

# **Molecular phylogenetic relationships within the subtribe Disinae (Orchidaceae) and their taxonomic, phytogeographic and evolutionary implications**

**Benny Bytebier**

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Stellenbosch University



Supervisors: Prof. Dirk U. Bellstedt, Stellenbosch University  
Prof. H. Peter Linder, University of Zurich

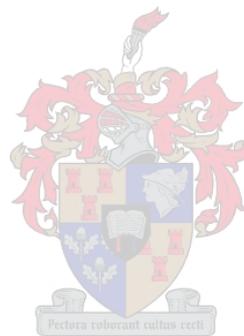
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## Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree

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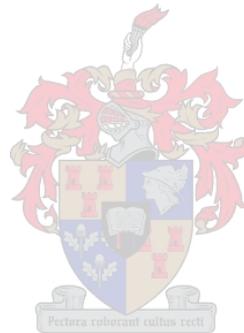
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## Abstract

Twenty five years after the last major morphological revision, phylogenetic relationships were inferred on the basis of a new DNA dataset for the African orchid subtribe Disinae, which includes the large genus *Disa* and the small genus *Schizodium*. One nuclear gene region (ITS) and two plastid gene regions (*trnLF* and *matK*) were sequenced for 136 ingroup, representing 70% of all known Disinae species, as well as for 7 outgroup taxa. The combined data matrix contained 4094 characters and was analysed using parsimony and Bayesian inference. The generic status of *Schizodium* can no longer be supported, as it is deeply embedded within the genus *Disa*. Furthermore, the currently recognised subgenera do not reflect the phylogenetic relationships. Several of the currently recognised sections are monophyletic, others contain misplaced elements, while some are polyphyletic. These results necessitate a re-classification of the Disinae. A monotypic subtribe Disinae and a subdivision of *Disa* into eighteen sections is formally proposed. These sections are monophyletic, well-supported, morphologically distinguishable and are delimited to maximize the congruence with the previous classification. All currently known species are enumerated and assigned to sections.

Likelihood optimisation onto a dated molecular phylogeny is subsequently used to explore the historical biogeography of *Disa*, as well as of three other Cape lineages (Irideae p.p., the *Pentaschistis* clade and Restionaceae), to find out where these lineages originated and how they spread through the Afrotropical region. Three hypotheses have been proposed: (i) a tropical origin with a southward migration towards the Cape; (ii) a Cape origin with a northward migration into tropical Africa and (iii) vicariance. None of these hypotheses, however, has been thoroughly tested. In all cases, tropical taxa are nested within a predominantly Cape clade and there is unidirectional migration from the Cape into the Drakensberg and from there northwards into tropical Africa. Dating estimates show that the migration into tropical East Africa has occurred in the last 17 million years, consistent with the Mio-Pliocene formation of the mountains in this area.

The same technique is then utilised to reconstruct the temporal occurrence of ancestral ecological attributes of the genus *Disa*. The first appearance of species in the grassland and savanna biomes, as well as in the subalpine habitat, are in agreement with the existing, reliable geological and paleontological information. This suggests that phylogenies can be used to date events for which other information is lacking or inconclusive, such as the age of the fynbos biome and the start of the winter rainfall regime in southern Africa. The results indicate that these are much older than what is currently accepted and date back to at least the Oligocene.

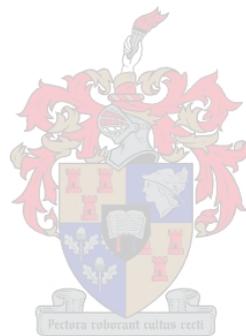


## Opsomming

Vyf-en-twintig jaar na die laaste groot morfologiese hersiening, is die filogenetiese verwantskappe van die Afrika orgideë subtribus Disinae, wat die groot genus *Disa* en die klein genus *Schizodium* insluit, in hierdie studie op grond van 'n nuwe DNA datastel afgelei. Daar is van 136 binnegroep, wat 70% van alle bekende Disinae spesies verteenwoordig, sowel as sewe buitegroep taksa geenopeenvolgings van een nukleêre geen streek (ITS) en twee plastiedgeen streke (*trnL* en *matK*) bepaal. Die gekombineerde data matriks het 4094 karakters bevat en is met die parsimonie en Bayesian metodes ontleed. Die generiese status van *Schizodium* kan nie hieruit ondersteun word nie, en is diep ingebed binne die genus *Disa*. Die huidige aanvaarde subgenera word ook nie deur hierdie filogenie ondersteun nie. Verskeie van die huidige herkende seksies is bevind om monofileties te wees, ander bevat verkeerd geplaasde spesies, terwyl ander polifileties blyk te wees. 'n Monotipiese subtribus Disinae en 'n onderverdeling van *Disa* in agtien seksies word formeel voorgestel. Dié seksies is monofileties, goed ondersteun, morfologies onderskeibaar en omskryf om maksimaal ooreen te stem met die vorige klassifikasie. Alle huidige bekende spesies word gelys en toegewys aan seksies.

Waarskynlikheidsoptimalisering op 'n gedateerde molekulêre filogenie is dan gebruik om die historiese biogeografie van *Disa* te ondersoek, tesame met drie ander Kaapse groepe (Irideae p.p., die *Pentasthictis* klade en Restionaceae), om te bepaal waar hierdie groepe hulle oorsprong gevind het en hoe hulle na die "Afrotemperate" streek versprei het. Drie hipoteses word voorgestel: (i) 'n tropiese oorsprong met 'n suidwaartse migrasie na die Kaap; (ii) 'n Kaapse oorsprong met 'n noordwaartse migrasie na tropiese Afrika, en (iii) vikariansie. Geen van hierdie hipoteses is egter vantevore deeglik getoets nie. In alle gevalle is bevind dat die tropiese taksa oorwegend binne 'n Kaapse klade gesetel is, en dat daar 'n eenrigting migrasie is van die Kaap na die Drakensberge en van daar noordwaarts na tropiese Afrika. Dateringsskattings toon dat die migrasie na tropiese Oos-Afrika in die laaste 17 miljoen jaar plaasgevind het, ooreenstemmend met die Mio-Plioseen vorming van die berge in die area.

Dieselfde tegniek is daarna aangewend om die temporale voorkoms van voorvaderlike ekologiese eienskappe van die genus *Disa* te rekonstrueer. Die eerste voorkoms van die spesies in die grasveld en savanna biome, sowel as die subalpiene habitat, is in ooreenstemming met bestaande, betroubare geologiese en paleontologiese informasie. Dit suggereer dat filogenieë gebruik kan word om gebeurtenisse te dateer waarvoor daar informasie ontbreek of nie beslissend is nie, soos die ouderdom van die Fynbos bioom en die begin van die winterreënval stelsel in suider-Afrika. Die resultate dui daarop dat dit heelwat ouer is as wat tans aanvaar word en terugdateer na ten minste die Oligoseen.



## Acknowledgments

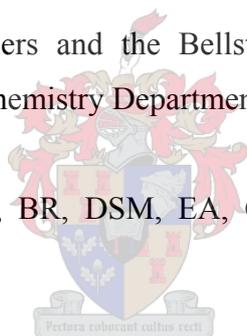
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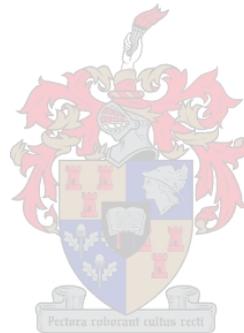


and Jane Sakwa for her precious love.

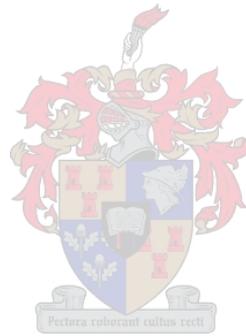
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# General Introduction



Orchidaceae, at an estimated 25,000 species (Chase et al., 2003; World Checklist of Monocots, 2004), is one of the largest plant families, and new species names are still being added at a rate of about 800 per year (Govaerts & Cribb, 2003). Orchids are perhaps best known for their diversity in flower form and for their peculiar pollination and ecological strategies, which makes them an attractive model system for the study of evolution. Indeed, Darwin devoted a whole book to the subject (Darwin, 1862).

For more than two centuries, various researchers have attempted to trace a pattern in the seemingly boundless vegetative and floral variation. Swartz (1800) was the first to produce an overview of the family, but undoubtedly the most comprehensive and influential work during its age, and even now, was that of Lindley (1830-40), who is often referred to as the father of orchidology. Lindley, followed by many others, based his classification mostly on an intuitive interpretation of the variation patterns in floral characters, especially those relating to anther configuration and pollinarium structure. Unfortunately, this often led to drastically different classification schemes (Lindley, 1830-40; Schlechter, 1926; Garay, 1960; Dressler, 1974; Dressler, 1981).

In the 1980's, a more explicit methodology in systematics started to replace the intuitive approach to phylogeny and classification. Linder (1982) was one of the first to introduce a cladistic approach to orchid systematics in his revision of the Disinae. The first cladistic study at family level was that of Burns-Balogh and Funk (1986), which, however, received considerable criticism for character choice and coding (Garay, 1986; Dressler, 1987) and was later improved upon by Freudenstein and Rasmussen (1999).

In the 1990's, the addition of molecular data helped to solve the position of some taxonomically difficult groups and allowed for a much finer-detailed phylogeny of the plant world (Angiosperm Phylogeny Group, 1998; 2003). The first molecular study, investigating relationships within the Orchidaceae, was that of Neyland and Urbatsch (1995), but it was Cameron et al. (1999) who presented a large and convincing *rbcL* analysis, spanning nearly all tribes in an effort to evaluate the monophyly and arrangement of the then recognised subfamilial and tribal groupings within the Orchidaceae. On the basis of this study, five subfamilies were proposed: Apostasioideae, Cyripedioideae, Vanilloideae, Orchidoideae and Epidendroideae.

Apostasioideae is the smallest subfamily with only 16 species, Cyripedioideae consists of 155 species, Vanilloideae of 249 species, Orchidoideae of 4704 species, while the Epidendroideae are by far the largest subfamily totalling 19,785 species (Chase et al., 2003). Further research (Cameron, 2002; 2004; Freudenstein et al., 2004) showed that the relationships amongst these subfamilies are best represented as follows: (Apostasioideae, (Vanilloideae, (Cyripedioideae, (Orchidoideae, Epidendroideae)))).

Relationships within the Orchidoideae were investigated by Kores et al. (1997), Pridgeon et al. (1997), Douzery et al. (1999), Kores et al. (2001), Salazar et al. (2003) and Freudenstein et al. (2004), amongst others. Douzery et al. (1999) showed that the tribe Diseae, of which the Disinae form part, is paraphyletic and that Orchideae are embedded within Diseae. The widely distributed Orchideae *s.l.* was shown to be sister to the monotypic genus *Codonorchis* (Kores et al., 2001; Freudenstein et al., 2004), which is confined to southern Chile, southern Argentina and the western Falkland Islands. As a result, it was put in the monotypic tribe Codonorchideae (Cribb & Kores, 2000). Although relationships amongst the major genera of the tribe Orchideae *s.l.* have been established (Douzery et al., 1999; Freudenstein et al., 2004), more work still needs to be done. In particular, well-sampled, species-level phylogenies are still few (Bateman et al., 2003; van der Niet et al., 2005) and are needed to gain more insight into the evolutionary processes that have made this family so large and so successful.

With 9,000 species in an area of 90,000 km<sup>2</sup> (Goldblatt & Manning, 2002), the Cape Floristic Region of southern Africa is considerably richer than other Mediterranean type floras (Cowling et al., 1996) and its diversity is comparable with that of neotropical floras (Myers et al., 2000; Goldblatt & Manning, 2002). The endemism reaches almost 70% (Goldblatt & Manning, 2002), a level more likely to be found in an island rather than a mainland flora. This high level of endemism can be attributed to the ecological and geographical isolation of the CFR, but an explanation for the high species richness is more difficult to find (Linder, 2005). More intriguing even is the fact that half of this richness is accounted for by only 33 Cape floral clades (Linder, 2003), of which the Disinae is one.

In a series of papers, published 25 years ago, Linder (1981a-f) revised the Disinae. Although several new species have been described since then, the circumscription of all others has remained virtually unchanged. The relationships amongst the constituent taxa of the Disinae has also received considerable attention (Linder, 1986; Linder & Kurzweil, 1990; Linder & Kurzweil, 1994; Kurzweil et al., 1995; Johnson et al., 1998; Linder & Kurzweil, 1999), but no clear or definite picture has emerged from this, and as a result the higher order taxonomy has remained unstable.

In this thesis, I investigate the phylogenetic relationships in Disinae utilising molecular systematic techniques and then draw on this phylogeny to make inferences about the phytogeography and evolutionary history of the group.

In **chapter 1**, I present a molecular phylogenetic analyses of 136 ingroup (70 % of all known entities) and seven outgroup taxa. I use one nuclear and two plastid gene regions, over 4000 characters in total, and two methods (parsimony and Bayesian inference) to deduce a robust phylogenetic hypothesis. I then relate previous morphological, anatomical and palynological research to the new phylogeny and investigate the discrepancies with previous classifications.

In **chapter 2**, I formally propose and justify a revised sectional classification for the genus *Disa*.

In **chapter 3**, I use the phylogeny and a calibrated molecular clock to explore the historical phytogeography of *Disa*. Where did it originate and how did it spread to attain its current distribution? However, rather than limiting this to a case study, I combine my results with those of Galley & Linder (in press), Goldblatt et al. (2003) and Hardy et al. (submitted) in order to try to discern a pattern of similarity. This chapter is part of the outcome of a three-week study visit to the University of Zurich and the interaction with the researchers of the Institute for Systematic Botany, in particular with Chloé Galley, who analysed the *Pentaschistis* clade, *Moraea* and the Restionaceae, whereas I analysed *Disa* and put the methodology in place. The paper was written by both of us.

Finally, in **chapter 4**, I investigate the evolutionary history of the genus and, in the absence of fossil evidence and reliable climatic reconstructions, ask the question as to what an ecological reconstruction can tell us about past climates.

As these chapters are written in the form of journal articles, a bibliography is given at the end of each chapter rather than at the end of the thesis. A final discussion is also not given as this is covered by the discussions in each of the relevant chapters.

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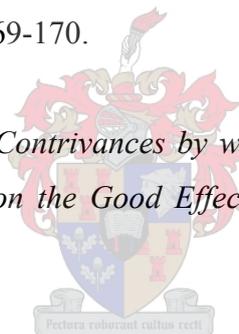
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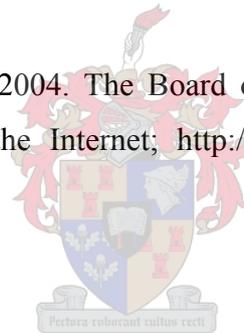
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# Chapter 1

## A molecular phylogeny for the large African orchid genus *Disa*





# A molecular phylogeny for the large African orchid genus *Disa*

Benny Bytebier<sup>a,\*</sup>, Dirk U. Bellstedt<sup>a</sup>, H. Peter Linder<sup>b</sup>

<sup>a</sup> *Biochemistry Department, Stellenbosch University, Private Bag X1, 7602 Stellenbosch, South Africa*

<sup>b</sup> *Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008, Switzerland*

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## Abstract

Phylogenetic relationships were inferred for the African subtribe Disinae (Orchidoideae, Orchidaceae), which include the large genus *Disa* and the small genus *Schizodium*. One nuclear (ITS) gene region and two plastid (*trnLF* and *matK*) gene regions were sequenced for 136 ingroup, representing 70% of all known Disinae species, as well as for 7 outgroup taxa. The combined data matrix contained 4094 characters and was analysed using parsimony and Bayesian inference. Our results show that the generic status of *Schizodium* can no longer be supported, as it is deeply embedded within the genus *Disa*. Furthermore, the currently recognised subgenera do not reflect the phylogenetic relationships and should be rejected. Several of the currently recognised sections are monophyletic, others contain misplaced elements, while some are polyphyletic. Morphological divergence, rather than convergence, has hampered previous attempts at a phylogenetic classification of the Disinae. On the basis of our molecular phylogenetic hypothesis, we propose a monotypic subtribe Disinae and a subdivision of the genus *Disa* into 18 sections.

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**Keywords:** Orchidaceae; Orchidoideae; Disinae; *Disa*; *Schizodium*; *matK*; *trnLF*; ITS; Morphological divergence

## 1. Introduction

The terrestrial orchid genus *Disa* P.J.Bergius currently includes 170 species (185 taxa) (World Checklist of Monocots, 2004), and is widely distributed throughout sub-Saharan Africa. Four species occur in Madagascar, one on the island of Réunion and one on the Arabian Peninsula (Kurzweil and Linder, 2001). The highest concentration of species, however, is in the Cape Floristic Region and with 92 species, 78 of which are endemic, it is the 15th largest genus represented there (Goldblatt and Manning, 2000). The genus *Schizodium* Lindl. consists of only six species (seven taxa), mainly distributed in the Cape Floristic Region (Kurzweil and Linder, 2001). Together these two genera form the subtribe Disinae Benth. as circumscribed by Kurzweil and Linder (2001). Disinae is one of the 33 Cape floral clades (Linder, 2003) that account for almost half of the species richness of the Cape flora.

The taxonomic history of the Disinae is complex. Lindley (1830–1840) recognised six genera: *Disa*, *Monadenia* Lindl., *Penthea* Lindl., *Forficaria* Lindl., *Herschelia* Lindl. and *Schizodium*. Bolus (1888, 1889), the first orchid taxonomist to study these plants in nature, reduced all of the above, except for the monotypic *Forficaria*, to just one genus, *Disa*. However, in his later works (Bolus, 1893–1896, 1911, 1913, 1918) he resurrected *Schizodium*, but sank *Forficaria* into *Disa*. Kraenzlin (1897–1904) recognised *Forficaria*, *Schizodium*, *Disa*, *Herschelia* and *Monadenia*, while Schlechter (1901) kept only the genera *Disa* and *Schizodium*. Rolfe (1912–1913), split up the group even further into *Orthopenthea* Rolfe, *Monadenia*, *Amphigena* (Bolus) Rolfe, *Herschelia*, *Penthea* Rolfe, *Disa* and *Schizodium*. This treatment was followed by Schelpe (1966).

Linder (1981a,b,c,d,e,f) revised the Disinae and upheld *Disa*, *Monadenia*, *Schizodium* and *Herschelia*. Initially, he included the genus *Brownleea* Harv. ex Lindl. as part of the Disinae, but this genus was later removed and put in the monotypical subtribe Brownleeinae H.P.Linder & Kurzweil (Linder and Kurzweil, 1994). For nomenclatural reasons

\* Corresponding author. Fax: +27 21 8085863.

E-mail address: bytebier@sun.ac.za (B. Bytebier).

the genus name *Herschelia* was later changed to *Herschelianthe* Rauschert (Rauschert, 1983). Subsequent morphological analyses (Kurzweil et al., 1995; Johnson et al., 1998) showed that *Monadenia* and *Herschelianthe* were better placed within *Disa*, leaving only the genera *Disa* and *Schizodium*. Douzery et al. (1999) and Bellstedt et al. (2001), using DNA sequence data, further corroborated the nesting of both *Herschelianthe* and *Monadenia* within *Disa*. This treatment has been followed by most (Linder and Kurzweil, 1999; Kurzweil, 2000; Kurzweil and Linder, 2001; Chase et al., 2003; Kurzweil and Archer, 2003) but not all (Olszewski and Szlachetko, 2003) recent authors.

Following Lindley (1830–1840), various attempts were made to subdivide the large genus *Disa* (e.g. Schlechter, 1901; Bolus, 1888, 1889, 1918; Kraenzlin (1897–1904)). The most recent one was by Linder (1981c,d, 1982) who proposed 5 subgenera and 15 sections, but kept *Monadenia* and *Herschelianthe* as distinct genera. The sections were largely distinguished by the structure of the rostellum, the structure and position of the leaves, the shape of the petals and lip, and the degree of development of the spur. The later inclusion of *Monadenia* and *Herschelianthe* in *Disa* increased the number of sections to 17 (Linder and Kurzweil, 1999). However, the morphological basis for the infrageneric groups was in some cases weak, consequently the monophyly of the subgenera and sections is questionable (Linder, 1982; Linder and Kurzweil, 1990; Kurzweil et al., 1995). In addition, the phylogenetic position of the morphologically aberrant genus *Schizodium* remained unclear, despite the use of palynological, anatomical and morphological characters.

Attempts to resolve relationships among the sections, based on morphological characteristics, were unsuccessful (Linder, 1982, 1986; Linder and Kurzweil, 1994; Kurzweil et al., 1995). A preliminary investigation of the phylogenetic

relationships between the sections, based on the *trnL*F sequences of 41 *Disa* taxa (Bellstedt et al., 2001) found similarities with the taxonomy established by Linder (1981c,d) but also many discrepancies, and the analysis raised more questions than it provided answers. The species sampling, however, was very uneven and heavily biased towards the subgenus *Disa*. Furthermore, no member of the genus *Schizodium* was included.

The objectives of this study, therefore, are: (1) to establish the phylogenetic position of *Schizodium*, and so establish the correct delimitation of the genus *Disa*; (2) to test the monophyly of the current subgenera and sections and to propose new ones, if necessary; and (3) to locate morphological markers for the sections to aid the diagnosis of the new sections, and by doing so facilitate the placement of species not sampled for sequence data.

## 2. Materials and methods

### 2.1. Taxon sampling

One hundred and twenty-six species, or 70% of all known Disinae, were collected. Intraspecific taxa were also included bringing the total to 136 ingroup taxa (Table 1). Each taxon was represented by a single specimen in this study. In addition to recognised taxa *sensu* World Checklist of Monocots (2004), we included *Disa maculomarronina* McMurtry, a taxon of presumed hybrid origin (Linder and Kurzweil, 1999) and three newly discovered species, *Disa* sp. nov. (section *Monadenia*), *Disa vigilans* McMurtry & T.J.Edwards (McMurtry et al., 2006) (section *Stenocarpa*) and *Disa remota* H.P.Linder (Linder and Hitchcock, 2006) (section *Disella*). The sampled species represent the full geographical range of the subtribe, all major habitats, both currently recognised genera and all sections. (Table 1). One

Table 1  
Genera, subgenera and sections of the Disinae following Kurzweil and Linder (2001), and the number of species and taxa sampled

Genus	Subgenus	Section	Abbrev.	No. of species/ no. of species sampled	% of species sampled	No. of taxa/ no. of taxa sampled	% of taxa sampled	
<i>Schizodium</i>			SCH	5/6	83.3	6/7	85.7	
<i>Disa</i>	<i>Micranthe</i>	<i>Micranthae</i>	MIC	13/27	48.1	14/30	46.7	
		<i>Falcipetalum</i>						
	<i>Disella</i>	<i>Disella</i>	DLA	7/14	50	8/16	50	
		<i>Intermediae</i>	INT	2/2	100	2/2	100	
		<i>Repandra</i>	REP	3/3	100	3/3	100	
		<i>Aconitoideae</i>	ACO	2/8	25	3/10	33.3	
		<i>Hircicornu</i>	<i>Hircicornes</i>	HIR	14/16	87.5	14/16	87.5
	<i>Monadenia</i>	<i>Monadenia</i>	MON	14/19	73.7	14/19	73.7	
		<i>Ovalifoliae</i>	OVA	1/1	100	1/1	100	
		<i>Stoloniferae</i>	STO	1/2	50	1/2	50	
		<i>Stenocarpa</i>	<i>Amphigena</i>	AMP	2/4	50	2/4	50
			<i>Coryphaea</i>	COR	9/9	100	10/10	100
	<i>Stenocarpa</i>	<i>Stenocarpa</i>	STE	14/17	82.4	18/21	85.7	
		<i>Herschelianthe</i>	HER	10/18	55.6	11/20	55	
		<i>Emarginatae</i>	EMA	6/6	100	6/7	85.7	
		<i>Austroalpinae</i>	AUS	1/3	33.3	1/3	33.3	
		<i>Disa</i>	<i>Disa</i>	DIS	19/22	86.4	19/22	86.4
<i>Phlebidia</i>	PHL		3/3	100	3/3	100		
		Total		126/180	70	136/196	69.4	

species of *Codonorchis* Lindl., four of *Disperis* Sw. and two of *Brownleea* were used as outgroups. The South American *Codonorchis* was used to root the tree following Freudenstein et al. (2004). Table 2 lists all the taxa included in this study complete with collector name and number, collection locality, herbaria where duplicates are deposited and GenBank accession numbers. All specimens were field collected except for *Disa salteri*, which was cultivated. All identifications were done by the first author and, in case of any potential doubt, were corroborated by H. Kurzweil of the Compton Herbarium (NBG). Specimen management was done with the help of the Brahms software (Botanical Research And Herbarium Management System, The BRAHMS Project, Department of Plant Sciences, University of Oxford).

