (2009)
<i>R. palustris</i>	Venter, H.J.T. 9194 (BLFU)	South Africa (1988)
<i>R. palustris</i>	Venter, H.J.T. 9004 (BLFU)	South Africa (1984)
<i>R. procumbens</i>	Pienaar, M. 10 (BLFU)	South Africa (2008)
<i>R. procumbens</i>	Miller, O.B. 3958A (BR)	Zimbabwe (1956)
<i>R. procumbens</i>	Venter, H.J.T. 9315 (BLFU)	South Africa (?)
<i>R. pulchella</i>	Williams, O.J. 89 (BLFU)	Mozambique (1965)
<i>R. pulchella</i>	Mare, D. 174687 (BLFU)	Zimbabwe (1965)
<i>R. splendens</i> subsp. <i>splendens</i>	Torre, A.R. & Correia, M.F. 16297 (LISC)	Mozambique (1967)
<i>R. splendens</i> subsp. <i>splendens</i>	Robertson, S.A. 6647 (MO)	Kenya (1992)
<i>R. splendens</i> subsp. <i>splendens</i>	Bruyns, P. 7700 (BOL)	Mozambique (1998)

Appendix 1: Continued...

<i>R. splendens</i> <i>subsp. bingeri</i>	Fotius, G. 1897 (G)	Chad (1970)
<i>R. splendens</i> <i>subsp. bingeri</i>	Sinsin, B. 2721 (WAG)	Benin (1999)
<i>R. utilis</i>	Sheppard, J.A. 308 (K)	Mozambique (1909)
<i>R. utilis</i>	Barros Machado, ANG XI 54-10 (LISC)	Angola (1954)
<i>R. velutina</i>	Pienaar, M. 28 (BLFU)	Botswana (2010)
<i>R. velutina</i>	Venter, F. 11299 (BLFU)	South Africa (1985)
<i>R. velutina</i>	Menezes, A. 4014 (LISC)	Angola (1971)
<i>R. vignei</i>	Santo, E. 2931 (LISC)	Guinea (1951)
<i>R. vignei</i>	Jongkind, C.C.H. & Nieuwenhuis, C.M.J. 2697 (WAG)	Ghana (1996)
<i>R. villicorona</i>	Venter, H.J.T. / Winter 9889/5899 (BLFU)	South Africa (?)
<i>R. welwitschii</i>	Stolz, A. 2307 (MO)	Tanzania (1913)
<i>R. welwitschii</i>	Milne-Redhead, E. 3806 (BR)	Zambia (1938)
<i>R. welwitschii</i>	Schaijes 2657 (BR)	Democratic Republic of the Congo (1985)
<i>R. zeyheri</i>	Bayer, M.B. s.n. (BLFU)	South Africa (?)
<i>R. zeyheri</i>	Giffen, M.H. 1202 (BLFU)	South Africa (1948)

Appendix 2: The morphological sub-matrix comprising 45 floral, fruit and vegetative characters with character states.