## 2.2. DNA extraction, amplification and sequencing

Fresh plant material was dried in silica gel and stored at  $-20^{\circ}\text{C}$ . DNA was extracted using the hexadecyltrimethylammonium bromide (CTAB) procedure of Doyle and Doyle (1987). Polymerase chain reactions (PCR) were performed in a Hybaid Thermal Cycler (Thermo Electron Corporation, Waltham, MA, USA) in a total volume of a 100  $\mu\text{l}$  containing 2.5 mM  $\text{MgCl}_2$ , 1  $\times$  JMR-455 buffer (Southern Cross Biotechnology, Cape Town, RSA), 1 U of Super-Therm *Taq* polymerase (Southern Cross Biotechnology, Cape Town, RSA), 200  $\mu\text{M}$  of each of the dNTP's and 0.5  $\mu\text{M}$  of each primer. When the yield of PCR product was low, 0.004% BSA (bovine serum albumin fraction V, Merck) was added and the amplification repeated. For amplification of the chloroplast *trnL* intron and *trnL-trnF* intergenic spacer region (hereafter named *trnLF*) primers c2 (Bellstedt et al., 2001) and f (Taberlet et al., 1991) were used. Part of the chloroplast *matK* gene and *trnK* intron (hereafter named *matK*) was amplified with the primers  $-19\text{F}$  and  $\text{R1}$  of Kocyan et al. (2004). The internal transcribed spacer region of the 18S–5.8S–26S nuclear ribosomal cistron (hereafter called ITS) was amplified with primers 17SE and 26SE of Sun et al. (1994), also called AB101 and AB102 by Douzery et al. (1999). Amplification profiles were as follows: for *trnLF*, 30 cycles with 1 min denaturation at  $94^{\circ}\text{C}$ , 1 min annealing at  $55^{\circ}\text{C}$ , 90 s extension at  $72^{\circ}\text{C}$ , followed by a final extension step of 6 min at  $72^{\circ}\text{C}$ ; for *matK*, 35 cycles with 30 s denaturation at  $95^{\circ}\text{C}$ , 1 min annealing at  $52^{\circ}\text{C}$ , 100 s extension at  $72^{\circ}\text{C}$  and a final extension round of 7 min at  $72^{\circ}\text{C}$ ; and for ITS, 30 cycles with 1 min denaturation at  $94^{\circ}\text{C}$ , 1 min annealing at  $59^{\circ}\text{C}$  and 90 s extension at  $72^{\circ}\text{C}$  followed by 1 round of extension of 6 min at  $72^{\circ}\text{C}$  were used. Although only single, clear bands were observed, PCR fragments were initially (for *trnLF* and ITS), separated on a 0.5% agarose gel for 2 h and 15 min at 400 V. They were then excised under UV light and purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corp., Madison, USA). At a later stage (for *matK*) the gel separation step was found to be unnecessary and was omitted. For cycle sequencing, the same primers

were used as above, except for *matK*, where three forward primers,  $-19\text{F}$ , 580F and 1082F were used (Kocyan et al., 2004). Cycle sequencing was done with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) in 10  $\mu\text{l}$  reactions consisting of an estimated 100 ng of DNA, 2  $\mu\text{l}$  5 $\times$  buffer (400 mM Tris–HCl, 10 mM  $\text{MgCl}_2$  at pH 9), 3.2 pmol primer, 2  $\mu\text{l}$  of Terminator Ready Reaction Mix and water. In the sequencing of *matK*, the Terminator Ready Reaction Mix was diluted fivefold with Half-Dye Mix (Bioline Ltd., London, UK). The cycle sequencing profile was 35 cycles consisting of 10 s at  $96^{\circ}\text{C}$ , 30 s at  $52^{\circ}\text{C}$  and 4 min at  $60^{\circ}\text{C}$ . Excess terminator dye was removed by gel filtration through Centri-Sep 96 Multi-well Filter Plates (Princeton Separation, Adelphia, USA). The cycle sequencing products were then analysed on an ABI Prism 3100 or 3130 XL 16-capillary Genetic Analyser (Applied Biosystems, Foster City, USA) in the Central Analytical Facility, University of Stellenbosch.

## 2.3. Sequence analysis and alignment

Electropherograms were edited in Chromas v1.45 (Technelysium Pty., Tewantin, Australia). Initial alignment was done with Clustal X v1.8 (Thompson et al., 1997) and further improved as described by Bateman et al. (2003) in Bioedit v7.0.1 (Hall, 1999). Indels were coded with the “simple indel coding” method of Simmons and Ochoterena (2000) as implemented in GapCoder (Young and Healy, 2003).

## 2.4. Phylogenetic analysis

Parsimony analyses were conducted in PAUP\*4.0b10 (Swofford, 2003) on an Apple G5 computer. Heuristic searches were done on datasets of the individual gene regions as well as on a combined sequence dataset. One thousand replicates of random stepwise taxon addition were performed to find islands of equally most parsimonious trees (Maddison, 1991), holding one tree at each step. This was followed by TBR swapping. To minimize the time spent on searching for large numbers of trees, a limit of ten trees was set for each replicate. However, exhaustive branch swapping was performed on the combined dataset. Clade support was assessed with 1000 bootstrap replicates (Felsenstein, 1985) with simple taxon addition and TBR branch swapping, but permitting only 10 trees per replicate to be held. For the combined dataset, the branch swapping was limited to 3 min/replicate.

The heterogeneity of the different gene regions used was assessed with the ILD test of Farris et al. (1995), implemented as the Partition Homogeneity (PH) test in PAUP\*. Each PH test was performed on 100 replicates.

MrBayes 3.0b4 or 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used to infer a phylogenetic hypothesis using a Bayesian approach. The three gene regions (*trnLF*, *matK* and ITS) were treated as separate partitions. The General Time Reversible model of

Table 2  
Species used in this analysis together with voucher information, origin of the plant material and GenBank accession numbers

Taxon <sup>a</sup>	Voucher information <sup>b</sup>	Origin <sup>c</sup>	GenBank Accession Nos.		
			<i>trnL</i> F	<i>matK</i>	ITS
<b>Outgroups</b>					
<i>Codonorchis lessonii</i> (d'Urv.) Lindl.	Rudall (Ref.: O-1398, Kew DNA Bank)	CLS	DQ415136	DQ414993	—
	Ryan 002 (K)	CLS	—	—	AF348005
<i>Brownleea parviflora</i> Harv. ex Lindl.	Kurzweil, 1972 (MAL, UZL, SRGH)	MLW, Nyika	DQ415137	DQ414994	DQ414851
<i>Brownleea macroceras</i> Sond.	Bytebier 2293 (NBG, BR, K, NU)	NAT, Sani Pass	DQ415138	DQ414995	DQ414852
<i>Disperis dicerochila</i> Summerh.	Kurzweil, 1983 (MAL, UZL)	MLW, Nyika	DQ415139	DQ414996	DQ414853
<i>Disperis capensis</i> (L.f.) Sw.	Bytebier 2362 (NBG, BR, K)	CPP-WC, Betty's Bay	DQ415142	DQ414999	DQ414856
<i>Disperis cucullata</i> Sw.	Bytebier 2032 (NBG, BR)	CPP-WC, Sir Lowry's Pass	DQ415141	DQ414998	DQ414855
<i>Disperis stenoplectron</i> Rchb.f.	Edwards & Bellstedt 2308 (NU)	CPP-EC, Ntsikeni	DQ415140	DQ414997	DQ414854
<b>Schizodium Lindl.</b>					
<i>Schizodium flexuosum</i> (L.) Lindl.	Linder 6963 (BOL)	CPP-WC, Romansrivier	DQ415168	DQ415026	DQ414883
<i>Schizodium bifidum</i> (Thunb.) Rchb.f.	Bytebier 2100 (NBG, BR, K)	CPP-WC, Gydo Pass	DQ415173	DQ415031	DQ414888
<i>Schizodium inflexum</i> Lindl.	Bytebier 2406 (NBG, BR)	CPP-WC, Kogelberg	DQ415171	DQ415029	DQ414886
<i>Schizodium obliquum</i> Lindl. ssp. <i>obliquum</i>	Bytebier 2384 (NBG, BR, K)	CPP-WC, Riverlands	DQ415170	DQ415028	DQ414885
<i>Schizodium obliquum</i> Lindl. ssp. <i>clavigerum</i> (Lindl.) H.P.Linder	Bytebier 2373 (NBG, BR, K, NY)	CPP-WC, Sir Lowry's Pass	DQ415172	DQ415030	DQ414887
<i>Schizodium satyrioides</i> (L.) Garay	Bytebier 2379 (NBG)	CPP-WC, Romansrivier	DQ415169	DQ415027	DQ414884
<b>Disa P.J.Bergius</b>					
<i>Micranthae</i> Lindl.					
<i>Disa polygonoides</i> Lindl.	Phillipson 5378 (GRA)	CPP-EC, Queensbury Bay	DQ415262	DQ415121	DQ414978
<i>Disa woodii</i> Schltr.	Bellstedt 671 (NBG)	CPP-EC, Mateku	DQ415261	DQ415120	DQ414977
<i>Disa chrysostachya</i> Sw.	Bytebier 2206 (NBG, BR)	CPP-EC, Gaika's Kop	DQ415259	DQ415118	DQ414975
<i>Disa ochrostachya</i> Rchb.f.	Bytebier 2535 (EA, BR, DSM, NY)	TAN, Matamba Plateau	DQ415250	DQ415109	DQ414966
<i>Disa satyriopsis</i> Kraenzl.	Bytebier 2510 (EA, BR, DSM)	TAN, Lusinga	DQ415254	DQ415113	DQ414970
<i>Disa fragrans</i> Schltr. ssp. <i>fragrans</i>	Oliver & Turner 12117 (NBG)	CPP-EC, Bastervoetpad	DQ415260	DQ415119	DQ414976
<i>Disa sankeyi</i> Rolfe	Johnson s.n. (NU)	NAT, Witsieshoek	DQ415258	DQ415117	DQ414974
<i>Disa celata</i> Summerh.	Bytebier 2499 (EA, BR, DSM)	TAN, Sao Hill	DQ415257	DQ415116	DQ414973
<i>Disa miniata</i> Summerh.	Van der Niet 194 (EA, Z)	MLW, Naganda Peak	DQ415251	DQ415110	DQ414967
<i>Disa ukingensis</i> Schltr.	Kurzweil 2009a (MAL)	MLW, Nyika	DQ415249	DQ415108	DQ414965
<i>Disa zombica</i> N.E.Br.	Kurzweil 2001 (MAL, UZL)	MLW, Nyika	DQ415252	DQ415111	DQ414968
<i>Disa ornithantha</i> Schltr.	Bytebier 2496 (EA, BR, DSM, K, NY)	TAN, Kibena	DQ415253	DQ415112	DQ414969
<i>Disa erubescens</i> Rendle ssp. <i>erubescens</i>	Bytebier 2537 (EA)	TAN, Dansland	DQ415255	DQ415114	DQ414971
<i>Disa erubescens</i> Rendle ssp. <i>carsonii</i> (N.E.Br.) H.P.Linder	Bytebier 2170 (EA, BR)	TAN, Sao Hill	DQ415256	DQ415115	DQ414972
<b>Disella Lindl.</b>					
<i>Disa neglecta</i> Sond.	Bytebier 2585 (BOL)	CPP-WC, Swartberg	DQ415192	DQ415050	DQ414907
<i>Disa obtusa</i> Lindl. ssp. <i>hottentotica</i> H.P.Linder	Mostert 384 (no voucher)	CPP-WC, Kleinmond	DQ415197	DQ415056	DQ414913
<i>Disa obtusa</i> Lindl. ssp. <i>picta</i> (Sond.) H.P.Linder	Bytebier 2403 (NBG, BR)	CPP-WC, Boskloof	DQ415196	DQ415055	DQ414912
<i>Disa telipogonis</i> Rchb.f.	Bytebier 2117 (NBG, BR, K, NY)	CPP-WC, Bain's Kloof	DQ415195	DQ415054	DQ414911
<i>Disa subtenuicornis</i> H.P.Linder	Bytebier 2598 (NBG, BR, K)	CPP-WC, Garcias Pass	DQ415193	DQ415051	DQ414908
<i>Disa ocellata</i> Bolus	Linder & Harley (no voucher)	CPP-WC, Table Mountain	AF360414 AF360471	DQ415053	DQ414910
<i>Disa uncinata</i> Bolus	Bytebier 2426 (NBG, BR)	CPP-WC, Bain's Kloof	DQ415194	DQ415052	DQ414909
<i>Disa remota</i> H.P.Linder ined.	Hitchcock 2057 (BOL)	CPP-WC, Hex River Mountains	DQ415190	DQ415048	DQ414905

Table 2 (continued)

Species used in this analysis together with voucher information, origin of the plant material and GenBank accession numbers

Taxon <sup>a</sup>	Voucher information <sup>b</sup>	Origin <sup>c</sup>	GenBank Accession Nos.		
			<i>trnL</i> F	<i>matK</i>	ITS
<b>Intermediae</b> H.P.Linder					
<i>Disa galpinii</i> Rolfe	Anderson s.n. (NU)	CPP-EC, Qacha's Nek	DQ415266	DQ415125	DQ414982
<i>Disa sanguinea</i> Sond.	Oliver & Turner 12158 (NBG)	LES, Sehlabathebe	DQ415267	DQ415126	DQ414983
<b>Repandra</b> Lindl.					
<i>Disa tysonii</i> Bolus	Bytebier 2274 (NBG)	NAT, Garden Castle	DQ415211	DQ415070	DQ414927
<i>Disa cornuta</i> (L.) Sw.	Bytebier 2128 (NBG)	CPP-WC, Groot Hagelkraal	DQ415213	DQ415072	DQ414929
<i>Disa hallackii</i> Rolfe	Bytebier 2129 (NBG)	CPP-WC, Groot Hagelkraal	DQ415212	DQ415071	DQ414928
<b>Aconitoideae</b> Kraenzl.					
<i>Disa similis</i> Summerh.	Bellstedt 922 (NBG, GRA)	CPP-EC, Mount Thesiger	DQ415245	DQ415104	DQ414961
<i>Disa aconitoides</i> Sond. ssp. <i>aconitoides</i>	Bellstedt 524 (no voucher)	CPP-EC, Toise	DQ415243	DQ415102	DQ414959
<i>Disa aconitoides</i> Sond. ssp. <i>goetziana</i> (Kraenzl.) H.P.Linder	Bytebier 2179 (EA)	TAN, Matamba Plateau	DQ415244	DQ415103	DQ414960
<b>Hircicornes</b> Kraenzl.					
<i>Disa crassicornis</i> Lindl.	Bytebier 2202 (NBG, BR)	CPP-EC, Gaika's Kop	DQ415265	DQ415124	DQ414981
<i>Disa thodei</i> Schltr. ex Kraenzl.	Bytebier 2291 (NBG)	NAT, Sani Pass	DQ415264	DQ415123	DQ414980
<i>Disa cooperi</i> Rchb.f.	Bytebier 2254 (NBG, BR, K)	TVL-MP, Verloren Vallei	DQ415271	DQ415130	DQ414987
<i>Disa scullyi</i> Bolus	Johnson s.n. (NU)	CPP-EC, Bastervoetpad	DQ415273	DQ415132	DQ414989
<i>Disa zuluensis</i> Rolfe	Krige 5 (NBG)	TVL-MP, Draaikraal	DQ415263	DQ415122	DQ414979
<i>Disa rhodantha</i> Schltr.	Bytebier 2256 (NBG, BR, K)	TVL-MP, Verloren Vallei	DQ415272	DQ415131	DQ414988
<i>Disa hircicornis</i> Rchb.f.	Kurzweil 2042 (MAL, UZL, BOL)	MLW, Nyika	DQ415269	DQ415128	DQ414985
<i>Disa perplexa</i> H.P.Linder	Van der Niet 201 (EA, Z)	MLW, Mount Mulanje	DQ415268	DQ415127	DQ414984
<i>Disa maculomarronina</i> McMurtry	Bytebier 2260 (NBG, BR)	TVL-MP, Verloren Vallei	DQ415276	DQ415135	DQ414992
<i>Disa caffra</i> Bolus	Bellstedt 672 (NBG, GRA)	CPP-EC, Mateku	DQ415270	DQ415129	DQ414986
<i>Disa walleri</i> Rchb.f.	Bytebier 2495 (EA, BR, DSM, NY)	TAN, Ifunda	DQ415248	DQ415107	DQ414964
<i>Disa robusta</i> N.E.Br.	Bytebier 2166 (EA, BR)	TAN, Mafinga	DQ415247	DQ415106	DQ414963
<i>Disa versicolor</i> Rchb.f.	Bytebier 2257 (NBG, BR, K)	TVL-MP, Verloren Vallei	DQ415275	DQ415134	DQ414991
<i>Disa extinctoria</i> Rchb.f.	Bytebier 2481 (NBG, BR, K)	TVL-MP, Witklipdam	DQ415274	DQ415133	DQ414990
<b>Monadenia</b> (Lindl.) Bolus					
<i>Disa conferta</i> Bolus	Bytebier 2157 (NBG, BR)	CPP-WC, Kogelberg	DQ415186	DQ415044	DQ414901
<i>Disa sabulosa</i> (Kraenzl.) Bolus	Bytebier 2624 (NBG, BR)	CPP-WC, Pringle Bay	DQ415177	DQ415035	DQ414892
<i>Disa pygmaea</i> Bolus	Bytebier 2136 (NBG, BR, K)	CPP-WC, Kogelberg	DQ415176	DQ415034	DQ414891
<i>Disa bracteata</i> Sw.	Bytebier 2086 (NBG)	CPP-WC, Rondeberg	DQ415187	DQ415045	DQ414902
<i>Disa densiflora</i> (Lindl.) Bolus	Lutzeyer s.n. (Grootbos herb.)	CPP-WC, Grootbos	DQ415188	DQ415046	DQ414903
<i>Disa cylindrica</i> (Thunb.) Sw.	Bytebier 2134 (NBG)	CPP-WC, Vogelgat	DQ415174	DQ415032	DQ414889
<i>Disa reticulata</i> Bolus	Bytebier 2132 (NBG)	CPP-WC, Vogelgat	DQ415182	DQ415040	DQ414897
<i>Disa comosa</i> (Rchb.f.) Schltr.	Bytebier 2050 (NBG, BR)	CPP-WC, Cederberg	DQ415180	DQ415038	DQ414895
<i>Disa bolusiana</i> Schltr.	Bytebier 2142 (NBG)	CPP-WC, Vogelgat	DQ415178	DQ415036	DQ414893
<i>Disa</i> sp. nov.	Bytebier 2595 (NBG, BR)	CPP-WC, Garcias Pass	DQ415184	DQ415042	DQ414899
<i>Disa atrorubens</i> Schltr.	Linder 7540 (NBG, Z)	CPP-WC, Galgeberg	DQ415179	DQ415037	DQ414894
<i>Disa ophrydea</i> (Lindl.) Bolus	Bytebier 2081 (NBG, BR, K)	CPP-WC, Table Mountain	DQ415185	DQ415043	DQ414900
<i>Disa rufescens</i> (Thunb.) Sw.	Bytebier 2082 (NBG)	CPP-WC, Table Mountain	DQ415175	DQ415033	DQ414890
<i>Disa brevicornis</i> (Lindl.) Bolus	Bytebier 2242 (NBG, BR)	NAT, Hlabeni	DQ415183	DQ415041	DQ414898
<b>Ovalifoliae</b> H.P.Linder					
<i>Disa ovalifolia</i> Sond.	Bytebier 2375 (NBG, BR, K)	CPP-WC, Nardouwberg Pass	DQ415167	DQ415025	DQ414882
<b>Stoloniferae</b> H.P.Linder					
<i>Disa stairsii</i> Kraenzl.	Luke 6913 (EA, K)	TAN, Udzungwa Mountains	DQ415246	DQ415105	DQ414962
<b>Amphigena</b> Bolus					
<i>Disa tenuis</i> Lindl.	Liltved s.n. (no voucher)	CPP-WC, Red Hill	DQ415218	DQ415077	DQ414934
<i>Disa salteri</i> G.J.Lewis	Holmes s.n. (no voucher)	CPP-WC, cultivated	DQ415217	DQ415076	DQ414933
<b>Coryphaea</b> Lindl.					
<i>Disa borbonica</i> Balf.f. & S.Moore	Pailler 130 (STCR, BR, K)	REU, Plaine des Cafres	DQ415181	DQ415039	DQ414896

(continued on next page)

Table 2 (continued)