Taxon/Node	1					2					3					4									
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
<i>R. brownii</i>	0	0	0	0	0	0	1	2	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0
<i>R. chimanimaniana</i>	0	0	?	?	?	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1
<i>R. dyeri</i>	0	0	0	0	0	1	1	1	2	0	1	0	0	0	1	1	0	0	0	0	1	2	0	0	0
<i>R. elsana</i>	0	0	0	0	0	1	0	0	2	1	1	0	0	0	0	0	0	1	0	0	1	0	0	1	1
<i>R. flanaganii</i>	0	2	0	0	0	0	0	0	0	1	2	1	1	1	0	0	0	1	0	0	1	0	0	0	1
<i>R. galpinii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	1
<i>R. globosa</i>	0	0	0	0	0	0	1	2	0	0	0	0	0	2	0	0	0	1	0	0	1	0	0	1	0
<i>R. grandiflora</i>	0	0	0	0	0	0	2	0	0	1	0	1	1	2	1	0	0	1	0	2	1	0	0	1	0
<i>R. haeneliae</i>	0	0	0	1	0	2	2	0	0	1	1	1	1	2	0	0	0	0	0	0	1	1	0	0	1
<i>R. hirsuta G1</i>	0	0	0	0	1	1	0	1	2	1	0	0	0	0	1	0	2	1	0	0	0	0	0	0	1
<i>R. hirsuta G2</i>	0	0	0	0	1	0	1	1	0	0	0	1	2	1	0	0	0	0	1	0	0	0	0	0	1
<i>R. keyii</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	0	1	1	?	?	?	?
<i>R. kubangensis</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	2	0	1	1	0	1
<i>R. lanceolata</i>	0	0	0	0	1	0	0	0	2	0	0	0	0	2	1	0	1	1	0	0	0	0	1	0	0
<i>R. longifolia</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	0	1	1	0	0
<i>R. longituba</i>	0	0	0	0	1	1	1	2	1	0	0	0	1	1	0	0	1	0	2	1	0	0	1	0	0
<i>R. lucens</i>	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
<i>R. madiensis</i>	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	2	0	0	1	0	0
<i>R. michelii</i>	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	1	1	0	2	0	0	0	1	?
<i>R. moyalica</i>	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	1	1	2	0	0	1	2	?	?	?
<i>R. namibiana</i>	0	0	1	0	0	0	2	0	0	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	1
<i>R. palustris</i>	0	0	0	1	0	0	0	0	1	2	1	1	1	0	2	1	0	0	1	2	1	0	0	0	0
<i>R. procumbens</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	0	2	0	0	0	1	0
<i>R. pulchella</i>	0	0	?	?	?	0	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	1	0	?	?
<i>R. splendens</i> subsp. <i>splendens</i>	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	1	1	1	1	0	?
<i>R. splendens</i> subsp. <i>bingeri</i>	0	0	0	0	0	0	2	0	0	1	0	1	0	1	1	1	0	0	1	1	1	1	1	0	0
<i>R. utilis</i>	0	0	0	0	0	0	2	0	1	0	0	1	0	2	0	0	1	1	2	0	0	1	0	?	?
<i>R. velutina</i>	0	0	0	0	1	0	0	2	0	0	0	0	0	0	1	1	0	2	1	2	0	0	1	0	0
<i>R. vignei</i>	0	0	0	0	0	1	2	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0
<i>R. villicorona</i>	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	2	0	0	0	0	1	0	?	?	?
<i>R. welwitschii</i>	0	1	0	0	0	0	2	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	2	1
<i>R. zeyheri</i>	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0
<i>Chlorocyathus lobulata</i>	1	1	1	0	1	1	2	0	0	1	1	0	2	0	0	1	0	1	1	2	0	1	1	1	0
<i>Schlechterella abyssinica</i>	0	1	0	0	1	1	2	0	0	0	1	2	1	1	1	1	0	1	0	0	0	1	0	0	0
<i>Stomatostemma monteiroae</i>	1	1	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	2	0

SUMMARY



SUMMARY

The phylogeny and biogeography of *Raphionacme* were investigated in this first comprehensive study which included virtually all the species. *Raphionacme* is the largest genus in the subfamily Periplocoideae (Apocynaceae *sensu lato*) and consists of 36 species and two subspecies. Sequence data from the nuclear ITS gene region and 45 morphological characters were used to determine relationships between 30 *Raphionacme* species.

Raphionacme is an African genus and widely distributed throughout this continent with the highest concentration of species in southern Africa, the Cape Floristic Region excluded. Only one species, *R. arabica*, is found outside Africa on the Arabian Peninsula. The most common habitat in which members of this genus occur is grassland or savanna. The majority of species are herbaceous geophytes, with four species that have a climbing habit. Four areas of species richness were identified. These are the Tongaland-Pondoland Mosaic in northeastern South Africa, which includes the Maputaland Centre of Endemism, the Zambezian Region, the Karoo-Namib Region and Kalahari-Highveld Transitional Zone both in the west of southern Africa. Nine endemic species were identified of which seven occur south of the Equator. These are *R. chimanimaniana*, *R. elsana*, *R. haeneliae*, *R. namibiana*, *R. sylvicola*, *R. villicorona* and *R. zeyheri*, with only *R. moyalica* occurring in the north of Kenya and *R. arabica* in Oman.

The cladistic results of this investigation indicated that the first and therefore oldest clade to diverge contain species from southern Africa, leading to the assumption that *Raphionacme* originated in this region. The geophytic habit of the species would probably be an indication that this coincided with the aridification of this region. Thereafter diversification and radiation may have occurred northwards following the savanna that developed east and north of the remaining Equatorial rain forest.