Taxon <sup>a</sup>	Voucher information <sup>b</sup>	Origin <sup>c</sup>	GenBank Accession Nos.		
			<i>trnL</i> F	<i>matK</i>	ITS
<i>Disa vaginata</i> Harv. ex Lindl.	Bytebier 2154 (NBG, BR)	CPP-WC, Groot Winterhoek	DQ415157	DQ415015	DQ414872
<i>Disa glandulosa</i> Burch. ex Lindl.	Bytebier 2629 (no voucher)	CPP-WC, Table Mountain	DQ415158	DQ415016	DQ414873
<i>Disa sagittalis</i> (L.f.) Sw.	Bytebier 2207 (NBG, BR, GRA)	CPP-EC, Gaika's Kop	DQ415164	DQ415022	DQ414879
<i>Disa triloba</i> Lindl.	Bytebier 2455 (NBG, BR, K)	CPP-WC, Krom Rivier	DQ415165	DQ415023	DQ414880
<i>Disa marlothii</i> Bolus	Bytebier 2310 (NBG)	CPP-WC, Ezelsfontein	DQ415156	DQ415014	DQ414871
<i>Disa draconis</i> (L.f.) Sw.	Bytebier 2089 (NBG, BR)	CPP-WC, Rondeberg	DQ415199	DQ415058	DQ414915
<i>Disa karooica</i> S.D.Johnson & H.P.Linder	Bytebier 2436 (NBG, BR, K, NY)	CPP-NC, Gannaga Pass	DQ415198	DQ415057	DQ414914
<i>Disa harveyana</i> Lindl. ssp. <i>harveyana</i>	Bytebier 2160 (NBG, BR, K)	CPP-WC, Du Toit's Kloof	DQ415201	DQ415060	DQ414917
<i>Disa harveyana</i> Lindl. ssp. <i>longicalcarata</i> S.D.Johnson & H.P.Linder	Bytebier 2106 (NBG, BR, K, NY, GRA, NU)	CPP-WC, Gydo Pass	DQ415200	DQ415059	DQ414916
<i>Stenocarpa</i> Lindl.					
<i>Disa tenella</i> (L.f.) Sw. ssp. <i>tenella</i>	Bytebier 2359 (NBG, BR, K, NY)	CPP-WC, Riverlands	DQ415203	DQ415062	DQ414919
<i>Disa tenella</i> (L.f.) Sw. ssp. <i>pusilla</i> H.P.Linder	Knox 4722 (NBG, BR)	CPP-WC, Skoonvlei	DQ415204	DQ415063	DQ414920
<i>Disa brachyceras</i> Lindl.	Bytebier 2607 (NBG, BR)	CPP-WC, Shaw's Pass	DQ415202	DQ415061	DQ414918
<i>Disa ferruginea</i> (Thunb.) Sw.	Bytebier 2307 (NBG)	CPP-WC, Silvermine	DQ415227	DQ415086	DQ414943
<i>Disa gladioliflora</i> Burch. ex Lindl. ssp. <i>gladioliflora</i>	Bytebier 2461 (NBG, BR, K)	CPP-WC, Outeniqua Pass	DQ415228	DQ415087	DQ414944
<i>Disa gladioliflora</i> Burch. ex Lindl. ssp. <i>capricornis</i> (Rchb.f.) H.P.Linder	Bytebier 2315 (NBG, BR)	CPP-WC, Langeberg	DQ415229	DQ415088	DQ414945
<i>Disa saxicola</i> Schltr.	Bytebier 2517 (EA, BR, DSM, K, NY)	TAN, Nyalwela	DQ415232	DQ415091	DQ414948
<i>Disa cephalotes</i> Rchb.f. ssp. <i>cephalotes</i>	Bytebier 2294 (NBG, BR, NU)	NAT, Sani Pass	DQ415233	DQ415092	DQ414949
<i>Disa cephalotes</i> Rchb.f. ssp. <i>frigida</i> (Schltr.) H.P.Linder	Van der Niet 166 (NBG, Z)	NAT, Mont-aux-Sources	DQ415231	DQ415090	DQ414947
<i>Disa oreophila</i> Bolus ssp. <i>oreophila</i>	Bytebier 2284 (NBG, NU)	NAT, Garden Castle	DQ415238	DQ415097	DQ414954
<i>Disa oreophila</i> Bolus ssp. <i>erecta</i> H.P.Linder	Bytebier 2300 (NBG, BR, GRA)	CPP-EC, Naude's Nek	DQ415240	DQ415099	DQ414956
<i>Disa nivea</i> H.P.Linder	Bytebier 2292 (NBG, BR, K, NU)	NAT, Sani Pass	DQ415239	DQ415098	DQ414955
<i>Disa stricta</i> Sond.	McMaster s.n. (NBG)	CPP-EC, Mount Kubusie	DQ415234	DQ415093	DQ414950
<i>Disa montana</i> Sond.	Johnson s.n. (NU)	CPP-EC, Bastervoetpad	DQ415241	DQ415100	DQ414957
<i>Disa vigilans</i> McMurtry & T.J.Edwards	Bellstedt 827 (NBG)	TVL-MP, Mokobulaan	DQ415236	DQ415095	DQ414952
<i>Disa pulchra</i> Sond.	Bytebier 2204 (NBG, BR, K, GRA)	CPP-EC, Gaika's Kop	DQ415235	DQ415094	DQ414951
<i>Disa amoena</i> H.P.Linder	Bytebier 2488 (NBG, BR, K)	TVL-MP, Long Tom Pass	DQ415237	DQ415096	DQ414953
<i>Disa aristata</i> H.P.Linder	Bytebier 2492 (NBG, BR)	TVL-NP, Wolkberg	DQ415230	DQ415089	DQ414946
<i>Herschelianthe</i> (Rauschert) H.P.Linder					
<i>Disa schlechteriana</i> Bolus	Bytebier 2456 (NBG)	CPP-WC, Garcias Pass	DQ415214	DQ415073	DQ414930
<i>Disa spathulata</i> (L.f.) Sw. ssp. <i>spathulata</i>	Steiner 3020 (NBG)	CPP-NC, Nieuwoudtville	DQ415215	DQ415074	DQ414931
<i>Disa spathulata</i> (L.f.) Sw. ssp. <i>tripartita</i> (Lindl.) H.P.Linder	Steiner s.n. (NBG)	CPP-WC, Lemoenpoort	DQ415216	DQ415075	DQ414932
<i>Disa graminifolia</i> Ker Gawl. ex Spreng.	Bytebier 2306 (NBG, BR, K)	CPP-WC, Silvermine	DQ415219	DQ415078	DQ414935
<i>Disa purpurascens</i> Bolus	Bytebier 2158 (NBG, BR, K)	CPP-WC, Cape Point	DQ415226	DQ415085	DQ414942
<i>Disa barbata</i> (L.f.) Sw.	Bytebier 2090 (NBG)	CPP-WC, Riverlands	DQ415221	DQ415080	DQ414937
<i>Disa venusta</i> Bolus	Bytebier 2165 (NBG, BR)	CPP-WC, Betty's Bay	DQ415223	DQ415082	DQ414939
<i>Disa lugens</i> Bolus var. <i>lugens</i>	McMaster s.n. (picture only)	CPP-EC, Bosberg	DQ415224	DQ415083	DQ414940
<i>Disa multifida</i> Lindl.	Bytebier 2630 (NBG, BR)	CPP-WC, Swartberg	DQ415225	DQ415084	DQ414941
<i>Disa hians</i> (L.f.) Spreng.	Bytebier 2457 (NBG)	CPP-WC, Garcias Pass	DQ415222	DQ415081	DQ414938
<i>Disa baurii</i> Bolus	Van Ede 25 (NBG)	TVL-MP, Dullstroom	DQ415220	DQ415079	DQ414936
<i>Emarginatae</i> H.P.Linder					
<i>Disa buchenaviana</i> Kraenzl.	Gehrke Af294 (BR)	MDG, Andingitra	DQ415205	DQ415064	DQ414921
<i>Disa stachyoides</i> Rchb.f.	Bytebier 2318 (NBG, BR)	NAT, Witsieshoek	DQ415207	DQ415066	DQ414923
<i>Disa alticola</i> H.P.Linder	Bytebier 2267 (NBG, BR, K)	TVL-MP, Long Tom Pass	DQ415206	DQ415065	DQ414922
<i>Disa patula</i> Sond. var. <i>transvaalensis</i> Summerh.	Bytebier 2245 (NBG, BR)	TVL-MP, Verloren Vallei	DQ415210	DQ415069	DQ414926

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Table 2 (continued)

Taxon <sup>a</sup>	Voucher information <sup>b</sup>	Origin <sup>c</sup>	GenBank Accession Nos.		
			<i>trnL</i> F	<i>matK</i>	ITS
<i>Disa intermedia</i> H.P.Linder	Bytebier 2472 (NBG, BR)	SWZ, Forbes Reef	DQ415208	DQ415067	DQ414924
<i>Disa nervosa</i> Lindl.	Bytebier 2296 (NBG)	CPP-EC, Ingeli	DQ415209	DQ415068	DQ414925
<i>Austroalpiniae</i> H.P.Linder					
<i>Disa zimbabweensis</i> H.P.Linder	Lotter sub McMurtry 12320 (PRE)	TVL-MP, Mount Formosa	DQ415242	DQ415101	DQ414958
<i>Disa</i>					
<i>Disa uniflora</i> P.J.Bergius	Harley s.n. (no voucher)	CPP-WC, Table Mountain	AF360435 AF360492	DQ415007	DQ414864
<i>Disa venosa</i> Sw.	Bytebier 2441 (NBG)	CPP-WC, Bain's Kloof	DQ415152	DQ415010	DQ414867
<i>Disa racemosa</i> L.f.	Bytebier 2448 (NBG, BR, K)	CPP-WC, Fernkloof	DQ415150	DQ415008	DQ414865
<i>Disa caulescens</i> Lindl.	Bytebier 2453 (NBG, BR, K, NY)	CPP-WC, Krom Rivier	DQ415149	DQ415006	DQ414863
<i>Disa tripetaloides</i> (L.f.) N.E.Br.	Bytebier 2460 (NBG, BR, K)	CPP-WC, Outeniqua Mountains	DQ415153	DQ415011	DQ414868
<i>Disa aurata</i> (Bolus) L.T.Parker & Koop.	Knox 4774 (NBG, BR)	CPP-WC, Langeberg	DQ415154	DQ415012	DQ414869
<i>Disa cardinalis</i> H.P.Linder	Bytebier 2311 (NBG, BR)	CPP-WC, Langeberg	DQ415155	DQ415013	DQ414870
<i>Disa vasselotii</i> Bolus ex Schltr.	Bytebier 2597 (NBG, BR, K)	CPP-WC, Garcias Pass	DQ415146	DQ415003	DQ414860
<i>Disa rosea</i> Lindl.	Bytebier 2423 (BR)	CPP-WC, Bain's Kloof	DQ415166	DQ415024	DQ414881
<i>Disa richardiana</i> Lem. ex Bolus	Bytebier 2435 (NBG)	CPP-WC, Table Mountain	DQ415145	DQ415002	DQ414859
<i>Disa pillansii</i> L. Bolus	Bytebier 2400 (NBG, BR, K)	CPP-WC, Boskloof	DQ415151	DQ415009	DQ414866
<i>Disa schizodioides</i> Sond.	Kurzweil 2095 (NBG)	CPP-WC, Swartberg	DQ415162	DQ415020	DQ414877
<i>Disa bodkinii</i> Bolus	Bytebier 2596 (NBG, BR)	CPP-WC, Garcias Pass	DQ415189	DQ415047	DQ414904
<i>Disa elegans</i> Sond.	Bytebier 2101 (NBG, BR)	CPP-WC, Gydo Pass	DQ415191	DQ415049	DQ414906
<i>Disa fasciata</i> Lindl.	Mostert 377 (no voucher)	CPP-WC, Lamlock Farm	DQ415163	DQ415021	DQ414878
<i>Disa bivalvata</i> (L.f.) T.Durand & Schinz	Bytebier 2438 (NBG, BR)	CPP-WC, Bain's Kloof	DQ415148	DQ415005	DQ414862
<i>Disa atricapilla</i> (Harv. ex Lindl.) Bolus	Bytebier 2316 (NBG, BR)	CPP-WC, Rawsonville	DQ415147	DQ415004	DQ414861
<i>Disa filicornis</i> (L.f.) Thunb.	Mostert 388 (no voucher)	CPP-WC, Kleinmond	DQ415143	DQ415000	DQ414857
<i>Disa tenuifolia</i> Sw.	Bytebier 2139 (NBG)	CPP-WC, Kogelberg	DQ415144	DQ415001	DQ414858
<i>Phlebidia</i> Lindl.					
<i>Disa maculata</i> L.f.	Bytebier 2402 (NBG)	CPP-WC, Boskloof	DQ415161	DQ415019	DQ414876
<i>Disa longicornu</i> L.f.	Bytebier 2434 (BR)	CPP-WC, Table Mountain	DQ415159	DQ415017	DQ414874
<i>Disa virginialis</i> H.P.Linder	Bytebier 2418 (NBG, BR)	CPP-WC, Bain's Kloof	DQ415160	DQ415018	DQ414875

<sup>a</sup> Order of taxa follows Kurzweil and Linder (2001) for the sections in *Disa*, and Linder (1981b,c,d,e,f) and Linder and Kurzweil (1999) for the species.

Author abbreviations follow Brummitt and Powell (1992).

<sup>b</sup> Herbarium acronyms are according to Holmgren et al. (1990).

<sup>c</sup> Abbreviations for geographical regions follow Brummitt (2001).

nucleotide distribution with gamma shape parameter and a proportion of invariant sites (GTR + I +  $\Gamma$ ) was selected for each partition with the help of MrModeltest v2.2 (Nylander, 2004). The chains were run for 1 million generations and sampled every 100 generations. This was repeated three times and these independent runs were compared to make sure that similar estimates of substitution model parameters, topology and branch lengths were obtained. Additionally, a 5 million generation run was performed. The number of “burn-in” generations needed before the various log-likelihood values reached stationarity was determined by a graphical plot and the first 2000 sampled generations were discarded. Swapping among chains and acceptance of proposed changes to model parameters were monitored to ensure that efficient mixing had occurred. Under the default 0.2 temperature parameter for heating the chains, swapping of chains proved to be below the recommended value of 10% and we therefore lowered this value to 0.075 to get an acceptable rate (> 10%) of chain swapping.

### 3. Results

#### 3.1. Length variation

Alignment was straightforward for the *matK* matrix, but proved to be more difficult for the *trnL*F and ITS matrices. There was extensive length variation in the *trnL*F region. The shortest sequence was of the outgroup species, *Disperis capensis*, which was only 408 bases long. The shortest ingroup sequence (*Schizodium flexuosum*) was 828 bases long, while the longest sequence (*Disa crassicornis*) was 1137 bases long. This makes the *trnL*F sequences of the species in *Disa* amongst the longest so far recorded (Shaw et al., 2005). The short outgroup sequences were due to a large deletion shared by both *Brownleea* and *Disperis* but not *Codonorchis* (or any other related genus for which sequence data is available on GenBank), while the length variation in the ingroup was due to extensive repeat regions within the *trnL* intron as described in detail by Bellstedt et al. (2001). These repeat sequences do not contribute any

phylogenetic information but make the matrix unwieldily in its length and introduce substantial amounts of missing data. They were therefore deleted. This reduced the aligned matrix length from 1892 to 1230 bases (Table 3). Sequence length variation was much less pronounced in the other two gene regions. The aligned matrix for *matK* was 1896 bases long with the shortest sequence being 1711 bases (*Disa similis*) and the longest 1768 bases (*Disa robusta*, *D. chrysoctachya* and *D. woodii*). The aligned sequence length of the ITS sequence matrix was 968 bases. The longest sequence was 842 bases (*Disperis dicerochila*), the longest ingroup sequence was 840 bases (*Disa cylindrica*) and the shortest sequence was 793 bases (*Disa schlechteriana*). However, the extensive sequence variation made it difficult to align, especially for the outgroups.

### 3.2. Parsimony analysis

The aligned *trnLF* matrix had 177 (14.4%) (Table 3) parsimony informative characters (PICs). The parsimony analysis resulted in a tree in which only 28 out of potentially 142 nodes had bootstrap (BS) support values over 75% (Table 3). Although the monophyly of the Disinae was retrieved with 94% BS, there was no resolution of the nodes directly above the subtribal node and the tree was basically a polytomy in which only a limited number of clades could be identified. The *matK* matrix had 412 (21.7%) PIC's, which resulted in a more resolved tree in which 55 nodes had over 75% BS support and several of the nodes above the subtribal node were resolved. The ITS data matrix yielded the highest number of PIC's, namely 507, which was 52.4% of all characters. This resulted in a tree in which 74 nodes received over 75% BS support.

The PH test between both plastid data sets gave a value of 0.641 indicating that the null hypothesis that they were sampled from the same phylogeny could not be rejected, and consequently they could be combined. The test between the combined plastid and the nuclear data set was significant with a *P* value of 0.01, indicating potential incongruence. However, the oversensitivity of the PH test is well documented (e.g., Reeves et al., 2001; Yoder et al., 2001; Barker and Lutzoni, 2002) and thus we used visual inspection to search the topologies for hard incongruences. No

incongruent clades in which both have a BS > 75% could be detected between the plastid and nuclear data sets. We, therefore, concatenated all data sets for a combined analysis. The combined data matrix had 4094 characters, 1096 (26.8%) of which were parsimony informative. The analysis retrieved 347 most parsimonious trees, one of which is presented as Supplementary Material. A bootstrap majority-rule consensus tree had 87 nodes with BS values over 75% (Table 3).

### 3.3. Bayesian inference analysis

A double run (Nruns=2) in MrBayes 3.1.1 gave an average standard deviation of split frequencies of 0.009332 after 2.5 million generations. The tree resulting from this analysis had 101 ingroup nodes with a posterior probability (PP) equal to or above 95% and is presented in Fig. 1. This tree is fully congruent with the strict consensus tree from the parsimony analysis. Although the strict consensus tree is more resolved than the Bayesian inference tree, all of these additional nodes on the parsimony tree receive less than 50% bootstrap support. We therefore prefer to present the slightly more conservative Bayesian inference tree.

### 3.4. Indel coding

Parsimony analysis of the individual indel-coded regions resulted in an increase of the number of nodes with more than 75% BS support for all three regions. For *trnLF* it increased the number of well-supported nodes from 28 to 34, for *matK* from 55 to 59 and for ITS from 74 to 80 (Table 3). Indel coding of the combined matrix increased the number of characters from 4094 to 4426, of which 1226 (27.6%) were parsimony informative (Table 3). The parsimony analysis resulted in 88 nodes with over 75% bootstrap support, just one more than the analysis without gaps. Moreover, this one extra node could have resulted from the stochastic nature of the BS test since it represented only a shift from 74 to 77%. The Bayesian inference analysis showed similar results. Whether analysed with or without indels coded, 101 nodes with more than 95% posterior probability were retrieved. In the

Table 3  
Matrix values and parsimony statistics of the *trnLF*, *matK* and ITS gene regions as well as of the combined dataset (All) without (–) and with (+) indels coded

	<i>trnLF</i>		<i>matK</i>		ITS			All		
	–	+	–	+	–	+	+	–	+	+
Aligned length	1230	1327	1896	1940	968	1169		4094	4436	
# Constant sites	933	933	1261	1284	365	368		2559	2585	
# Variable, non-informative sites	120	189	223	241	96	195		439	625	
# Parsimony informative sites	177	205	412	415	507	606		1096	1226	
% Informative sites	14.4	15.4	21.7	21.4	52.4	51.8		26.8	27.6	
CI	0.664	0.698	0.543	0.550	0.382	0.441		0.453	0.477	
RI	0.812	0.827	0.791	0.791	0.726	0.732		0.745	0.750	
# Steps on best tree	572	683	1471	1492	2993	3267		5126	5533	
# Nodes >75% BS	28	34	55	59	74	80		87	88	

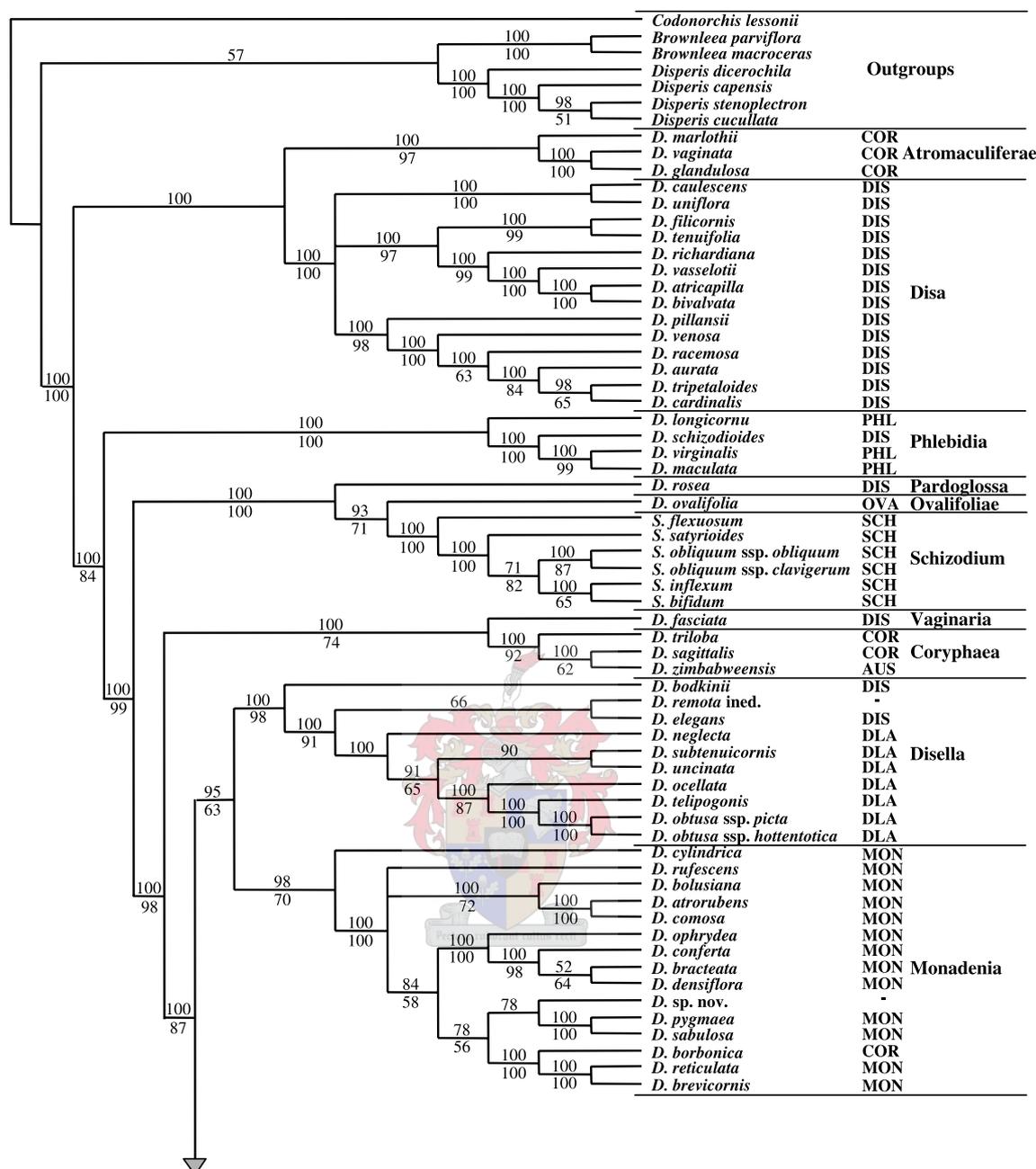


Fig. 1. Bayesian inference fifty percent majority tree based on a run of five million MCMC generations, sampling every 100th generation. Posterior probabilities are given above branches; below branches are bootstrap values from the parsimony analysis. The three-letter abbreviation to the right of the species name indicates in which section it is currently placed (see Table 1 for the section number code). The proposed delimitation and names of the revised sections, based on this analysis, are on the far right.

analysis with indels, the PP value of two nodes increased to above the 95% level, but two other nodes decreased to below the threshold values so that the net result was a status quo. Although we did not test this rigorously, our interpretation from a visual inspection of the sequence alignment was that the indels were indeed informative but support nodes that were already well corroborated by the substitution sequence data anyway. Since the indels did not contribute any additional information, the rest of this discussion is based on the analyses of substitution sequence data only.

### 3.5. Phylogenetic relationships

The monophyly of the Disinae is firmly supported by 100% BS and 100% PP. The genus *Schizodium*, however, is deeply embedded within the genus *Disa* and recognition of *Schizodium* would render *Disa* paraphyletic. Furthermore, according to the molecular data, the subgenera defined by Linder (1981c); see Table 1) do not represent the phylogenetic relationships amongst the sections.

We identified eighteen clades (Fig. 1) on the basis of the following criteria: (i) that they are monophyletic and well-

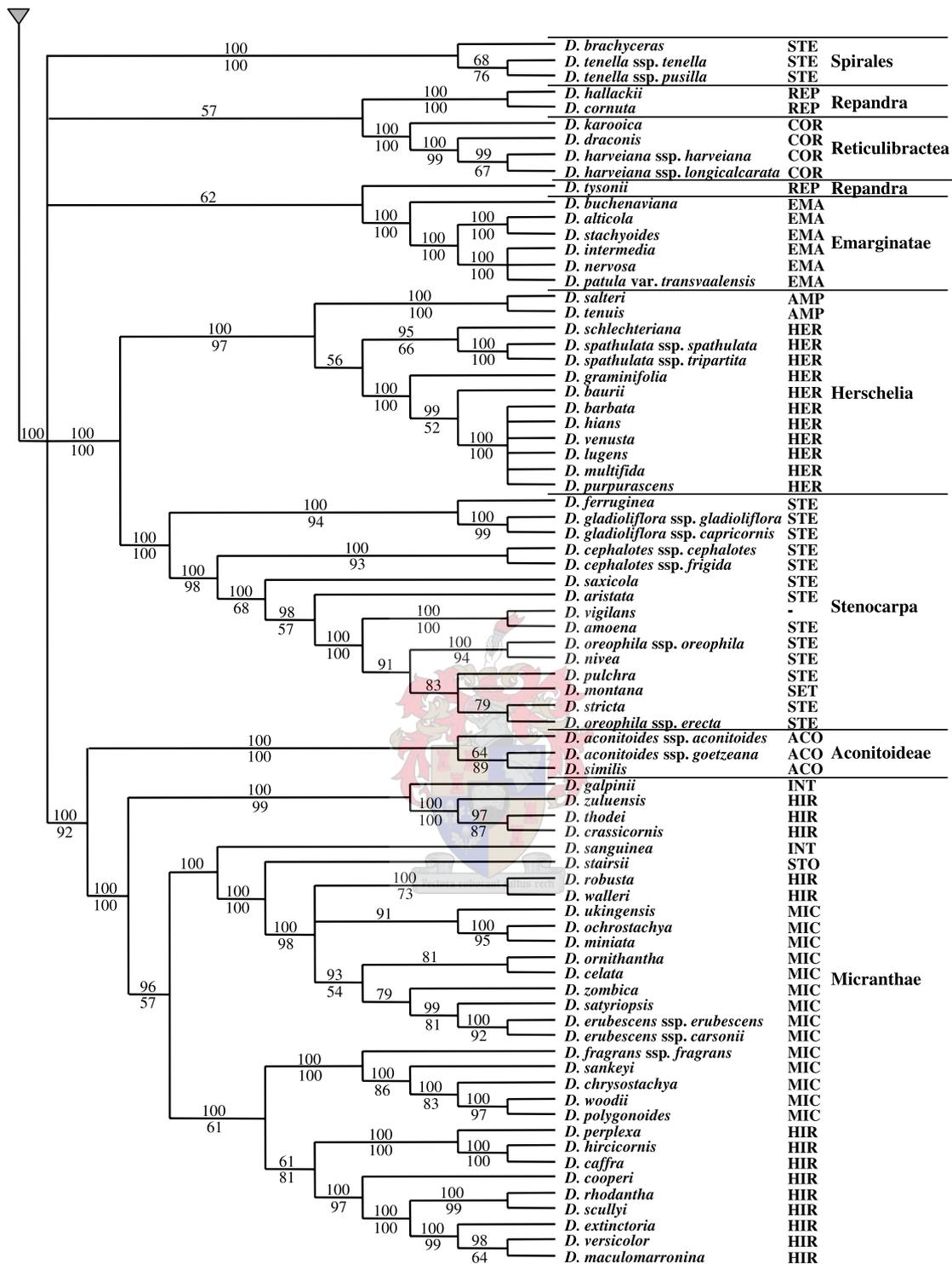


Fig. 1 (continued)

supported; (ii) that they are morphologically distinguishable (Fig. 2); and (iii) that there is maximum overlap with the classification of Linder (1981c,d) and Linder and Kurzweil (1999). These eighteen clades, for which we will use informal names here, will be formalised as sections in a separate paper, where we will discuss their delimitation in more detail. Thirteen of these clades bear resemblance to

the current classification based on morphology, and the sectional names as circumscribed by Linder (1981c,d) and Linder and Kurzweil (1999) can be used with the same circumscription, or in a slightly reduced or expanded sense. But while there is a good deal of overlap between the classification based on morphology and the results of this study, there are also some clear discrepancies and differences.

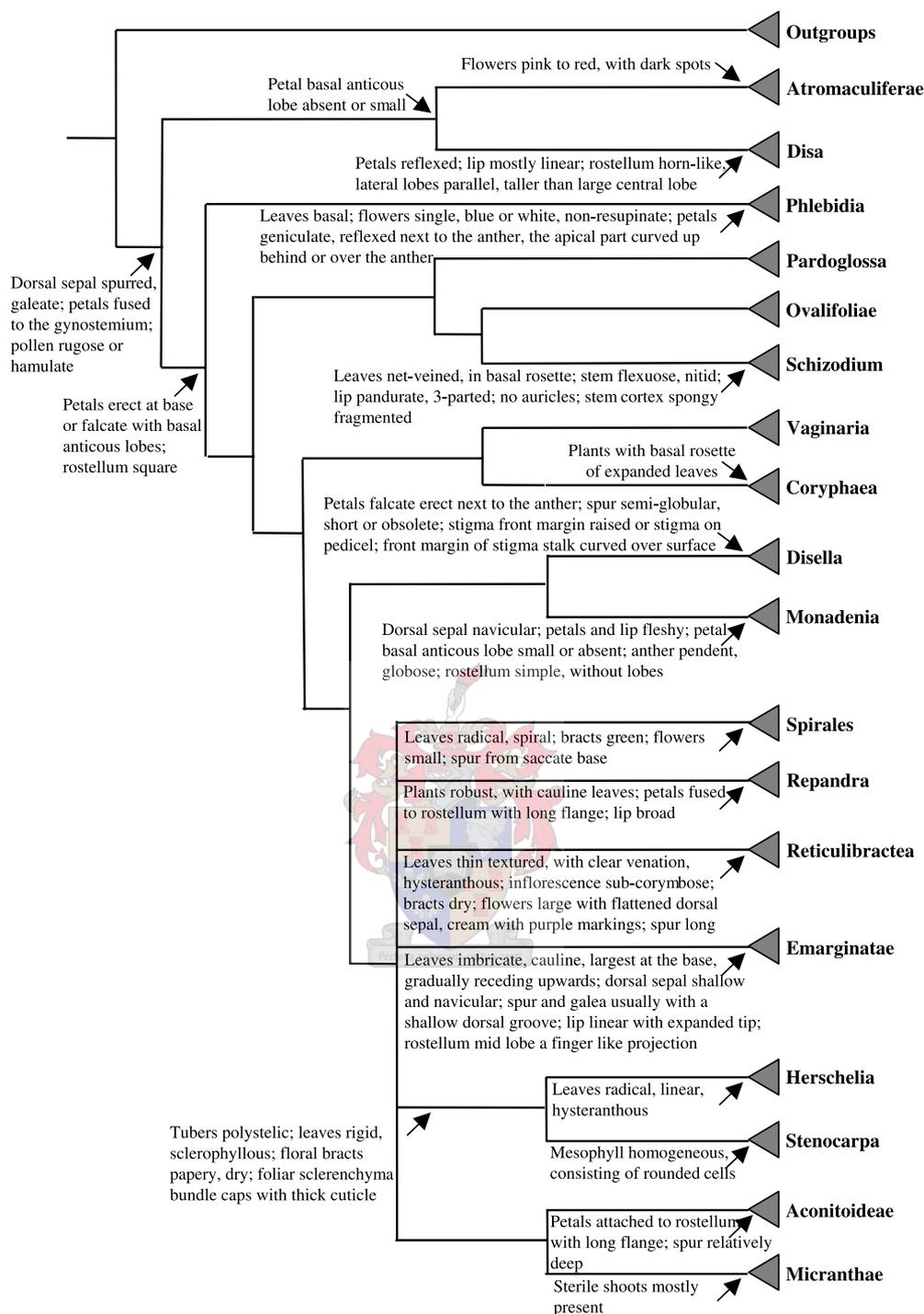


Fig. 2. Key morphological and anatomical synapomorphies mapped on a cladogram with sections as terminals.

Five species previously included in section *Disa* seem to be better placed elsewhere. *D. elegans* and *D. bodkinii* are the only two that are closely related, and together with the newly discovered *D. remota* fit better in section *Disella*. *D. rosea* and *D. fasciata* are better placed in monotypic sections while *D. schizoidioides* is embedded in section *Phlebidia*.

Section *Stenocarpa* is polyphyletic and the sister species, *D. brachyceras* and *D. tenella*, should form a separate section. Also polyphyletic is section *Coryphaea*, which consists

of three clades and one species that are not closely related. The position of one of these, is not certain. In the Bayesian inference analysis of the combined dataset, “*Atromaculiferae*” is sister to section *Disa* with 100% PP, and a parsimony analysis of the plastid dataset alone confirms this position with 100% BS. However, a parsimony analysis of the nuclear dataset retrieves “*Atromaculiferae*” in two possible positions: one as sister to section *Disa*, and the other as (*Disa*, (“*Atromaculiferae*”, rest of subtribe)). The three-species section *Austroalpiniae* is embedded in the remainder

of *Coryphaea* and is thus better merged with it. Section *Repandra* is polyphyletic in the Bayesian inference tree, but monophyletic (albeit without bootstrap support) in the strict consensus tree of the parsimony analysis. The Bayesian tree places *D. tysonii* as sister to section *Emarginatae* but also without support. Consequently we decided to retain the section with all three species based on the morphological data. Sections *Herschelianthe* and *Amphigena* are also retrieved as circumscribed by Linder (1981c,f), but the exact nature of the relationships between them could not be elucidated as the basal node of Linder's section *Herschelianthe* lacks meaningful support. It is, therefore, probably better to treat them as one section rather than two. Lastly, species from sections *Micranthae*, *Hircicornes*, *Intermediae* and *Stoloniferae* are intermingled. Subdividing this clade seems ill-advised at this time as only one subclade (*D. galpinii*, *D. zuluensis*, *D. crassicornis*, *D. thodei*) is 100% supported, but its relationship to the rest, although clear from a Bayesian inference analysis, receives only weak bootstrap support under parsimony.

## 4. Discussion

### 4.1. Congruence between chloroplast and nuclear datasets

We could not detect any “soft” or “hard” incongruence (Seelanan et al., 1997) between the plastid and nuclear datasets. Recent studies in the orchids by Gravendeel et al. (2004) on *Pleione* D. Don and by Van der Niet et al. (2005) on *Satyrium* Sw., a genus closely related to *Disa* (Douzery et al., 1999; Freudenstein et al., 2004), have found incongruence between the nuclear and plastid genomes. Artificial hybrids, even at the generic level, are common in orchids and form the basis of a worldwide horticultural trade. Although at least seven natural hybrids have been reported so far within the genus *Disa* (Stewart and Manning, 1982; Linder, 1981c, 1985, 1990), five are only known from single plants, ephemeral or small populations, while two (*D. patula* var. *patula*, *D. maculomarronina*) appear to have established larger populations (Linder, 1990). Genome mixing is thus certainly a possibility and will be manifested as inter-locus phylogenetic conflict, if the two loci are obtained from different parent taxa. While the absence of conflict is not evidence that there is no hybridization and more extensive sampling may reveal additional cases, it seems that hybridisation is rare and most probably not a factor in the speciation of this particular genus.

### 4.2. Molecules versus morphology—reasons for discrepancies

The evidence presented here supports a recircumscription of the genus *Disa* in the broadest possible sense, i.e. with the inclusion of *Monadenia* and *Herschelianthe*, as was already argued by several authors (Kurzweil et al., 1995; Johnson et al., 1998), and implemented by Linder and Kurzweil (1999), but also with *Schizodium*. The monophyly of *Schizodium* as such, has never been in doubt (Linder,

1981b; Linder and Kurzweil, 1990; Kurzweil et al., 1995), but it is a highly autapomorphic clade and as a result of this, its phylogenetic placement within the Disinae could not be determined, even though it has all the characters of the subtribe. For this reason most authors kept it as a separate genus.

The genus *Disa* is currently divided into five subgenera (Table 1) (Linder, 1981c; Linder and Kurzweil, 1999; Kurzweil and Linder, 2001). According to our findings the current subgenera are artificial since they do not reflect phylogenetic relationships and we, therefore, reject them. It seems that at this level in the phylogeny the relationships are very complex, and no taxa can be readily established that would group the sections.

A comparison of the sectional classification of Linder (1981c) and Linder and Kurzweil (1999) with our molecular analysis reveals that some sections are monophyletic, some contain misplaced elements, while some are polyphyletic. There are various reasons for these discrepancies.

Section *Disa* contains five misplaced species. Linder (1981c) commented that “*Disa fasciata* and *D. rosea* are morphologically isolated, while *D. bodkinii* and *D. elegans* are ecologically peculiar”. It is exactly these species, together with *D. schizodioides*, which turn out to be misplaced. The transfer of the latter species to *Phlebidia* is supported by several morphological characters (Fig. 2) and its previous placement in section *Disa* can thus only be seen as a taxonomic misjudgment. *D. bodkinii* and *D. elegans*, two morphologically closely related species, are better placed in section *Disella*. They share falcate petals which are erect next to the anther and a stigma front margin which is raised or a stigma on a pedicel and the front margin of the stigma stalk curved over the surface, with the other members of *Disella*. The morphologically isolated position of *D. fasciata* has long been recognised. Both Lindley (1830–1840) and Bolus (1888, 1914) assigned it to the monotypic section *Vaginarina* Lindl. and we suggest that this section be resurrected. The semi-actinomorphic flowers are very dissimilar from any other flower shape in the genus and resemble those of the genus *Adenandra* Willd. in the family Rutaceae. Its floral morphology may be pollinator driven towards mimicking plants in a different family, thus obscuring its evolutionary relationships. Similarly, *D. rosea* was morphologically isolated within section *Disa*, and our analysis shows it does not belong there. As sister to *D. ovalifoliae* and *Schizodium* it seems better placed in its own section (*Pardoglossa* Lindl.) as originally suggested by Lindley (1830–1840). All the above mentioned species (including *D. schizodioides* which is single-flowered) were placed in section *Disa* series *Corymbosae* H.P. Linder, on the basis of the non-resupinate flowers and corymbose inflorescence, yet they are morphologically extremely diverse species whose grouping together was artificial.

Section *Monadenia* as circumscribed by Linder and Kurzweil (1999) is retrieved virtually unchanged. Only one species, *Disa borbonica*, an endemic of the Island of Réunion, needs to be added to this section. It was previ-

ously placed in section *Coryphaea* (Linder, 1981c) as sister to *D. vaginata* and *D. glandulosa* on the basis of shared characters such as self-pollination and an undifferentiated flower structure. These characters appear to be of a homoplasious nature. *Disa borbonica* must have dispersed fairly recently from mainland Africa to Réunion, which is about 2 million years old (McDougall, 1971) and undergone speciation. It is known that in self-compatible island colonists, insufficient pollination caused by a paucity of pollinators favours floral traits that promote increased levels of selfing (Barrett, 1996). This often leads to a reduction in flower size and the loss of floral adaptations that promote cross-pollination (e.g., Barrett, 1985; Barrett and Shore, 1987; Inoue et al., 1996). This is the case in *Disa borbonica*, which has developed reduced and cleistogamous flowers. Moreover, the fact that very few herbarium specimens were available for taxonomic study, may have led Linder (1981c) to misplace *D. borbonica* in section *Coryphaea* rather than in section *Monadenia*. It is interesting to note that *D. borbonica* is closely related to *D. brevicornis*, the only other species in *Monadenia* that grows in a summer-rainfall regime. Unlike most species in this section these two species do not need fire as stimulus to induce flowering.

Section *Stenocarpa* contains two misplaced species. Linder (1981c) included *D. tenella* and *D. brachyceras* as series *Spirales* H.P.Linder in section *Stenocarpa* but remarked that this series was taxonomically difficult. Kurzweil et al. (1995) presented several anatomical arguments as to why *Spirales* was probably not part of *Stenocarpa*. *Disa tenella* is the only species that lacks both foliar sclerenchyma bundle caps and polystelic tubers. Furthermore, the arm-like mesophyll cells are quite unlike those of all the other members of *Stenocarpa*, which have a homogenous mesophyll consisting of rounded cells. From our results it is clear that *Spirales* needs to be raised to sectional level. Both species share radical, spiral leaves which are unique within the genus, as well as small flowers with green bracts and a spur from a saccate base. Both also flower in winter. This new section is phylogenetically isolated and appears on an unusually long branch (Supplementary Figure F1), something for which no immediate explanation can be found.

Three sections (*Coryphaea*, *Micranthae* and *Hircicornis*) were found to be polyphyletic. Section *Coryphaea* was originally established by Lindley (1830–1840) and recognised essentially unchanged by Linder (1981c). Both based it on very general characters such as an erect galea at the base funnel-shaped or saccate, a filiform or acuminate lip and erect petals, yet the species that make up this section are morphologically very diverse. Johnson and Linder (1995) expressed doubt about the monophyly of *Coryphaea* and our results indicate that the section is artificially assembled from three unrelated clades and one species. The first clade, which is sister to section *Disa*, consists of *D. vaginata*, *D. glandulosa* and *D. marlothii*. *D. vaginata* and *D. glandulosa* are autogamous and, as a result, have relatively undifferentiated flowers which makes them difficult to place on the basis of floral morphology. The second group consists of *D.*

*sagittalis* (the sectional type) and *D. triloba*, two morphologically quite similar species, which are closely related to *D. zimbabweensis* of section *Austroalpinae*, even though they bear little morphological resemblance to the latter. The third clade groups the species in the *D. draconis* complex (Johnson and Linder, 1995). The four taxa in this clade all share thin-textured, hysteranthous leaves, bracts with a clear reticulate venation pattern which are dry at anthesis, a subcorymbose inflorescence with large, white to cream flowers with purple markings, a flattened dorsal sepal and a long spur. The recognition of *Coryphaea s.l.* as a section by Linder (1981c) and Kurzweil and Linder (2001) was thus probably based more on the historical legacy left by Lindley's classification, than on a discernable pattern of synapomorphies.

Undoubtedly, the most surprising result from this analysis is phylogenetic intermingling of the large sections *Micranthae* (27 species) and *Hircicornes* (16 species). Both form part of a bigger clade that also includes the small sections *Intermediae* and *Stoloniferae*. The two species in section *Intermediae* only share some fairly general characters like cauline leaves, a dense cylindrical inflorescence with small flowers and, falcate and broadly ovate sepals. In section *Stoloniferae* the two species have no tubers and spread through stolons. The latter characters are also present in several species in section *Disa* and may thus be of a homoplasious nature. Section *Micranthae* has three floral synapomorphies: a pendent spur inserted above the base of the galea, small rostellum structures and an erect anther which is regarded as secondarily derived (Linder and Kurzweil, 1990). *Micranthae* also differs from the rest of the genus in its leaf anatomy (Kurzweil et al., 1995). It has a heterogeneous mesophyll, which is a definite apomorphy and a reticulate cuticle, possibly also apomorphic (Kurzweil et al., 1995). The substantial number of synapomorphies led Linder and Kurzweil (1990) to suggest that *Disa* section *Micranthae* should be treated as a distinct genus within the Disinae. The same conclusion was drawn from a palynological analysis of the group (Chesselet, 1989, 1993). This suggestion was, however, never formalised. Although plants in *Hircicornes* share an elliptic to spatulate lip and an elongated spur, these characters are also found in other sections, and consequently *Hircicornes* lacks clear synapomorphies. It is therefore understandable that species from section *Micranthae* are embedded in it, since there is no strong morphological evidence for the monophyly of *Hircicornes*. There is also some morphological support that section *Micranthae* (sensu Linder, 1981d) consists of two groups, even though it was not used to further subdivide the section into series (Linder, 1981d). The majority of species in section *Micranthae* have bilobed petals, which are unique in the genus. Nonetheless, there is, as yet, no molecular support for the monophyly of this group of species. The five species of section *Micranthae* with unlobed petals (*D. polygonoides*, *D. woodii*, *D. chrysostachya*, *D. sankeyi*, *D. fragrans* ssp. *fragrans*) are mostly distributed in southern Africa. Although unlobed petals are the plesiomorphic

state and the group lacks any other clear synapomorphies, on molecular data they form a clade. Nonetheless, the polyphyly of section *Micranthae* remains surprising. Section *Micranthae s.l.* (as defined here) has some morphological support in that nearly all species produce sterile shoots, a character that is also infrequently seen in the sister group *Aconitoideae*. In the phylogeny proposed by Linder (1982) and Linder and Kurzweil (1999) this was thought to be a homoplasious attribute, however, our results clearly shows that the presence of sterile shoots is an informative taxonomic character. Interestingly, sterile shoots also occur in members of the closely related genus *Satyrium*, although this character is not phylogenetically informative there (Kurzweil and Linder, 1998; Van der Niet et al., 2005). In both genera it is only found in summer rainfall species but contrary to *Disa*, sterile shoots seem to have evolved several times in *Satyrium* (Van der Niet et al., 2005; van der Niet, pers comm.).

The sections in *Disa* and the relationships among them were largely based on a combination of the lip, rostellum, column and petal structures (Linder, 1982). The combination of these is strongly influenced by the pollination syndrome and this might obscure the correct phylogenetic relationships. Vegetative characters were thought to be inferior to deduce the higher relationships and several of the vegetative specialisations such as the occurrence of sterile shoots or the presence of hysteranthous basal leaves were thought to cut across groups and be the result of adaptations to certain climatic regions (Linder, 1982). Our analysis suggests otherwise and shows that the above-mentioned vegetative characters are synapomorphies and thus good indicators of phylogenetic relationships (Fig. 2). A similar observation was recently made by Cameron (2005) who performed a molecular phylogenetic analysis of Malaxidae, a tribe of epidendroid orchids that has been taxonomically problematic. He found that the tribe could be split into two major clades: one of terrestrial species and the other of epiphytes. Within the terrestrial clade, species with conduplicate leaves were clearly separated from species with plicate leaves, and within the epiphytes, species with laterally compressed leaves also formed a clade.

Taxonomists erecting a classification have to assign all taxa in the group, even if there is little clarity on the relationships of some of them. They may be reluctant to create multiple monotypic groups for problematic taxa, as monotypic taxa do not provide any information about the phylogenetic relationships of the immediately subordinate taxon (Backlund and Bremer, 1998). Consequently, monotypic taxa will be avoided to minimise redundancy in classification (Backlund and Bremer, 1998), even though monotypic taxa may be natural entities and should therefore be recognised as such (Stace, 1989). Within this framework, errors are possible when synapomorphies are not obvious. While, in general, the sectional classification based on morphology agrees well the molecular analysis, we identified several misplaced elements and polyphyletic clades. Several reasons were identified for these discrepancies. In the case of *D.*

*schizodioides*, it was a mere taxonomic misjudgement. For the polyphyletic section *Coryphaea*, perhaps the historic legacy played a major role. Lindley included eight morphologically highly divergent species in his section *Coryphaea*, which was recognised essentially unchanged by Bolus (1888), Kraenzlin, 1897–1904 and Schlechter (1901). Although Linder (1981c) realised that the synapomorphies for this section were very general, he could not associate the constituent species with any of the other sections and thus kept it as was originally suggested. Another reason for the misplacement of taxa was reduced or undifferentiated flowers, which obscure relationships. *D. borbonica*, *D. vaginata* and *D. glandulosa* all have reduced flowers as a result of being autogamous, thus they were grouped together. However, by far the most important reason why species were misplaced is the extraordinary morphological diversity of the Disinae and the fact that many taxa have become highly autapomorphic. The evolutionary diversification of the Disinae may be pollinator driven. Johnson et al. (1998) found 19 different specialised pollination systems in only 27 species of *Disa*. Since then, several more have been documented (Johnson, 2000; Johnson et al., 2003; Johnson and Brown, 2004; Anderson et al., 2005; Johnson, 2005; Johnson et al., 2005). At least seven species offer no reward to pollinators, but mimic rewarding species. The models are not orchids, but are taxonomically and morphologically divergent, including *Watsonia*-Iridaceae (Johnson, 2000), *Zaluzianskya*-Scrophulariaceae (Anderson et al., 2005), *Adenandra*-Rutaceae (Johnson, 1992), *Tritonopsis*-Iridaceae (Johnson, 1994), *Kniphofia*-Asphodelaceae (Johnson, 1994) and *Pelargonium*-Geraniaceae (Johnson and Steiner, 1997). In addition, adaptation to pollination by several different orders of insects (Johnson et al., 1998) as well as birds (Johnson and Brown, 2004) have led to a diversity of flower forms within a single genus that is almost unmatched. As a result, several autapomorphic taxa have evolved for which relationships have been difficult to infer. In some cases these autapomorphic taxa were assigned to separate genera such as *Schizodium*, *Monadenia*, *Herschelia(nthe)*, *Forficaria*, *Amphigena*, *Penthea* and *Orthopenthea*. In other cases they were assigned to monotypic sections, such as *Ovalifoliae*, or repeatedly placed in different sections by different (or even the same) taxonomists. It is thus divergence in the morphology, more than convergence, which has led to the misplacement of taxa and the discrepancies in the circumscription of the sections between the morphological and molecular analysis.

#### 4.3. Conclusion

Although considerable advance has been made in delimiting natural sections, the relationships amongst them could not be unequivocally determined as some internal nodes remain unresolved or unsupported. Due to the complex nature of these relationships and the unbalanced topology of the tree, taxa (subgenera) that would group the sections could thus not be established. Nevertheless, the addition of

a new dataset has allowed significant progress in clarifying the sectional delimitation in *Disa*, one of Africa's largest ground orchid genera.