Some taxonomic implications, based on the phylogenetic results, have been included. The outgroup *Schlechterella abyssinica* was found to be nested in the *Raphionacme* clade, in both the molecular and combined cladograms. The removal of *Raphionacme abyssinica* to the genus *Schlechterella* would seem to have been incorrect and the species *S. abyssinica* may have to be returned to *Raphionacme*. Three *Raphionacme* species, *R. bingeri*, *R. excisa* and *R. splendens* were combined under the name *R. splendens*. Subsequently *R. splendens* was subdivided into two subspecies. The cladistical results of this study support the creation of *Raphionacme splendens* subsp. *splendens* and *R. splendens* subsp. *bingeri*.

Keywords: African flora, biogeography, geophyte, ITS, molecular taxonomy, *Raphionacme*, phylogeny, *Schlechterella* affinity

OPSOMMING

Die filogenie en biogeografie van *Raphionacme* was ondersoek in hierdie eerste omvattende studie wat naastenby al die spesies ingesluit het. *Raphionacme* is die grootste genus in die subfamilie Periplocoideae (Apocynaceae *sensu lato*) en bestaan uit 36 spesies en twee subspecies. Data van basispaaropeenvolgingsbepaling van die kern ITS geenstreek en 45 morfologiese kenmerke is gebruik om die verwantskappe tussen 30 *Raphionacme* spesies vas te stel.

Raphionacme is 'n Afrika genus en kom wydversprei oor hierdie kontinent voor met die hoogste spesiekonsentrasie in suidelike Afrika, maar is afwesig in die Kaap Floristiese Streek. Slegs een spesie, *R. arabica*, word buite Afrika op die Arabiese Skiereiland aangetref. Die meeste lede van die genus word in savanna of grasveld aangetref. Die meerderheid spesies is kruidagtige geofiete, maar vier spesies is klimplantagtig. Vier gebiede met 'n hoë spesiekonsentrasie is geïdentifiseer, naamlik die Tongaland-Pondoland Mosaiek in die noordooste van Suid-Afrika, wat die Maputaland Sentrum van Endemisme insluit, die Zambeziestreek, die Karoo-Namibstreek en Kalahari-Hoëveldoorgangsones, beide in die weste van suidelike Afrika. Nege endemiese spesies is geïdentifiseer waarvan sewe suid van die ewenaar voorkom. Hierdie spesies is *R. chimanimaniana*, *R. elsana*, *R. haeneliae*, *R. namibiana*, *R. sylvicola*, *R. villicorona* en *R. zeyheri*, met slegs *R. moyalica* wat in die noorde van Kenia voorkom en *R. arabica* in Oman.

Die kladistiese resultate van hierdie ondersoek toon aan dat die eerste en dus oudste klade wat gevorm het, spesies van suidelike Afrika bevat, wat daarop dui dat *Raphionacme* in hierdie gebied ontstaan het. Die geofitiese groeivorm is waarskynlik 'n aanduiding dat dit saamgeval het met die uitdroging van die streek. Daarna het diversifisering en noordwaartse verspreiding van *Raphionacme* spesies moontlik die ontwikkeling van savanna oos en noord van die oorblywende ekwatoriale reënwood gevolg.

Etlke taksonomiese toepassings, gegrond op die filogenetiese resultate, is aangedui. Die buitegroep *Schlechterella abyssinica* het in beide die molekulêre en gekombineerde kladogramme deel van die *Raphionacme* klade gevorm. Dit wil dus voorkom asof die oorplasing van *Raphionacme abyssinica* na die genus *Schlechterella* foutief was en dat die spesie *S. abyssinica* weer na *Raphionacme* oorgedra moet word. Drie *Raphionacme* spesies, *R. bingeri*, *R. excisa* en *R. splendens* is onder die naam *R. splendens* saamgevoeg. Daarna is *R. splendens* weer in twee subspesies verdeel. Die kladistiese resultate van hierdie studie ondersteun die skepping van *Raphionacme splendens* subsp. *splendens* en *R. splendens* subsp. *bingeri*.

Sleutelwoorde: Afrika flora, biogeografie, geofiet, ITS, molekulêre taksonomie, *Raphionacme*, filogenie, *Schlechterella*-affiniteit