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### Appendix A. Supplementary data

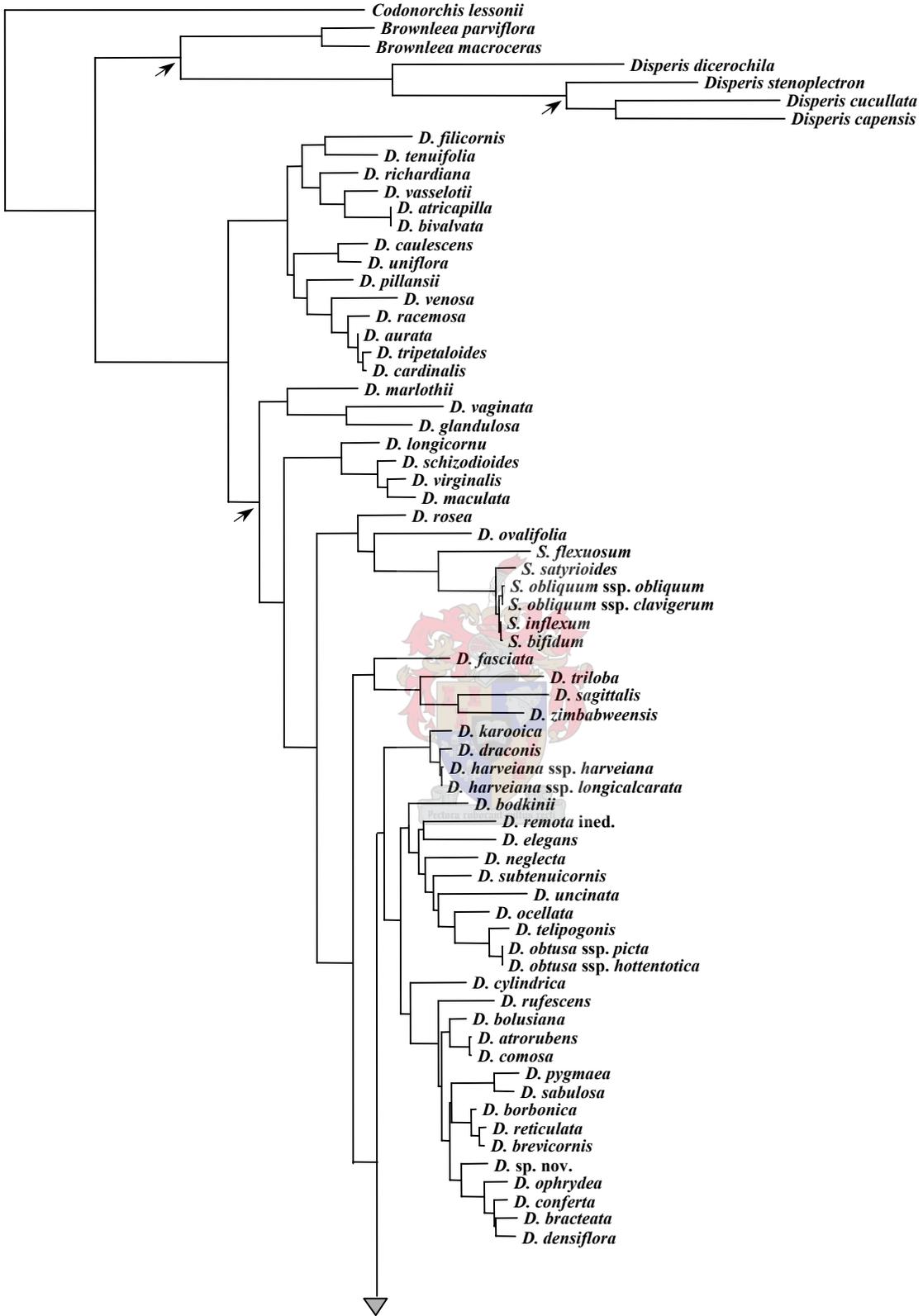
Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympcv.2006.08.014](https://doi.org/10.1016/j.ympcv.2006.08.014).

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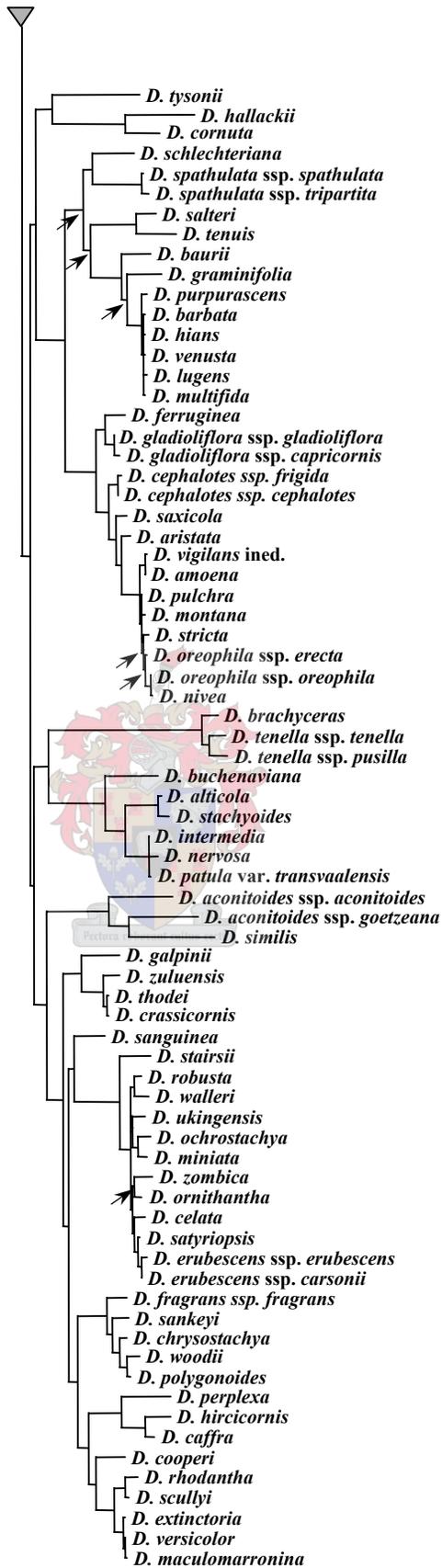
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Supplementary Material



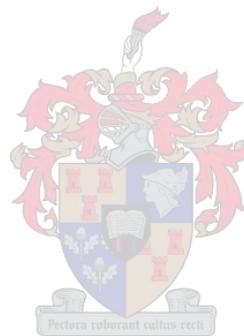
Supplementary Figure F1. One out of 347 most parsimonious trees, presented as a phylogram under Acctran optimization. Arrows indicate branches which collapse in the strict consensus tree.



— 10 changes

## Chapter 2

### A new phylogeny-based sectional classification for the large African orchid genus *Disa*



# A new phylogeny-based sectional classification for the large African orchid genus *Disa*

Benny Bytebier<sup>1,\*</sup>, Dirk U. Bellstedt<sup>1</sup> and H. Peter Linder<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Stellenbosch University, Private Bag X1, 7602 Matieland, South Africa

<sup>2</sup>Institute for Systematic Botany, University of Zurich, Zollikerstrasse 107, CH 8008, Zurich, Switzerland

\*Author for correspondence (bytebier@sun.ac.za)



Keywords: Orchidaceae, Orchidoideae, Disaceae, Disinae, *Disa*, *Schizodium*, molecular systematics, phylogenetic classification, Africa.

Running title: Sectional classification of *Disa*

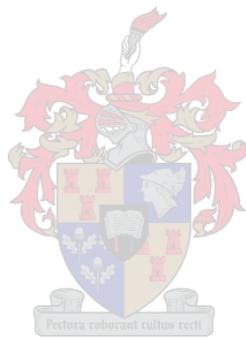
(prepared for Taxon)

## Abstract

Twenty five years after the last major revision, the results of a molecular phylogenetic analysis have necessitated a re-classification of the Disinae (Orchidoideae, Orchidaceae). The small genus *Schizodium*, endemic to the Cape Floristic Region, is deeply embedded in the genus *Disa* and its generic status can thus no longer be maintained. We propose a monotypic subtribe Disinae and a subdivision of *Disa* into eighteen sections. The sections are monophyletic, well-supported, morphologically distinguishable and are delimited to maximize the congruence with the previous classification. All currently known species are enumerated and assigned to sections.

## Introduction

The Disinae were last subject of an extensive revision twenty five years ago. Linder (1981a-f) divided the subtribe into five genera: *Brownleea* Harv. ex Lindl., *Disa* P.J.Bergius, *Monadenia* Lindl., *Herschelia* Lindl. and *Schizodium* Lindl.. *Brownleea*, included at first because it shares a spurred dorsal sepal with the other genera, was later transferred to its own subtribe Brownleeinae H.P.Linder & Kurzweil (Linder & Kurzweil, 1994). Further investigations (Kurzweil & al., 1995; Johnson & al, 1998) showed that *Monadenia* and *Herschelia*, the latter in the meanwhile renamed *Herschelianthe* Rauschert (Rauschert, 1983), were better placed within *Disa*. *Schizodium*, while sharing many characters with *Disa*, was kept as a separate genus because of its highly autapomorphic nature. This changing generic division has characterised the taxonomic history of the Disinae. Different authors (Lindley, 1830-1840; Bolus, 1888, 1889; Kraenzlin, 1897-1904; Schlechter, 1901; Rolfe, 1912-1913, Bolus, 1918) had entirely different views on where generic boundaries ought to be drawn (for an overview see Table 1; Bytebier & al, in press) and the extensive morphological diversity (see Fig. 1) made it difficult to reconcile botanical opinion into a stable taxonomy.



The most recent classification (Linder & Kurzweil, 1999; Kurzweil and Linder, 2001) recognized two genera, *Disa* and *Schizodium*, with the genus *Disa* subdivided in five subgenera and 17 sections.

Table 1: Historical subdivision of the Disinae

Lindley 1830-1840	Bolus 1889	Kraenzlin 1897-1902	Schlechter 1901	Rolfe 1912-1913
Disa	Disa	Disa	Disa	Disa
§Macranthae	§Monadenia	§Scutelliferae	§Monadenia	
§Micranthae	§Eudisa	§Vexillata	§Calostachys	
§Repandra	§Vexillata	§Polygonoideae	§Macro-Disa	
§Phlebidia	§Coryphaea	§Hircicornis	§Penthea	
§Vaginaria	§Schizodium	§Corymbosae	§Corypnaea	
§Pardoglossa	§Orthocarpa	§Macranthae	§Aegoceratium	
§Coryphaea	§Vaginaria	§Coryphaea	§Disella	
§Stenocarpa	§Herschelia	§Aconitoideae	§Eu-Disa	
§Oregura	§Oregura	§Disella	§Orthocarpa	
§Trichochila	§Aristaria	§Spathulatae	§Herschelia	
§Disella	§Amphigena	§Vaginaria	§Oregura	
			§Forficaria	
				Orthopenthea
				Amphigena
Penthea				Penthea
Monadenia		Monadenia		Monadenia
Schizodium		Schizodium	Schizodium	Schizodium
Forficaria	Forficaria	Forficaria		Forficaria
Herschelia		Herschelia		Herschelia

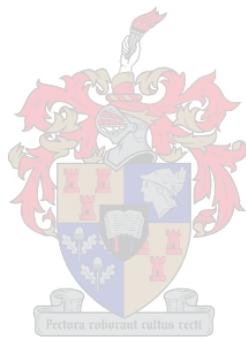
The advent of molecular taxonomy has allowed us to add a completely new dataset to the morphological, anatomical, and palynological data previously available on the Disinae. Early work into the tribe Diseae (Douzery & al, 1999) showed that species formerly included in *Monadenia* and *Herschelia* are indeed closely related to *Disa*. Bellstedt & al. (2001) confirmed that the above two genera are nested within *Disa* and that *Brownleea* did not form part of the subtribe Disinae. Corroboration of the subgeneric classification (Linder & Kurzweil, 1999; Kurzweil & Linder, 2001) and of position of *Schizodium* was not possible because of the limited sampling.

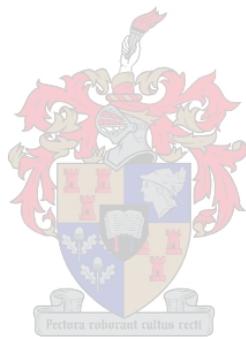
In an accompanying chapter (Bytebier & al., in press) we present a detailed molecular phylogenetic analysis of the Disinae, based on sampling of over 70 % of all known species. Taxa from both genera, all sections, the full geographic range, and all major habitats were included. The objectives of this paper are (i) to discuss the taxonomic implication of our molecular phylogeny, (ii) to propose a new formal classification, (iii) to justify the basis for this classification, and (iv) to assign all known *Disa* species to their appropriate sections.

## **Material and methods**

Phylogenetic relationships were inferred for 7 outgroup and 136 ingroup taxa, representing 70% of all recognised *Disa* taxa. One nuclear and two plastid gene regions were sequenced and compiled in a matrix with 4094 characters, 1096 (26.8%) of which were parsimony informative. In a parsimony analysis, 87 nodes of 142 (61%) were supported with a bootstrap value of 75% or higher, and the topology resulting from a Bayesian inference analysis had 101 (71%) nodes with a posterior probability of at least 95% (Fig. 2). The detailed phylogenetic analysis, which forms the basis of the currently proposed classification, can be found in Bytebier & al. (in press). Eighteen sections were identified on the basis of the following criteria: (i) that they are monophyletic and well-supported; (ii) that they are morphologically distinguishable; and (iii) that there is maximum overlap with the classification of Linder (1981b-f) and Linder & Kurzweil (1999). Here we discuss these sections in more detail and outline our arguments for recognizing them.

Several of the new sections are large and a further subdivision in subsections and series may be desirable. Although some patterns are emerging on which further subdivisions could be based, we refrain from doing so. Firstly, some of these clades are phylogenetically not firmly supported. Secondly, our species sampling has only reached 70%. While we are confident that the missing elements can be comfortably housed within the proposed sectional classification, we have reasons to believe that some of them might be crucial to reveal the phylogenetic relationships within the sections. Subdivision of the sections should be based on a more complete species sampling, as well as the inclusion of more sequence data to produce a more robust phylogenetic hypothesis.





We list all currently recognised species, subspecies and varietal names in accordance with the World Checklist of Monocots (2004). We have, however, treated *D. deckenii* Rchb.f. as a subspecies of *D. fragrans* following Linder (1981c) and added two newly described species i.e. *D. remota* (Linder & Hitchcock, 2006) and *D. vigilans* (McMurtry & al., 2006), as well as one as yet undescribed species. We have not included synonymy as this is available from the World Checklist of Monocots (2004), except for § *Schizodium*, which has been treated as a separate genus for over 100 years and for which several new combinations have to be made. Taxa for which sequence data were not available were placed to their appropriate sections on the basis of morphological data (Linder, 1981b-f; Linder & Kurzweil, 1990; Chesselet & Linder, 1993; Johnson & Linder, 1995, Kurzweil & al, 1995, Linder & Kurzweil, 1999).

### **Justification for the new classification**

The genus *Disa* is currently divided into five subgenera: *Micranthae*, *Falcipetalum*, *Hircicornes*, *Stenocarpa* and *Disa* (Linder, 1981c; Linder, 1986; Linder & Kurzweil, 1999; Kurzweil & Linder, 2001). These subgenera do not reflect phylogenetic relationships and we therefore propose not to recognise them any longer. At this level in the phylogeny the relationships are very complex, and no obvious taxa can be readily established that would group the sections, such that they are morphologically defined and not excessive in number.

Section *Disa* sensu Linder (1981c) is largely retained, but several misplaced elements are removed and placed in other, or in monotypic, sections. The newly circumscribed section is morphologically very variable and several elements included here have in the past been assigned generic status i.e. *Penthea* Lindl. and *Orthopenthea* Rolfe (Lindley, 1830-40; Schlechter, 1901; Rolfe 1912-13). Except for *D. tripetaloides*, which extends northeast into Pondoland, this section is restricted to the Cape Floristic Region (CFR) of South Africa. Three clades can be provisionally identified within the section. *D. uniflora*, the type species for the genus, forms a clade with *D. caulescens*. This clade groups with a second clade of mostly rhizomatous species, which includes *D. tripetaloides* and *D. cardinalis* amongst others. The third clade consists of the two members of Linder's (1981c) series *Egaleatae* (*D. filicornis*-*D. tenuifolia*) sister to a

number of species that share non-resupinate flowers and a corymbose inflorescence (mostly *Orthopenthea* Rolfe).

Sister to § *Disa* are three species (*D. vaginata*, *D. glandulosa*, *D. marlothii*) that were previously placed in § *Coryphaea* (Linder, 1981c). They form a clade that is unrelated to the clade containing the type species of § *Coryphaea* and are consequently placed in a section of their own. All three species share a preference to growing along stream sides or in moist places with the members of § *Disa*. *Disa vaginata* and *D. glandulosa* are autogamous and as a result are morphologically relatively undifferentiated, but all three species have pink to red flowers marked with dark spots and the section is therefore named *Atromaculiferae*. This section is restricted to the CFR.

Section *Phlebidia* sensu Linder & Kurzweil (1999) is expanded by the addition of *D. schizodioides*, which was previously placed in § *Disa*. All species share a cluster of basal green, cauline leaves that are often dry at anthesis, and a single white to blue and non-resupinate flower. Ecologically, they are almost completely restricted to horizontal, south-facing rock ledges. Section *Phlebidia* is restricted to the CFR.

The composition of the clade which includes *Schizodium* is morphologically unexpected. Because of its highly autapomorphic nature, most authors, except for Bolus in his early work, retained *Schizodium* as a separate genus. With a rosette of basal leaves, spotted adaxially and often purple abaxially, a nitid, flexuose and wiry stem and a pandurate lip, it is easily recognisable in the field. Yet it is deeply embedded within *Disa*, thus confirming the opinion of Bolus that it is part of a larger genus. As the former genus *Schizodium* forms such a well-defined morphological group, and because we cannot establish any characters linking § *Schizodium* morphologically to *D. ovalifolia* and *D. rosea*, which are placed as sequential sisters to it, we retain *Schizodium* as a section. *D. ovalifolia* was already placed in a monotypic section (§ *Ovalifoliae*) by Linder (1981c), and we retain this. Linder (1981c) placed *D. rosea* in § *Disa*, whereas Lindley (1830-40) assigned it to the monotypic § *Pardoglossa* because of its peculiar morphology. We resurrect the latter section. All species in these three sections are endemic to the CFR.

The second morphologically unexpected, but well-supported clade, consists of *D. fasciata*, *D. sagittalis*, *D. triloba* and *D. zimbabweensis*. *D. fasciata*, with its semi-actinomorphic flowers that mimic those of the genus *Adenandra* Willd. (Rutaceae), was assigned by Lindley (1830-40) to the monotypic § *Vaginarina*. This treatment was followed by Bolus (1888, 1889, 1918) and Kraenzlin (189-1904), but not by Linder (1981c), who placed it in § *Disa*. To accommodate this unusual species we propose to resurrect Lindley's § *Vaginarina*. It shares its white flowers and a subcorymbose inflorescence with *D. sagittalis* and *D. triloba*, two species which are morphologically clearly related. Embedded, however, within the *D. sagittalis* - *D. triloba* clade is *D. zimbabweensis*, one of three species that form Linder's § *Austroalpinae*. They share a basal rosette of expanded leaves and grow mostly lithophytically or on shallow or gravelly soil. While this not entirely satisfactory, we propose to sink Linder's § *Austroalpinae* into § *Coryphaea* pending further investigation and the inclusion of *D. basutorum* and *D. rungweensis* in the molecular sampling. Section *Vaginarina* is endemic to the CFR, while § *Coryphaea* is widespread and ranges from the CFR to southern Tanzania.

Section *Disella* is expanded to include *D. bodkinii* and *D. elegans*, previously placed in § *Disa*. The synapomorphies for this section include a short, semi-globular or an obsolete spur, falcate petals which are erect next to the anther, stigma front margin raised or the stigma on a pedicel and the front margin of the stigma stalk curved over the surface. The recently discovered *D. remota* (Linder & Hitchcock, 2006) with its unusual trilobed lip also belongs to this clade. It is interesting to note that *D. obtusa* and *D. neglecta*, which resemble each other morphologically, are not sister species. Section *Disella* is entirely restricted to the CFR.

Section *Monadenia* as circumscribed by Linder & Kurzweil (1999) is retrieved virtually unchanged, and only *D. borbonica*, an endemic from Réunion previously placed in § *Coryphaea*, needs to be included in this section. Within § *Monadenia*, *D. cylindrica* is sister to all other species. *Disa cylindrica* has in the past been difficult to place. Lindley (1830-40) kept it in genus *Disa* following Swartz (1800), but did not assign it to any of his sections because he did not have any material to examine. Nevertheless, he comments “an *Monadenia*, *densiflorae* affinis?”, a statement which proved close to the truth. Bolus (1888) placed it in his rather unnatural § *Eu-disa*, but

comments that “owing to peculiarities of perianth or column” it was not satisfactorily referred there but “that it appeared undesirable to add sections containing single species”. Linder (1981b) placed it in § *Coryphaea*, but Linder & Kurzweil (1999) later transferred it to § *Monadenia*. It lacks many of the typical features of § *Monadenia*, most notably the single viscidium and the unlobed rostellum, and seems genetically isolated, but is better placed within § *Monadenia* than in a monotypic section. The relationships between the other species in § *Monadenia* are unclear as several nodes are unresolved or poorly supported, in spite of the fact that 74% of the taxa were sampled. Perhaps the inclusion of *D. macrostachya*, which Linder (1981e) thought to be a "link" between various subgroups will clarify relations. Section *Monadenia* is mostly centered in the CFR, but occurs as far north as Zimbabwe, Malawi and Réunion. *D. bracteata* has been introduced to Australia.

The anatomical evidence of Kurzweil & al. (1995) that series *Spirales* (*D. tenella* and *D. brachyceras*) was not part of § *Stenocarpa* sensu Linder (1981c) is corroborated by the variation in the DNA sequence data. Consequently we raise *Spirales* to sectional level. The relationship of this section to the other sections remains unclear. Section *Spirales* is endemic to the CFR.

The three members of § *Repandra* were retrieved as a clade in the strict consensus tree based on the parsimony analysis, but without any bootstrap support. *Disa cornuta* and *D. hallackii* are always retrieved as sister species with a 100% support, but their relationship to *D. tysonii* remains unconfirmed. We prefer a conservative approach and maintain § *Repandra* as circumscribed by Linder (1981b) and Linder & Kurzweil (1999). All three species are very robust plants with cauline leaves and dense, many-flowered inflorescences. *D. cornuta* is one of the very few species that occurs both in the winter- and summer-rainfall areas of southern Africa. Not surprisingly, it is a very variable species and a form occurring on the sandveld of the Cape West Coast was described as a separate species (*D. aemula* Bolus). While Linder (1981b) was of the opinion that the variation was continuous and thus preferred a wider circumscription of the species, the possibility exists that it consists of several incipient taxa. This needs further investigation, as does the status of *D. tysonii*. Section *Repandra* occurs from the CFR to as far north as Zimbabwe.

The last clade in the polyphyletic § *Coryphaea* sensu Linder (1981b) is formed by the species in the *D. draconis* complex (Johnson & Linder, 1995). The four taxa in this clade are morphologically closely related to each other, and as they are isolated from the rest of § *Coryphaea* they are best treated as a separate section. They share thin-textured, hysteranthous leaves and bracts with a clear reticulate venation pattern, hence the new sectional name *Reticulibractea*. *Disa karooica*, the only species in the genus that occurs in the dry Succulent Karoo Biome is sister to the three other taxa. Section *Reticulibractea* is restricted to the Greater Cape Floristic Region (Jürgens, 1991; Born & al., in press).

Section *Emarginatae* was already strongly supported in the preliminary analysis of Bellstedt & al. (2001), even though only one gene region was sequenced for three out of six species in the section. We have sampled all six species and confirm the strong support for this section. Several synapomorphies, such as the imbricate cauline leaves which are widest at the base and gradually narrowed upwards, a dorsal groove on the spur and galea, and the finger-like projection on the rostellum mid lobe, characterize this clade. *Disa buchenaviana*, endemic to the central highlands of Madagascar, is sister to the species occurring on the summer-rainfall, high-altitude grasslands of southern Africa and must have resulted from a dispersal event to Madagascar. Two other, fully supported clades can be identified: one formed by the diminutive *D. alticola* and *D. stachyoides* and the other by the robust *D. nervosa*, *D. patula* and *D. intermedia*. Section *Emarginatae* occurs from the Eastern Cape of South Africa to Zimbabwe and in Madagascar.

Only two out of four species in § *Amphigena* sensu Linder & Kurzweil (1999) were sampled and they form a strongly supported clade closely related to § *Trichochila*. Unfortunately the exact nature of the relationships could not be elucidated. This might be due to the incomplete sampling. In particular, *D. newdigateae* and *D. forficaria*, two morphologically quite isolated members of § *Trichochila* might play a pivotal role in revealing the exact relationships (Linder, 1981e). However, fresh material could not be obtained since both species are extremely rare. The former one is only known from two collections and was last seen in 1932, while the latter is known from five collections and was last seen in 1966, despite intensive efforts over the last ten years to trace it. As the relationships between these two sections are unresolved, we

merge them into § *Trichochila*. Species in this section all share radical, linear, hysteranthous leaves. Our results do not support the suggestion of Linder & Kurzweil (1999) that *D. schlechteriana*, which differs from the rest by having a distinct spur and an entire lip, might be better placed in § *Stenocarpa*. It is also noteworthy that the relationships between *D. barbata*, *D. hians*, *D. lugens*, *D. multifida*, *D. purpurascens* and *D. venusta* could not be resolved. Most of these occur on the sandy flats of the western and southern Cape, which were all below sea level as recently as the late Pliocene (Sieser & Dingle, 1981). This could indicate that these species are of recent origin, which would be supported by the fact that they show almost no molecular variation. Section *Trichochila* is widespread and occurs from the CFR to southern Tanzania.

Within the reduced § *Stenocarpa*, the species occurring in the CFR are sister to the species occurring in the Drakensberg and further north. *Disa porrecta*, which occurs between the CFR and the Drakensberg, was unfortunately not sampled, although morphology places it as sister to *D. ferruginea*, which occurs in the CFR. The species in the CFR all flower in autumn, the driest period of the year. They share radical, linear hysteranthous leaves, while species occurring in the summer-rainfall areas share cauline leaves which are green at the time of flowering. Linder (1981c) subdivided the above group into series *Radicales* (the CFR species plus *D. porrecta*) and series *Natalenses* (the Drakensberg species). Pending the confirmation of the position of *D. porrecta*, this subdivision still holds. Although it was not the objective of this study to evaluate species as such, it is worthy to note that the two subspecies of *D. oreophila* are not sister taxa. Section *Stenocarpa* is restricted to South Africa, except for *D. saxicola* which ranges from the Eastern Cape to southern Tanzania.

Section *Aconitoideae* sensu Linder (1981c) is the most poorly sampled section in our analysis, since we managed to collect only two species out of eight (three taxa out of ten). Nevertheless, this is a morphologically homogenous section and we have no reason to assume that it would not be monophyletic. This section is very widespread throughout Africa. The type species, *D. aconitoides*, for instance, ranges from the Eastern Cape in South Africa to Ethiopia. *D. pulchella* is found in both Ethiopia and the mountains of Yemen.

Section *Micranthae* sensu Linder (1981d), § *Hircicornes* sensu Linder (1981c) and § *Intermediae* (Linder 1981c) are polyphyletic, with their species, as well as the only species sampled from § *Stoloniferae*, being phylogenetically intermingled. The two species in § *Intermediae* only share some fairly general characters like cauline leaves, a dense cylindrical inflorescence with small flowers and falcate, broadly ovate sepals. The two species in § *Stoloniferae* have no tubers and spread by means of stolons. Section *Micranthae* has three floral synapomorphies: a pendent spur inserted above the base of the galea, small rostellum structures and an erect anther. It also differs from the rest of the genus in its leaf anatomy (Kurzweil & al., 1995). Section *Hircicornes* lacks clear synapomorphies. We combine these four sections into one enlarged § *Micranthae*, within which several groupings can be detected. The well supported *D. galpinii* clade consists of four southern African species, previously placed in § *Hircicornes* and § *Intermediae*, while the *D. stairsii* clade, also well supported, consists solely of species that occur in south-central and eastern Africa. The latter is sister to a weakly supported clade of species, which are either restricted to southern Africa or are amongst the most widespread species in the genus. As only 31 out of 50 taxa were sampled and only one of the more basal nodes is firmly supported, subdivision seems ill-advised. Nearly all species in § *Micranthae* produce sterile shoots, a character that is also infrequently seen in the sister § *Aconitoideae*. In the phylogeny proposed by Linder (1981c,d) and Linder & Kurzweil (1999) this was interpreted as a homoplasious attribute, however, our results suggest that the presence of sterile shoots is an informative taxonomic character. Section *Micranthae* is very widespread in Africa and also occurs on Madagascar.

### **Taxonomic treatment**

***Disa*** P.J.Bergius, Descript. Pl. Cap.: 348 (1767) - Type: *Disa uniflora* P.J.Bergius

=*Repandra* Lindl., Orchid. Scelet.: 12 (1826).

=*Penthea* Lindl., Intr. Nat. Syst. Bot., ed. 2: 446 (1836). Lectotype: *Penthea patens* (L.f.) Lindl. (≡*Disa tenuifolia* Sw.). Assigned by Linder in Contr. Bolus Herb. 9: 12 (1981).

=*Herschelia* Lindl., Gen. Sp. Orchid. Pl.: 362 (1838), non T.E.Bowdich, Exc. Madeira: 159 (1825) et ex Rchb.f., Handb. Nat. Pfl.-Syst.: 201 (1837) (as “*Herschellia*” orth. var.), nom. illeg. Type: *Herschelia coelestis* Lindl. (= *Disa*

*graminifolia* Ker Gawl. ex Spreng.). The only species assigned to this genus by Lindley.

=*Forficaria* Lindl., Gen. Sp. Orchid. Pl.: 362 (1838). Type: *Forficaria graminifolia* Lindl. (≡*Disa forficaria* Bolus). The only species assigned to this genus by Lindley.

=*Monadenia* Lindl., Gen. Sp. Orchid. Pl.: 356 (1838). Lectotype: *M. brevicornis* Lindl. (≡*Disa brevicornis* (Lindl.) Bolus) Assigned by Linder in Bothalia 13: 342 (1981), even though Kurzweil & Linder in Gen. Orchid. 2(1): 33 (2001) erroneously indicate that no type has been assigned.

=*Schizodium* Lindl., Gen. Sp. Orchid. Pl.: 358 (1838). Lectotype: *Schizodium flexuosum* (L.) Lindl (≡*Disa flexuosa* (L.) Sw.). Assigned by H.P.Linder in J. S. African Bot. 47: 342 (1981).

=*Gamaria* Raf., Fl. Tellur. 4: 49 (1838). Type: *Gamaria cornuta* (L.) Raf. (≡*Disa cornuta* (L.) Sw.). The only species assigned to this genus by Rafinesque.

=*Orthopenthea* Rolfe in W. T. Thiselton-Dyer, Fl. Cap. 5(3): 179 (1912) Lectotype: *Orthopenthea bivalvata* (L.f.) Rolfe (≡*Disa bivalvata* (L.f.) T.Durand & Schinz). Assigned by Linder in Contr. Bolus Herb. 9: 12 (1981)

=*Amphigena* (Bolus) Rolfe in W. T. Thiselton-Dyer, Fl. Cap. 5(3): 197 (1913). Type: *Amphigena tenuis* (Lindl.) Rolfe (≡*Disa tenuis* Lindl.). Established as a section by Bolus in Trans. S. Afr. Phil. Soc. 5: 139 (1888) with one species (*D. tenuis* Lindl.). Raised to genus level by Rolfe and including two species (*A. tenuis* (Lindl.) Rolfe and *A. leptostachya* (Sond.) Rolfe). The type therefore ought to be *A. tenuis* (Lindl.) Rolfe, as indicated by Linder in Contr. Bolus Herb. 9: 12 (1981), but Kurzweil & Linder in Gen. Orchid. 2(1): 33 (2001) erroneously indicate *A. leptostachya* (Sond.) Rolfe as the type.

=x *Herschelioidisa* H.P.Linder, S. African Orchid J. 16: 102 (1985). Type: x *Herschelioidisa vogelpoelii* H.P.Linder. The only species in this genus of hybrid origin. This name has not yet been transferred to *Disa* and the new combination is made here.

*xDisa vogelpoelii* (H.P.Linder) Bytebier, **comb. nov.**

≡ x*Herschelioidisa vogelpoelii* H.P.Linder, S. African Orchid J. 16: 102 (1985). Type: South Africa, Western Cape, *Vogelpoel s.n.* (BOL, holo; K, (preserved flower), iso)

=*Herschelianthe* Rauschert, Feddes Repert. 94: 434 (1983). Type: *Herschelia coelestis* Lindl. (= *Disa graminifolia* Ker Gawl. ex Spreng.). The only species assigned to the genus *Herschelia* by Lindley.

Kurweil & Linder in Gen. Orchid. 2(1): 37-38 (2001) subdivided the genus into five subgenera as follows:

*Disa* subg. *Disa*, Contr. Bol. Herb. 9: 270 (1981). Type: *D. uniflora* P.J.Bergius. Sections included: *Disa* and *Phlebidia* Lindl.

*Disa* subg. *Falcipetalum* H.P.Linder, Contr. Bol. Herb. 9: 30 (1981). Type: *D. longifolia* Lindl. Sections included *Disella* Lindl., *Intermediae* H.P.Linder, *Repandra* Lindl. and *Aconitoideae* Kraenzl.

*Disa* subg. *Hircicornes* (Kraenzl.) H.P.Linder, Contr. Bol. Herb. 9: 105 (1981) as “*Hircicornu*”. Type: *D. hircicornis* Rchb.f. Sections included: *Hircicornes* Kraenzl., *Monadenia* (Lindl.) Bolus, *Ovalifoliae* H.P.Linder and *Stoloniferae* H.P.Linder

*Disa* subg. *Stenocarpa* (Lindl.) H.P.Linder, Contr. Bol. Herb. 9: 155 (1981). Type: *D. gladioliflora* Burch. ex Lindl. Lindley (1838) established § *Stenocarpa* Lindl. for one species, *D. gladioliflora* Burch. ex Lindl.. Linder (1981c) used this as the basionym for the subgenus, but erroneously assigned *Disa porrecta* Sw. as the type for the subgenus. Since this is an autonym, it should be based on the same type as for § *Stenocarpa* Lindl. i.e. *D. gladioliflora* Burch. ex Lindl.. Sections included: *Amphigena* Bolus, *Coryphaea* Lindl., *Stenocarpa* Lindl., *Herschelianthe* (Rauschert) Bolus, *Emarginatae* H.P.Linder and *Austroalpinae* H.P.Linder

*Disa* subg. *Micranthae* (Lindl.) H.P.Linder, Bothalia 16: 56 (1986). Lectotype: *D. chrysostachya* Sw. Section included: *Micranthae* Lindl.

Our analysis indicates that these subgenera do not reflect phylogenetic relationships. It seems difficult and premature to subdivide the genus into subgenera and we therefore chose not to assign this rank.

On the basis of our phylogeny we propose the following sectional classification. We have assigned lectotypes to sections not typified before, in line with circumscription of the original authors. Taxa preceded by an \* were not sampled in the molecular phylogeny (Bytebier & al., in press) and were assigned to sections based on morphology.

1-**Disa** sect. **Disa**, Contr. Bol. Herb. 9: 270 (1981). Type: *D. uniflora* P.J.Bergius

≡*Disa* sect. *Macrantha* Lindl., Gen. Sp. Orchid. Pl.: 347 (1838). Lectotype: *D. grandiflora* L. (≡*D. uniflora* P.J.Bergius) (here assigned).

≡*Disa* sect. *Macro-Disa* Schltr., Bot Jahrb. 31: 224 (1901). Lectotype: *D. uniflora* P.J. Bergius (here assigned).

Schechter assigned two species to this section: *D. uniflora* P.J.Bergius and *D. racemosa* L.f.. The latter included *D. venosa* Sw. as a variety. They were grouped together on the basis of the big size of the flowers. *D. uniflora* P.J.Bergius is here assigned as the lectotype.

=*Disa* sect. *Vexillata* Bolus, J. Linn. Soc.-Bot. 20: 479 (1884). Lectotype: *D. venosa* Sw. (here assigned).

When Bolus established this section, he assigned *D. venosa* Sw., *D. secunda* Sw. (= *D. racemosa* L.f.), *D. filicornis* (L.f.) Thunb., *D. patens* (L.f.) Thunb. (≡ *D. tenuifolia* Sw.) and *D. reflexa* (Lindl.) Rchb.f. (= *D. filicornis* (L.f.) Thunb.) to it. However, he gives an extensive Latin description for *D. venosa* Sw., even though this species was published before, and only mentions the names of the other species stating that together they form a natural section. The emphasis put on *D. venosa* Sw. seems like an implicit typification “avant la lettre” and we therefore here chose it as the lectotype.

=*Disa* sect. *Orthocarpa* Bolus, J. Linn. Soc.-Bot. 20: 480 (1884). Lectotype: *D. richardiana* Lehm. ex Bolus. (here assigned)

Similarly and in the same paper, Bolus also gives an extensive Latin description for *D. richardiana* Lem. ex Bolus, and then mentions that it forms the above section together with *D. melaleuca* (Thunb.) Sw. (= *D. bivalvata* (L.f.) T.Durand & Schinz), *D. minor* (Sond.) Rchb.f., *D. atricapilla* (Harv. ex Lindl.) Bolus, *D. rosea* Lindl. and *D. fasciata* Lindl. For this reason we assign *D. richardiana* Lehm. ex Bolus as the lectotype.

=*Disa* sect. *Corymbosae* Kraenzl., Orchid Gen. Sp. 1: 761 (1900). Lectotype: *D. richardiana* Lehm. ex Bolus. (here assigned).

Kraenzlin assigned 5 species to this section on the basis of their corymbose inflorescence namely, *D. rosea* Lindl., *D. richardiana* Lehm. ex Bolus, *D. bodkinii* Bolus, *D. bivalvata* (L.f.) T.Durand & Schinz and *D. atricapilla*

(Harv. ex Lindl.) Bolus. *D. richardiana* Lehm. ex Bolus is here assigned as lectotype.

*Disa* sect. *Eu-Disa* Bolus, Trans. S. Afr. Phil. Soc. 5: 137 (1888), nom. illeg.

According to article 21.3 of the Saint Louis Code this name is illegitimate. In Bolus' circumscription it contained the type for the genus, *D. uniflora* P.J.Bergius.

*Disa atricapilla* (Harv. ex Lindl.) Bolus, J. Linn. Soc., Bot. 19: 344 (1882).

*Disa aurata* (Bolus) L.T.Parker & Koop., Biochem. Syst. Ecol. 21: 807 (1993).

*Disa begleyi* L.Bolus, Ann. Bolus Herb. 1: 195 (1915).

*Disa bivalvata* (L.f.) T.Durand & Schinz, Consp. Fl. Afric. 5: 100 (1894).

*Disa cardinalis* H.P.Linder, J. S. African Bot. 46: 213 (1980).

*Disa caulescens* Lindl., Gen. Sp. Orchid. Pl.: 351 (1838).

*Disa filicornis* (L.f.) Thunb., Fl. Cap. 1: 87 (1807).

\**Disa minor* (Sond.) Rchb.f., Flora 48: 182 (1865).

\**Disa oligantha* Rchb.f., Flora 48: 182 (1865).

*Disa pillansii* L.Bolus, Ann. Bolus Herb. 2: 32 (1916).

*Disa racemosa* L.f., Suppl. Pl.: 406 (1782).

*Disa richardiana* Lehm. ex Bolus, J. Linn. Soc., Bot. 20: 480 (1884).

*Disa tenuifolia* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 214 (1800).

*Disa tripetaloides* (L.f.) N.E.Br., Gard. Chron. 1889(1): 360 (1889).

*Disa uniflora* P.J.Bergius, Descr. Pl. Cap.: 348 (1767).

*Disa vasselotii* Bolus ex Schltr., Bot. Jahrb. Syst. 31: 274 (1901).

*Disa venosa* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 213 (1800).

**2-*Disa* sect. *Atromaculiferae* Bytebier sect. nov.**, a sectione *Coryphaea* floribus roseis vel rubris maculis atris differt. Type: *Disa glandulosa* Burch. ex Lindl.

*Disa glandulosa* Burch. ex Lindl., Gen. Sp. Orchid. Pl.: 351 (1838).

*Disa marlothii* Bolus, Trans. S. African Philos. Soc. 16: 148 (1906).

*Disa vaginata* Harv. ex Lindl., London J. Bot. 1: 15 (1842).

**3-*Disa* sect. *Phlebidia* Lindl.**, Gen. Sp. Orchid. Pl., 347: 350 (1838). Type *D. longicornu* L.f. The only species assigned to this section by Lindley.

*Disa longicornu* L.f., Suppl. Pl.: 406 (1782).

*Disa maculata* L.f., Suppl. Pl.: 407 (1782).

*Disa schizodioides* Sond., Linnaea 19: 92 (1846).

*Disa virginalis* H.P.Linder, Novon 8: 405 (1998).

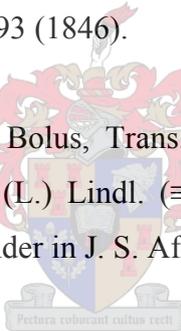
**4-Disa sect. *Pardoglossa*** Lindl., Gen. Sp. Orchid. Pl.: 347, 350 (1838). Type: *D. rosea* Lindl. The only species assigned to this section by Lindley.

*Disa rosea* Lindl., Gen. Sp. Orchid. Pl.: 350 (1838).

**5-Disa sect. *Ovalifoliae*** H.P.Linder, Contr. Bol. Herb. 9: 147 (1981). Type: *D. ovalifolia* Sond.

*Disa ovalifolia* Sond., Linnaea 19: 93 (1846).

**6-Disa sect. *Schizodium*** (Lindl.) Bolus, Trans. S. Afr. Phil. Soc. 5: 138 (1888).  
Lectotype: *Schizodium flexuosum* (L.) Lindl. ( $\equiv$  *Disa flexuosa* (L.) Sw.; basionym: *Orchis flexuosa* L.; assigned by Linder in J. S. African Bot. 47: 342 (1981))



*Disa bifida* (Thunb.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 212 (1800).

Basionym: *Satyrium bifidum* Thunb., Prodr. Fl. Cap.: 5 (1794).

$\equiv$  *Schizodium bifidum* (Thunb.) Rech.f., Flora 66: 460 (1883).

$\equiv$  *Schizodium rigidum* Lindl., Gen. Sp. Orchid. Pl.: 360 (1838).

*Disa flexuosa* (L.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 212 (1800).

Basionym: *Orchis flexuosa* L., Pl. Rar. Afr.: 26 (1760).

$\equiv$  *Satyrium flexuosum* (L.) Thunb., Prodr. Pl. Cap.: 5 (1794).

$\equiv$  *Schizodium flexuosum* (L.) Lindl., Gen. Sp. Orchid. Pl.: 359 (1838).

*Disa inflexa* (Lindl.) Bolus, Trans. S. African Philos. Soc. 5(1): 162 (1888).

Basionym: *Schizodium inflexum* Lindl., Gen. Sp. Orchid. Pl.: 360 (1838).

\**Disa longipetala* (Lindl.) Bolus, J. Linn. Soc., Bot. 25: 201 (1889).

Basionym: *Schizodium longipetalum* Lindl., Gen. Sp. Orchid. Pl.: 359 (1838).

$\equiv$  *Schizodium antenniferum* Schltr., Bot. Jahrb. Syst. 24: 426 (1897).

*Disa obliqua* (Lindl.) Bolus, Trans. S. African Philos. Soc. 5(1): 162 (1888).

Basionym: *Schizodium obliquum* Lindl., Gen. Sp. Orchid. Pl.: 359 (1838).

*Disa obliqua* subsp. *clavigera* (Lindl.) Bytebier, **comb.nov.**

Basionym: *Schizodium clavigerum* Lindl., Gen. Sp. Orchid. Pl.: 360 (1838).

Type: South Africa, Western Cape, *Drége 1231d* (K, holo; P, S, iso).

≡ *Disa clavigera* (Lindl.) Bolus, Trans. S. African Philos. Soc. 5(1): 140 (1888).

≡ *Schizodium obliquum* subsp. *clavigerum* (Lindl.) H.P.Linder, J. S. African Bot. 47: 356 (1981).

= *Schizodium obtusatum* Lindl., Gen. Sp. Orchid. Pl.: 359 (1838).

= *Schizodium gueinzii* Rchb.f., Linnaea 20: 694 (1847).

= *Disa gueinzii* (Rchb.f.) Bolus, J. Linn. Soc., Bot. 25: 201 (1889).

= *Schizodium modestum* L.Bolus, Fl. Pl. South Africa 19: t. 752 (1939).

*Disa satyrioides* (L.) Bytebier, **comb. nov.**

Basionym: *Orchis satyrioides* L., Pl. Afr. Rar. 27: No 92 (1760).

≡ *Orchis biflora* L., Sp. Pl. ed. 2: 1330 (1763), nom. illeg. *Schizodium biflorum* (L.) T.Durand & Schinz, Consp. Fl. Afr. 5: 113 (1895). Type: South Africa, Caput Bona Spei, *Oldenland s.n.* (G, Herb. Burman, holo).

≡ *Orchiodes biflorum* (L.) Kuntze, Revis. Gen. Pl. 2: 675 (1891).

≡ *Schizodium biflorum* (L.) T.Durand & Schinz, Consp. Fl. Afric. 5: 113 (1894).

≡ *Disa biflora* (L.) Druce, Rep. Bot. Exch. Club Brit. Isles 3: 417 (1913 publ. 1914).

≡ *Schizodium satyrioides* (L.) Garay, Harvard Pap. Bot. 2: 49 (1997).

= *Orchis flexicaulis* L.f., Suppl. Pl.: 398 (1782).

= *Satyrium tortum* Thunb., Prodr. Pl. Cap.: 5 (1794).

= *Disa torta* (Thunb.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

= *Schizodium arcuatum* Lindl., Gen. Sp. Orchid. Pl.: 359 (1838).

= *Schizodium tortum* (Thunb.) Steud., Nomencl. Bot., ed. 2, 2: 530 (1841).

**7-Disa sect. *Vaginaris*** Lindl., Gen. Sp. Orchid. Pl.: 347, 350 (1838). Type: *D. fasciata* Lindl. The only species assigned to this section by Lindley

*Disa fasciata* Lindl., Gen. Sp. Orchid. Pl.: 350 (1838).

8-*Disa* sect. *Coryphaea* Lindl., Gen. Sp. Orchid. Pl.: 347, 350 (1838). Lectotype:  
*D. sagittalis* (L.f.) Sw.; assigned by Linder in Contr. Bolus Herb. 9: 165 (1981)  
=*Disa* sect. *Austroalpinae* H.P.Linder, Contr. Bol. Herb. 9: 263 (1981). Type:  
*D. basutorum* Schltr.

\**Disa basutorum* Schltr., Bot. Jahrb. Syst. 20(50): 17 (1895).

*Disa rungweensis* Schltr., Bot. Jahrb. Syst. 53: 543 (1915).

*Disa sagittalis* (L.f.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 212 (1800).

*Disa triloba* Lindl., Gen. Sp. Orchid. Pl.: 351 (1838).

*Disa zimbabweensis* H.P.Linder, Contr. Bolus Herb. 9: 267 (1981).

9-*Disa* sect. *Disella* Lindl., Gen. Sp. Orchid. Pl., 347: 354 (1838). Lectotype:  
*D. obtusa* Lindl. subsp. *obtusa*; assigned by Linder in Contr. Bolus Herb. 9: 31 (1981)  
=*Disa* sect. *Aristaria* Rchb.f., Linnaea 20: 689 (1847). Type: *D. telipogonis*  
Rchb.f.

*Disa bodkinii* Bolus, J. Linn. Soc., Bot. 22: 74 (1885).

\**Disa brevipetala* H.P.Linder, Contr. Bolus Herb. 9: 42 (1981).

\**Disa cedarbergensis* H.P.Linder, S. African J. Bot. 54: 497 (1988).

*Disa elegans* Sond. ex Rchb.f., Flora 48: 182 (1865).

\**Disa introrsa* Kurzweil, Liltved & H.P.Linder, Nordic J. Bot. 17: 353 (1997).

\**Disa lineata* Bolus, J. Linn. Soc., Bot. 22: 74 (1885).

\**Disa longifolia* Lindl., Gen. Sp. Orchid. Pl.: 349 (1838).

\**Disa micropetala* Schltr., Bot. Jahrb. Syst. 20(50): 7 (1895).

*Disa neglecta* Sond., Linnaea 19: 100 (1846).

*Disa obtusa* Lindl., Gen. Sp. Orchid. Pl.: 355 (1838).

*Disa obtusa* subsp. *hottentotica* H.P.Linder, Contr. Bolus Herb. 9: 40 (1981).

*Disa obtusa* subsp. *picta* (Sond.) H.P.Linder, Contr. Bolus Herb. 9: 41 (1981).

*Disa ocellata* Bolus, J. Linn. Soc., Bot. 20: 477 (1884).

*Disa remota* H.P.Linder, S. African J. Bot. 72: 627 (2006).

*Disa subtenuicornis* H.P.Linder, Contr. Bolus Herb. 9: 53 (1981).

*Disa telipogonis* Rchb.f., Linnaea 20: 689 (1847).

\**Disa tenuicornis* Bolus, J. Linn. Soc., Bot. 22: 68 (1885).

*Disa uncinata* Bolus, J. Linn. Soc., Bot. 20: 478 (1884).

10-*Disa* sect. **Monadenia** (Lindl.) Bolus, Trans. S. Afr. Phil. Soc. 5: 137 (1888).  
Lectotype: *D. brevicornis* (Lindl.) Bolus; assigned by Linder in Bothalia 13: 342  
(1981)

*Disa atrorubens* Schltr., Bot. Jahrb. Syst. 24: 427 (1897).

*Disa bolusiana* Schltr., Bot. Jahrb. Syst. 24: 426 (1897).

*Disa borbonica* Balf.f. & S.Moore, J. Bot. 14: 293 (1876).

*Disa bracteata* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

*Disa brevicornis* (Lindl.) Bolus, J. Linn. Soc., Bot. 25: 196 (1889).

\**Disa cernua* (Thunb.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

*Disa comosa* (Rchb.f.) Schltr., Bot. Jahrb. Syst. 31: 206 (1901).

*Disa conferta* Bolus, Icon. Orchid. Austro-Afric. 1(1): t. 28 (1893).

*Disa cylindrica* (Thunb.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 213 (1800).

*Disa densiflora* (Lindl.) Bolus, J. Linn. Soc., Bot. 25: 197 (1889).

\**Disa ecalcarata* (G.J.Lewis) H.P.Linder in H.P.Linder & H.Kurzweil, Orchids S. Africa: 224 (1999).

*Disa macrostachya* (Lindl.) Bolus, J. Linn. Soc., Bot. 25: 197 (1889).

\**Disa nubigena* H.P.Linder, Fl. Pl. South Africa 56: t. 2148 (1999).

*Disa ophrydea* (Lindl.) Bolus, Trans. S. African Philos. Soc. 5(1): 142 (1888).

\**Disa physodes* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

*Disa pygmaea* Bolus, J. Linn. Soc., Bot. 22: 72 (1885).

*Disa reticulata* Bolus, J. Linn. Soc., Bot. 22: 73 (1885).

*Disa rufescens* (Thunb.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 210 (1800).

*Disa sabulosa* (Kraenzl.) Bolus, Icon. Orchid. Austro-Afric. 1(1): t. 27 (1893).

*Disa* sp. nov. (Bytebier 2595; NBG, BR).

11-*Disa* sect. **Reticulibractea** Bytebier, sect. nov., in genere singularis propter folia et bracteas papyraceas venis reticulatis. Type: *D. draconis* (L.f.) Sw.

*Disa draconis* (L.f.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 210 (1800).

*Disa harveyana* Lindl., London J. Bot. 1: 15 (1842).

In various publications the specific epithet has been spelled "harveiana". The species is named in honour of the botanist W. H. Harvey and thus, according to Article 60,

Recommendation 60C.1.(c) of the Saint Louis Code, the correct spelling should be "harveyana".

*Disa harveyana* subsp. *longicalcarata* S.D.Johnson & H.P.Linder, Bot. J. Linn. Soc. 118: 305 (1995).

*Disa karooica* S.D.Johnson & H.P.Linder, Bot. J. Linn. Soc. 118: 304 (1995).

12-*Disa* sect. *Repandra* Lindl., Gen. Sp. Orchid. Pl.: 347, 349 (1838). Lectotype: *D. cornuta* (L.) Sw.; assigned by Linder in Contr. Bolus Herb. 9: 70 (1981)

*Disa cornuta* (L.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 210 (1800).

*Disa hallackii* Rolfe in W.T. Thiselton-Dyer (ed.), Fl. Cap. 5(3): 227 (1913).

*Disa tysonii* Bolus, J. Linn. Soc., Bot. 25: 172 (1890).

13-*Disa* sect. *Trichochila* Lindl., Gen. Sp. Orchid. Pl.: 347, 353 (1838). Lectotype: *D. barbata* (L.f.) Sw.; assigned by Linder in Bothalia 13: 368 (1981).

=*Disa* sect. *Herschelia* (Lindl.) Bolus, J. Linn. Soc., Bot. 20: 481 (1884), nom. superfl.. Type: *Herschelia coelestis* Lindl. (= *D. graminifolia* Ker Gawl. ex Spreng.). The only species assigned to the genus by Lindley.

=*Disa* sect. *Amphigena* Bolus, Trans. S. Afr. Phil. Soc. 5: 139 (1888). Type: *Disa tenuis* Lindl.. The only species assigned to this section by Bolus.

=*Disa* sect. *Spathulatae* Kraenzl., Orchid. Gen. Spec 1: 793 (1900). Lectotype: *D. spathulata* (L.f.) Sw.; assigned by Linder in Bothalia 13: 368 (1981)

=*Disa* sect. *Forficaria* (Lindl.) Schltr. in Bot Jahrb. 31: 297 (1901). Type: *D. forficaria* Bolus. This is a nom. nov. for *Forficaria graminifolia* Lindl., the only species assigned to this genus by Lindley, which thus becomes the type.

=*Disa* sect. *Microperistera* Bolus in Trans. S. Afr. Phil. Soc. 16: 149 (1907). Type species: *D. schlechteriana* Bolus (as "*schlechterana*"). The only species assigned by Bolus to this section.

=*Disa* sect. *Herschelianthe* (Rauschert) H.P.Linder in Linder & Kurzweil, Orchids S. Afr.: 255 (1999), ), nom. superfl.. Type: *Disa graminifolia* Ker Gawl. ex Spreng.

In 1838, Lindley established *Disa* sect. *Trichochila* for the species *D. spathulata*, *D. tripartita* ( $\equiv$  *D. spathulata* subsp. *tripartita*), *D. multifida*, *D. barbata* and *D. lacera* Sw. ( $\equiv$  *D. hians*), as well as the genus *Herschelia* for *H. coelestis* Lindl. ( $\equiv$  *D. graminifolia*). In 1884, Bolus proposed to refer *Herschelia* into *Disa* and grouped *D. graminifolia* with Lindley's section *Trichochila*. However, he did not name this section *Trichochila* but transferred the generic name *Herschelia* to sectional rank. Schlechter (1901) followed Bolus and cites section *Trichochila* in the synonymy of section *Herschelia*. In 1983, Rauschert pointed out that Lindley's generic name *Herschelia*, was a later homonym of *Herschelia* T.E.Bowdich, a genus in Solanaceae, and changed the name to *Herschelianthe* Rauschert. Linder (1981f) at first recognised the genus *Herschelia(nthe)*, but later Linder & Kurzweil (1999) transferred it to the genus *Disa* as section *Herschelianthe* (Rauschert) H.P.Linder. Under the current code the sectional names *Herschelia* and *Herschelianthe* are superfluous, since the name *Trichochila* has priority.

*Disa barbata* (L.f.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 212 (1800).

*Disa baurii* Bolus, J. Linn. Soc., Bot. 25: 174 (1889).

\**Disa chimanimaniensis* (H.P.Linder) H.P.Linder in Linder & Kurzweil, Orchids S. Africa: 470 (1999).

\**Disa cochlearis* S.D.Johnson & Liltved, S. African J. Bot. 63: 291 (1997).

\**Disa esterhuyseniae* Schelpe ex H.P.Linder, Contr. Bolus Herb. 9: 161 (1981).

\**Disa forcipata* Schltr., Bot. Jahrb. Syst. 24: 428 (1897).

\**Disa forficaria* Bolus, Icon. Orchid. Austro-Afric. 1: t. 87 (1896).

*Disa graminifolia* Ker Gawl. ex Spreng., Syst. Veg. 3: 699 (1826).

*Disa hians* (L.f.) Spreng., Syst. Veg. 3: 698 (1826).

\**Disa longilabris* Schltr., Bot. Jahrb. Syst. 38: 150 (1906).

*Disa lugens* Bolus, J. Linn. Soc., Bot. 20: 483 (1884).

\**Disa lugens* var. *nigrescens* (H.P.Linder) H.P.Linder in Linder & Kurzweil, Orchids S. Africa: 263 (1999).

*Disa multifida* Lindl., Gen. Sp. Orchid. Pl.: 353 (1838).

\**Disa newdigateae* L.Bolus, Fl. Pl. South Africa 11: t. 415 (1931).

\**Disa praecox* (H.P.Linder) H.P.Linder in Linder & Kurzweil, Orchids S. Africa: 470 (1999).

\**Disa procera* H.P.Linder ex Bytebier, **nom. nov.** pro *D. excelsa* sensu Lindl., Gen. Sp. Orchid. Pl.: 356 (1838), non (Thunb.) Sw., *Herschelia excelsa* sensu Rolfe in W.T. Thiselton-Dyer (ed.), Fl. Cap. 5(3): 200 (1913). Type: South Africa, Western Cape, *Thunberg 21443* (UPS, lecto).

*Disa purpurascens* Bolus, J. Linn. Soc., Bot. 20: 482 (1884).

*Disa salteri* G.J.Lewis, J. S. African Bot. 7: 78 (1941).

*Disa schlechteriana* Bolus, Trans. S. African Philos. Soc. 16: 149 (1906).

*Disa spathulata* (L.f.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 213 (1800).

*Disa spathulata* subsp. *tripartita* (Lindl.) H.P.Linder in Linder & Kurzweil, Orchids S. Africa: 259 (1999).

*Disa tenuis* Lindl., Gen. Sp. Orchid. Pl.: 354 (1838).

*Disa venusta* Bolus, J. Linn. Soc., Bot. 20: 482 (1884).

*Disa walteri* Schltr., Bot. Jahrb. Syst. 53: 544 (1915).

14-***Disa* sect. *Stenocarpa*** Lindl., Gen. Sp. Orchid. Pl.: 347, 352 (1838). Type:

*D. gladioliflora* Burch. ex Lindl. The only species assigned to this section by Lindley

=*Disa* sect. *Oregura* Lindl., Gen. Sp. Orchid. Pl.: 347, 352 (1838). Type:

*D. porrecta* Sw. The only species assigned to this section by Lindley.

*Disa amoena* H.P.Linder, Contr. Bolus Herb. 9: 236 (1981).

\**Disa arida* Vlok, S. African J. Bot. 51: 335 (1985).

*Disa aristata* H.P.Linder, Fl. Pl. South Africa 46: t. 1825 (1981).

*Disa cephalotes* Rchb.f., Otia Bot. Hamburg.: 106 (1881).

*Disa cephalotes* subsp. *frigida* (Schltr.) H.P.Linder, Contr. Bolus Herb. 9: 218 (1981).

\**Disa dracomontana* Schelpe ex H.P.Linder, Contr. Bolus Herb. 9: 226 (1981).

*Disa ferruginea* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 210 (1800).

*Disa gladioliflora* Burch. ex Lindl., Gen. Sp. Orchid. Pl.: 352 (1838).

*Disa gladioliflora* subsp. *capricornis* (Rchb.f.) H.P.Linder, Contr. Bolus Herb. 9: 208 (1981).

*Disa montana* Sond., Linnaea 19: 90 (1846).

*Disa nivea* H.P.Linder, Contr. Bolus Herb. 9: 223 (1981).

*Disa oreophila* Bolus, J. Linn. Soc., Bot. 25: 170 (1889).

*Disa oreophila* subsp. *erecta* H.P.Linder, Contr. Bolus Herb. 9: 223 (1981).

\**Disa porrecta* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

*Disa pulchra* Sond., Linnaea 19: 94 (1846).

*Disa saxicola* Schltr., Bot. Jahrb. Syst. 20(50): 41 (1895).

*Disa stricta* Sond., Linnaea 19: 91 (1846).

*Disa vigilans* McMurtry & T.J.Edwards, S. African J. Bot. 72, 551 (2006).

15-***Disa* sect. *Emarginatae*** H.P.Linder, Contr. Bol. Herb. 9: 240 (1981). Type: *D. stachyoides* Rchb.f.

*Disa alticola* H.P.Linder, Contr. Bolus Herb. 9: 252 (1981).

*Disa buchenaviana* Kraenzl., Abh. Naturwiss. Vereine Bremen 7: 261 (1882).

*Disa intermedia* H.P.Linder, Contr. Bolus Herb. 9: 259 (1981).

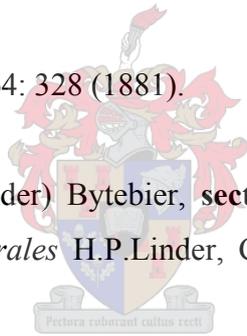
*Disa nervosa* Lindl., Gen. Sp. Orchid. Pl.: 352 (1838).

\**Disa patula* Sond., Linnaea 19: 94 (1846).

*Disa patula* var. *transvaalensis* Summerh., Bull. Misc. Inform. Kew 1938: 148 (1938).

*Disa stachyoides* Rchb.f., Flora 64: 328 (1881).

16-***Disa* sect. *Spirales*** (H.P.Linder) Bytebier, **sect. et stat. nov.** Type: *D. tenella* Lindl. Basionym: *Disa* ser. *Spirales* H.P.Linder, Contr. Bol. Herb. 9: 192 (1981). Type: *D. tenella* Lindl.



*Disa brachyceras* Lindl., Gen. Sp. Orchid. Pl.: 355 (1838).

*Disa tenella* (L.f.) Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 212 (1800).

*Disa tenella* subsp. *pusilla* H.P.Linder, Contr. Bolus Herb. 9: 197 (1981).

17-***Disa* sect. *Aconitoideae*** Kraenzl., Orchid Gen. Sp. 1: 736, 776 (1900). Type species: *D. aconitoides* Sond.; assigned by Summerhayes in Fl. Trop. E. Africa, Orchidaceae (Part 1): 154 (1968)

*Disa aconitoides* Sond., Linnaea 19: 91 (1846).

\**Disa aconitoides* subsp. *concinna* (N.E.Br.) H.P.Linder, Contr. Bolus Herb. 9: 91 (1981).

*Disa aconitoides* subsp. *goetzeana* (Kraenzl.) H.P.Linder, Contr. Bolus Herb. 9: 92 (1981).

\**Disa aperta* N.E.Br. in W.T. Thiselton-Dyer (ed.), Fl. Trop. Afr. 7: 286 (1898).

\**Disa dichroa* Summerh., Kew Bull. 17: 549 (1964).

\**Disa equestris* Rchb.f., Flora 48: 181 (1865).

\**Disa nigerica* Rolfe, Bull. Misc. Inform. Kew 1914: 214 (1914).

\**Disa nyikensis* H.P.Linder, Contr. Bolus Herb. 9: 96 (1981).

\**Disa pulchella* Hochst. ex A.Rich., Tent. Fl. Abyss. 2: 301 (1850).

*Disa similis* Summerh., Kew Bull. 17: 552 (1964).

18-***Disa* sect. *Micranthae*** Lindl., Gen. Sp. Orchid. Pl.: 347, 348 (1838). Lectotype: *D. chrysostachya* Sw.; assigned by Summerhayes in Kew Bull. 17: 537 (1964)

=*Disa* sect. *Polygonoideae* Kraenzl., Orchid Gen. Sp. 1: 736, 743 (1900).

Lectotype: *D. chrysostachya* Sw. (here assigned)

=*Disa* sect. *Scutelliferae* Kraenzl., Orchid Gen. Sp. 1: 736, 737 (1900).

Lectotype: *D. scutellifera* A.Rich. (here assigned)

=*Disa* sect. *Hircicornes* Kraenzl., Orchid Gen. Sp. 1: 757 (1900). Lectotype: *D. hircicornis* Rchb.f.; assigned by Summerhayes in Fl. Trop. E. Africa, Orchidaceae (Part 1): 154 (1968)

=*Disa* sect. *Calostachys* Schltr., Bot Jahrb. 31: 201, 216 (1901). Lectotype: *D. chrysostachya* Sw. (here assigned)

=*Disa* sect. *Aegoceratium* Schltr. in Bot Jahrb. 31: 235 (1901). Lectotype: *D. hircicornis* Rchb.f. (here assigned)

=*Disa* sect. *Intermediae* H.P. Linder, Contr. Bol. Herb. 9: 65 (1981). Type: *D. galpinii* Bolus

=*Disa* sect. *Stoloniferae* H.P.Linder, Contr. Bol. Herb. 9: 150 (1981). Type: *D. stairsii* Kraenzl.

Lindley (1830-1840) included four species in § *Micranthae* namely *D. incarnata* Lindl., *D. gracilis* Lindl., *D. chrysostachya* Sw. and *D. polygonoides* Lindl. All of these are also included in Kraenzlin's § *Polygonoideae* and in Schlechter's § *Calostachys*. Kraenzlin (1897-1904) assigned the big-flowered species *D. carsoni*, *D. erubescens*, *D. zombica* and *D. scutellifera* to § *Scutelliferae*, which Schlechter (1901) incorporated in § *Calostachys*. Summerhayes (1964) agreed with Schlechter in uniting Kraenzlin's § *Polygonoideae* and § *Scutelliferae* and corrected the name to the oldest sectional name, § *Micranthae*. The concept of § *Micranthae* is here extended with the

inclusion of § *Hircicornes* Kraenzl. sensu Linder, Contr. Bolus Herb. 9: 105 (1981) as well as § *Intermediae* H.P.Linder and § *Stoloniferae* H.P.Linder.

\**Disa aequiloba* Summerh., Bull. Misc. Inform. Kew 1927: 419 (1927).

\**Disa alinae* Szlach., Fragm. Florist. Geobot. 39: 543 (1994).

\**Disa andringitrana* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 98 (1924).

*Disa caffra* Bolus, J. Linn. Soc., Bot. 25: 171 (1889).

*Disa celata* Summerh., Kew Bull. 17: 535 (1964).

*Disa chrysostachya* Sw., Kongl. Vetensk. Acad. Nya Handl. 21: 211 (1800).

\**Disa clavicornis* H.P.Linder, J. S. African Bot. 50: 261 (1984).

*Disa cooperi* Rchb.f., Flora 64: 328 (1881).

*Disa crassicornis* Lindl., Gen. Sp. Orchid. Pl.: 348 (1838).

=*D. oliveriana* Rchb.f., Flora 69: 547 (1886), **syn. nov.**

\**Disa cryptantha* Summerh., Kew Bull. 17: 537 (1964).

\**Disa danielae* Geerinck, Bull. Jard. Bot. Natl. Belg. 52: 142 (1982).

\**Disa eminii* Kraenzl., Bot. Jahrb. Syst. 19: 248 (1894).

*Disa engleriana* Kraenzl., Bot. Jahrb. Syst. 33: 58 (1902).

*Disa erubescens* Rendle, J. Bot. 33: 297 (1895).

*Disa erubescens* subsp. *carsonii* (N.E.Br.) H.P.Linder, Bull. Jard. Bot. Natl. Belg. 51: 340 (1981).

\**Disa facula* P.J.Cribb, C.Herrm. & Sebsebe, Lindleyana 17: 180 (2002).

*Disa extinctoria* Rchb.f., Flora 64: 328 (1881).

*Disa fragrans* Schltr., Bot. Jahrb. Syst. 20(50): 40 (1895).

\**Disa fragrans* subsp. *deckenii* (Rchb.f.) H.P.Linder, Bull. Jard. Bot. Natl. Belg. 51: 290 (1981).

*Disa galpinii* Rolfe in W.T. Thiselton-Dyer (ed.), Fl. Cap. 5(3): 230 (1913).

*Disa hircicornis* Rchb.f., Otia Bot. Hamburg.: 105 (1881).

*Disa incarnata* Lindl., Gen. Sp. Orchid. Pl.: 348 (1838).

\**Disa katangensis* De Wild., Ann. Mus. Congo Belge, Bot., IV, 1: 25 (1902).

\**Disa lisowskii* Szlach., Fragm. Florist. Geobot. 39: 546 (1994).

*Disa maculomarronina* McMurtry, S. African Orchid J. 15: 91 (1984).

*Disa miniata* Summerh., Kew Bull. 17: 539 (1964).

*Disa ochrostachya* Rchb.f., Flora 48: 181 (1865).

*Disa ornithantha* Schltr., Bot. Jahrb. Syst. 53: 538 (1915).

- Disa perplexa* H.P.Linder, Contr. Bolus Herb. 9: 128 (1981).
- Disa polygonoides* Lindl., Gen. Sp. Orchid. Pl.: 349 (1838).
- \**Disa renziana* Szlach., Fragm. Florist. Geobot. 39: 545 (1994).
- Disa rhodantha* Schltr., Bot. Jahrb. Syst. 20(50): 40 (1895).
- Disa robusta* N.E.Br. in W.T. Thiselton-Dyer (ed.), Fl. Trop. Afr. 7: 282 (1898).
- Disa roeperocharoides* Kraenzl., Bot. Jahrb. Syst. 51: 379 (1914).
- Disa sanguinea* Sond., Linnaea 19: 97 (1846).
- Disa sankeyi* Rolfe in W.T. Thiselton-Dyer (ed.), Fl. Cap. 5(3): 225 (1913).
- Disa satyriopsis* Kraenzl., Bot. Jahrb. Syst. 28: 177 (1900).
- Disa scullyi* Bolus, J. Linn. Soc., Bot. 22: 70 (1885).
- \**Disa scutellifera* A.Rich., Ann. Sci. Nat., Bot., II, 14: 272 (1840).
- Disa stairsii* Kraenzl., Gard. Chron., III, 12: 728 (1892).
- Disa thodei* Schltr. ex Kraenzl., Orchid. Gen. Sp. 1: 796 (1900).
- Disa ukingensis* Schltr., Bot. Jahrb. Syst. 53: 539 (1915).
- \**Disa verdickii* De Wild., Ann. Mus. Congo Belge, Bot., IV, 1: 26 (1902).
- Disa versicolor* Rchb.f., Flora 48: 181 (1865).
- Disa walleri* Rchb.f., Otia Bot. Hamburg.: 105 (1881).
- Disa welwitschii* Rchb.f., Flora 48: 181 (1865).
- Disa welwitschii* subsp. *occultans* (Schltr.) H.P.Linder, Bull. Jard. Bot. Natl. Belg. 51: 306 (1981).
- Disa woodii* Schltr., Ann. Transvaal Mus. 10: 247 (1924).
- Disa zombica* N.E.Br. in W.T. Thiselton-Dyer (ed.), Fl. Trop. Afr. 7: 278 (1898).
- Disa zuluensis* Rolfe in W.T. Thiselton-Dyer (ed.), Fl. Cap. 5(3): 233 (1913).

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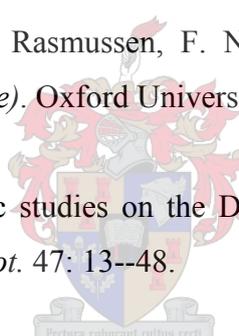
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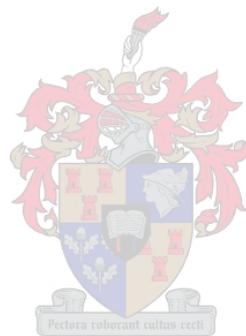
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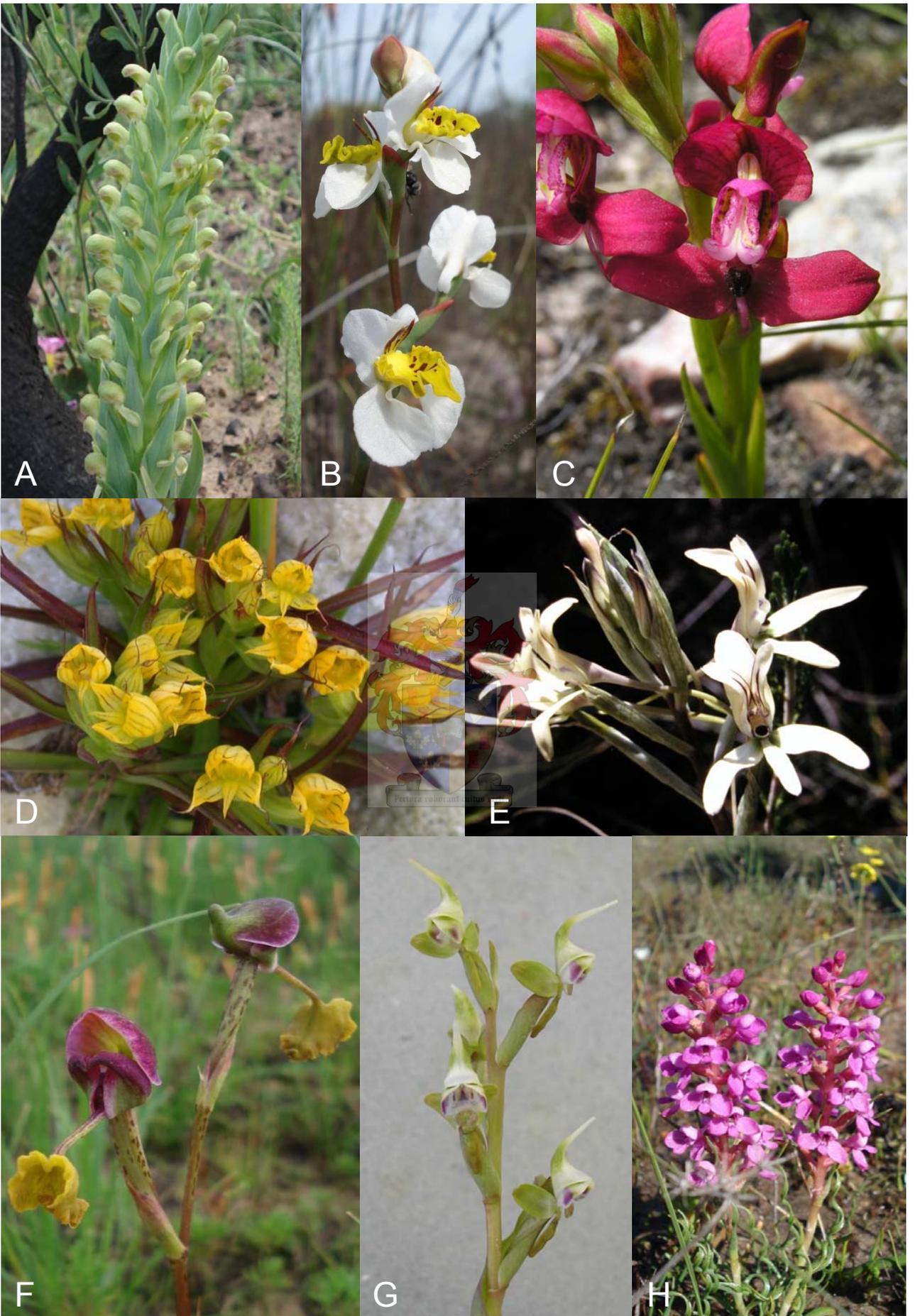


Figure 1: Morphological diversity in the Disinae. A § *Monadenia*, *D. physodes*; B § *Schizodium*, *D. flexuosa*; C § *Disa*, *D. filicornis*; D § *Disella*, *D. telipogonis*; E § *Reticulibractea*, *D. harveyana*; F § *Trichochila*, *D. spathulata*; G § *Ovalifoliae*, *D. ovalifolia*; H § *Spirales*, *D. tenella*.

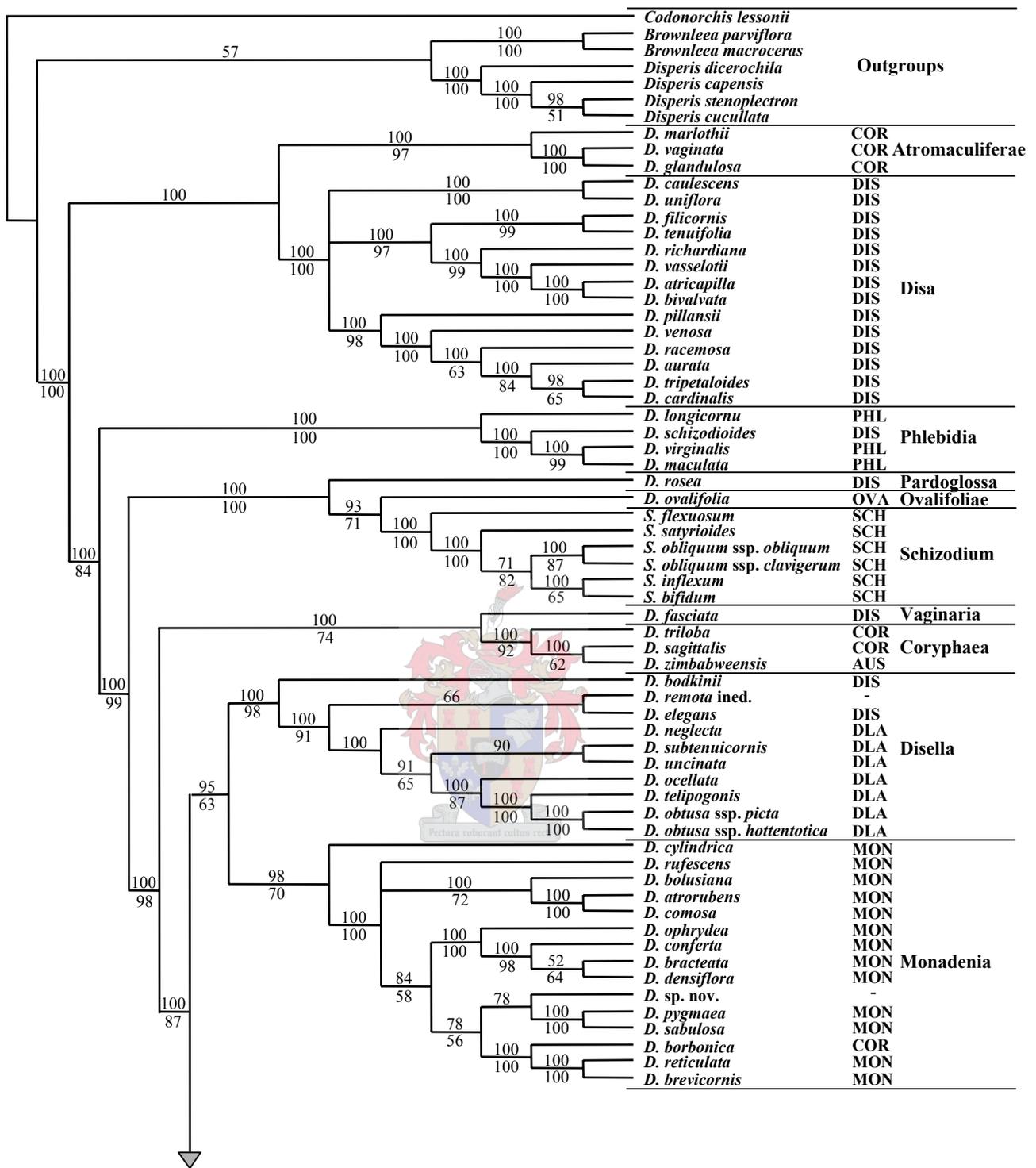


Figure 2: Phylogenetic hypotheses for the Disinae (from Bytebier et al. (in press)). Posterior probabilities from a Bayesian inference analysis are given above branches; below branches are bootstrap values from a parsimony analysis. The three letter abbreviation to the right of the species name indicates in which section it is currently placed (Linder & Kurzweil, 1999). AMP=Amphigena, AUS= Austroalpiniae, COR=Coryphaea, DIS=Disa, DLA=Disella, EMA=Emarginatae, HER=Herschelianthe, HIR=Hircicornes, INT=Internediae, MIC=Micranthae, MON= Monadenia, OVA=Ovalifoliae, PHL=Phlebidia, REP=Repandra, SCH=Schizodium, STE=Stenocarpa, STO= Stoloniferae. On the far right is the newly proposed delimitation with the names of the new sections.

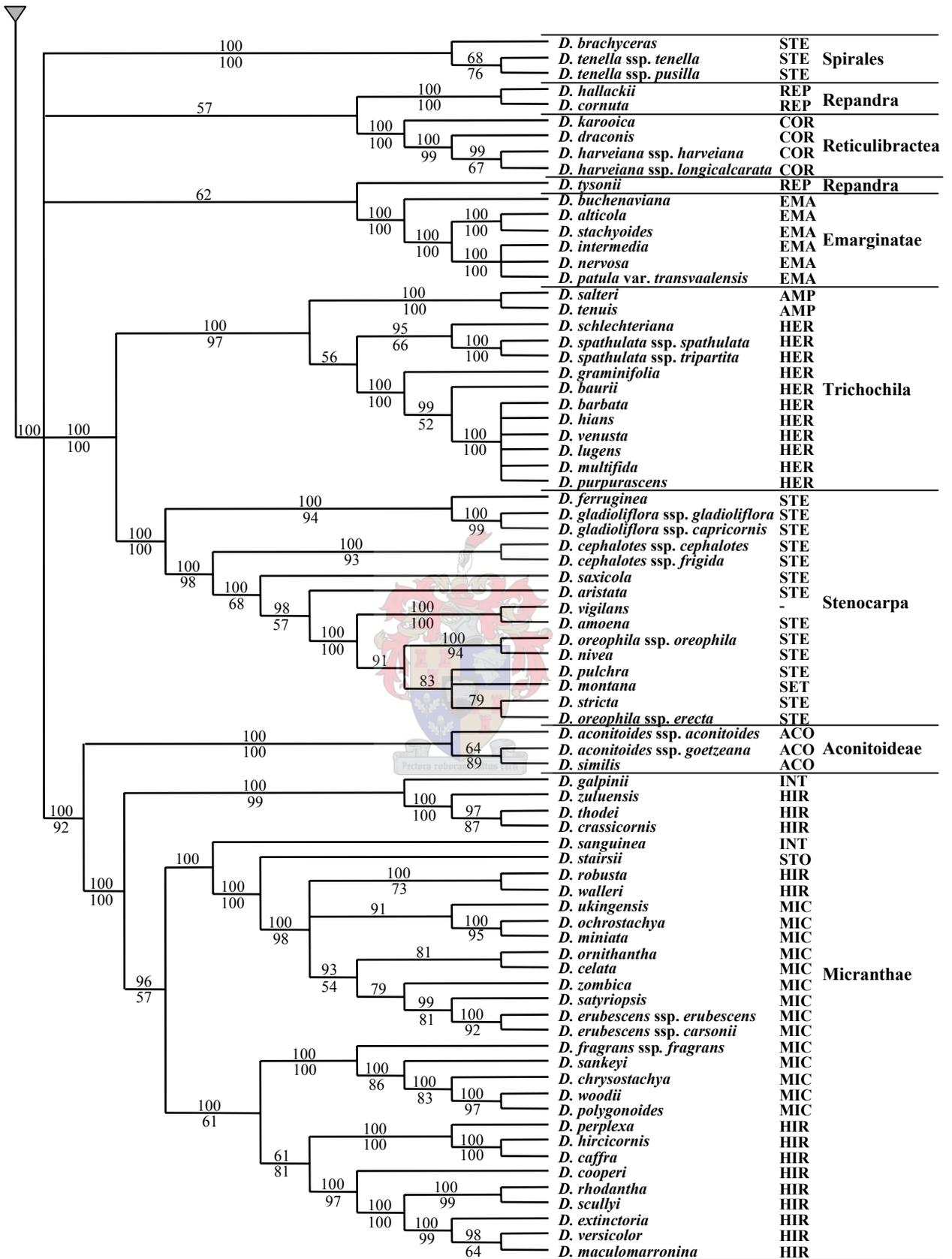


Figure 2 (continued)

# The Cape element in the Afrotropical flora: from Cape to Cairo?

Chloé Galley<sup>1,\*</sup>, Benny Bytebier<sup>2,†</sup>, Dirk U. Bellstedt<sup>2</sup>  
and H. Peter Linder<sup>1</sup>

<sup>1</sup>*Institute for Systematic Botany, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland*

<sup>2</sup>*Department of Biochemistry, Stellenbosch University, Private Bag XI, 7602 Matieland, South Africa*

The build-up of biodiversity is the result of immigration and *in situ* speciation. We investigate these two processes for four lineages (*Disa*, Irideae p.p., the *Pentaschistis* clade and Restionaceae) that are widespread in the Afrotropical flora. These four lineages may be representative of the numerous clades which are species rich in the Cape and also occur in the highlands of tropical Africa. It is as yet unclear in which direction the lineages spread. Three hypotheses have been proposed: (i) a tropical origin with a southward migration towards the Cape, (ii) a Cape origin with a northward migration into tropical Africa, and (iii) vicariance. None of these hypotheses has been thoroughly tested. We reconstruct the historical biogeography of the four lineages using likelihood optimization onto molecular phylogenies. We find that tropical taxa are nested within a predominantly Cape clade. There is unidirectional migration from the Cape into the Drakensberg and from there northwards into tropical Africa. The amount of *in situ* diversification differs between areas and clades. Dating estimates show that the migration into tropical East Africa has occurred in the last 17 Myr, consistent with the Mio-Pliocene formation of the mountains in this area.

**Keywords:** historical biogeography; ancestral character reconstruction; phytogeography; molecular dating; Africa

## 1. INTRODUCTION

Local floras and faunas accumulate diversity by the recruitment of new lineages as well as by *in situ* speciation. The sourcing of lineages has long occupied biogeographers: historical biogeographers list the diverse ‘elements’ for a biot (Wulff 1950); panbiogeographers assemble ‘tracks’ showing the shared elements between biota (Craw *et al.* 1999); and cladistic biogeographers compile sets of ‘components’ or three-area statements (Nelson & Ladiges 1996; Humphries & Parenti 1999). The increasing availability of dated phylogenies has made it possible to understand how and when biomes were assembled (Crisp 2006). No region is isolated from immigration, consequently the relative roles of immigration and *in situ* diversification are more difficult to untangle for regions which share many lineages. Well sampled phylogenies are needed to determine whether an endemic species in a biota speciated locally or recruited from a ‘source area’.

Here, we explore the phytogeographical patterns in the Afrotropical region (Weimarck 1936; Wild 1964; Linder 1990). The region is an archipelago of isolated areas ranging from the highlands of Ethiopia in the northeast, to the southern tip of Africa and to the Fouta Djallon in Guinea in the west (White 1978). It combines the Afrotropical and Cape phytochoria of White (1983).

These patches of temperate vegetation, often separated by thousands of kilometres, can be grouped into three centres of endemism: the Cape region; the greater Drakensberg range; and the Afrotropical Centre (Linder 1990). Although the floristic affinities between these centres have frequently been explored (Weimarck 1941; Hedberg 1965; Wild 1968; Killick 1978; White 1978; Linder 1990), the historical biogeography remains enigmatic.

Many of the very diverse Cape clades (Linder 2003) also occur in the Afrotropical region (Cowling 1983; Carbutt & Edwards 2002) although their species richness decreases to the west and the north. Their contribution to the floras of these regions is substantial: Hilliard & Burtt (1987) considered an estimated 22% of the genera recorded in the southern Drakensberg as ‘centred in the Cape region’, while Hedberg (1965) showed that 4% of Afroalpine flora elements form a ‘Cape element’. These constitute part of the austral element in the Afrotropical flora and complement the boreal element which has more north temperate affinities.

Three main hypotheses for the origin and migration of these taxa have been postulated to explain their current distribution: (i) an origin in tropical Africa and migration through the Afrotropical region southwards into the Cape (Levyns 1938, 1952, 1964), (ii) an origin in the Cape and migration northwards into tropical Africa (Linder 1994), and (iii) vicariance, with the floras in each region representing relics from a once widespread African flora that has receded with climatic changes (Adamson 1958; Wild 1968; King 1978). Although the disjunctions are well documented and the historical processes have been discussed (Levyns 1952; Adamson 1958; Levyns 1962,

\* Author for correspondence (galley@systbot.unizh.ch).

† These authors contributed equally to the work.

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1964; Wild 1968; King 1978; Van Zinderen Bakker 1978), few authors have tested these hypotheses. Linder (1994) rejected a north to south migration on the basis of a cladistic biogeographical analysis of Disinae (Orchidaceae) and Griswold (1991) found it difficult to reconcile a Pleistocene vicariance scenario with the current distribution of Afrotropical spiders. McGuire & Kron (2005) inferred a north to south migration for the Cape mega genus *Erica*, but did so on the basis of an under-sampled and poorly supported molecular phylogenetic tree.

Here, we reconstruct ancestral distributions using likelihood optimization on phylogenetic hypotheses, for four clades with a classical Afrotropical distribution pattern, with the majority of their species restricted to the Cape. We test the directionality of migrations through Africa and address the following questions: (i) Is migration between the Cape and the regions north of the Limpopo River direct, or do we infer movement via the Drakensberg? (ii) How does the temporal sequence and dating of the migration events relate to the geological history of Africa? and (iii) Did taxa that are found outside the Cape speciate *in situ* or are they derived from separate migration events?

## 2. MATERIAL AND METHODS

### (a) Phylogenetic trees

The phylogenetic hypotheses for the four study groups and sequence data for the dating were derived from the following sources: *Disa*, cpDNA and nrDNA (Bytebier *et al.* 2006); Irideae s.p., cpDNA (Goldblatt *et al.* 2002, fig. 3); the *Pentaschistis* clade, cpDNA (Galley & Linder submitted, fig. 3); the African Restionaceae, cpDNA (Hardy *et al.* submitted). In the cases of *Disa* and the Restionaceae, the topology of the tree with the highest likelihood score from the set of Bayesian trees was used.

### (b) Age estimations

Trees were made ultrametric and the ages of the disjunctions in the four study groups were estimated using a Bayesian relaxed clock (Renner 2005; Rutschmann 2006) as implemented in MULTIDIVTIME (Thorne & Kishino 2002). There are no fossils available for the study groups, so the crown node of each study group was constrained with a calibration point derived from a separate 'global analysis' which had four fossil calibration points. Details of the MULTIDIVTIME analysis and the global analysis can be found in the electronic supplementary material.

### (c) Areas and taxon scoring

Taxa were scored as present or absent for six regions, shown in figure 1: the Greater Cape Floristic Region (hereafter referred to as the 'Cape'; Jürgens 1991; Born *et al.* 2006); the Drakensberg range comprising the Drakensberg escarpment (Partridge & Maud 1987), upland areas south to Elliot and north to Tzaneen (Carbutt & Edwards 2004); Zimbabwe overlap region between the Limpopo and the Zambesi rivers; South Central African centre including Mount Mulanje, the Nyika plateau and the southern Tanzanian highlands; 'Eastern Africa' including the Central and the East African uplands, as well as the Ethiopian plateau; and 'Western Africa' comprising the Cameroon highlands and the uplands westward to the Fouta Djallon. Other areas were also scored where applicable (Réunion, Madagascar, the Mediterranean and Amsterdam Island/St. Paul Island). Widespread taxa were coded as present

in all relevant areas. An exception to this is *Pentaschistis natalensis* for which the three accessions (from Natal, Madagascar and South Central Africa) did not form a clade, consequently each accession was coded according to the area in which it was collected.

### (d) Reconstruction of biogeographical history

The distribution ranges of ancestral nodes were reconstructed using likelihood optimization as implemented in MESQUITE v. 1.1 (Maddison & Maddison 2006) using the rate-corrected branch lengths. Each node was optimized as present versus absent for each of the six areas. A threshold value of 2 log-likelihood (lnL) units was used to indicate statistical significance for the ancestral state optimization of each node (Mooers & Schluter 1999; Maddison & Maddison 2006). We compared the lnL scores of a two-rate (forward and backward rates independent) and a one-rate (forward and backward rates constrained to be equal) model for each character, for each taxon. The accuracy of parameter estimation depends on the amount of data available and the frequency of the minority character state, as well as model complexity (Mooers & Schluter 1999). All taxa had several characters for which the use of the two-rate model did not result in a significantly improved fit (sometimes a worse fit was obtained) and we therefore used the one-rate model for optimization. This handles trees with few transitions and an imbalance of character states better than the two-rate model (Schluter *et al.* 1997; Mooers & Schluter 1999).

To infer the ancestral distribution of a node, the optimization for each area was taken into account separately. The node was optimized as the area which was significantly 'present' at that node. In most cases, the node was optimized as 'absent' for all other areas and was thus optimized unambiguously. In a number of cases, a node was significantly optimized to more than one area. In nine cases, a node was optimized as absent to all areas except one for which support was not significant but above 0.75 proportional likelihood (see figure 2, also Figs. 1 and 2 of electronic supplementary material). In these cases we assigned this area to the node. Nodes that were not assigned any area were omitted from further calculations.

A migration event on a branch was counted when the daughter node optimized to a different area than that of the parent node. Three types of migration were recognized: range expansion, when a node or taxon is present in the same area as its parent node, but occurs in an additional area; vicariance, when a parent node is optimized to two areas but the two daughter nodes each to only one of these; and dispersal, when the parent and the daughter nodes optimize to different areas.

### (e) Calculations

We calculated the age of the dispersal events by estimating the age of the ancestral node of the branch which has the change in distribution. This assumes that the migration accompanied speciation. The same method was used to date range expansions (migration without accompanied speciation). Since no speciation accompanied range expansions, they cannot be dated precisely; the date could be any time between the node subtending the taxon and the present. This approach therefore estimates a maximum age.

Migration is a function of movement from an area plus persistence, the former of which is related to the number of species in the source area. This needs to be taken into account to test for unidirectionality of migration and we divided the

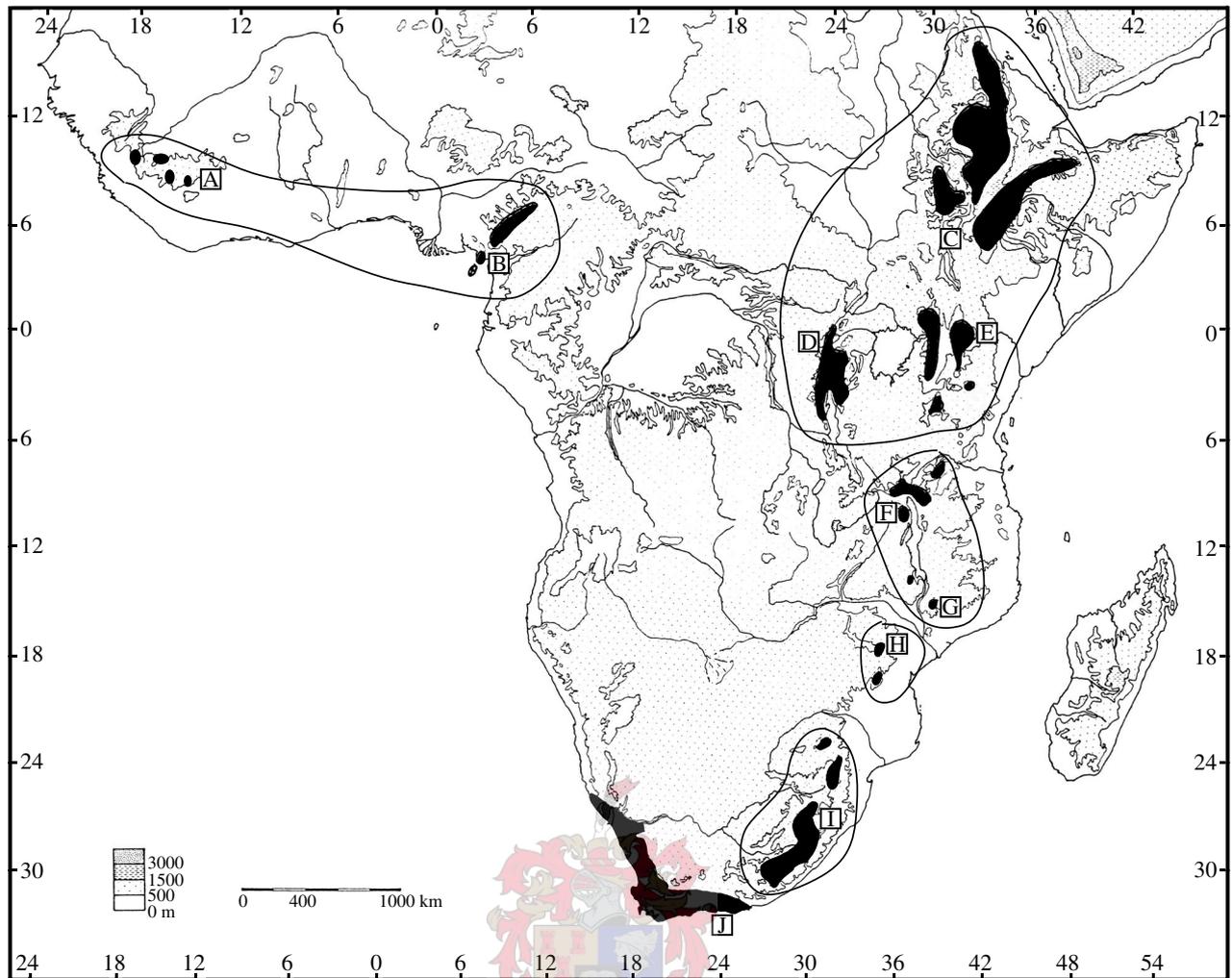


Figure 1. Map indicating areas used in analysis. ‘Western Africa’ including: A the Fouta Djallon in Guinea and B Mount Cameroon; ‘Eastern Africa’ including: C Ethiopian highlands, D Virunga and Rwenzori mountains and E North Tanzanian/West and Central Kenyan/East Ugandan mountains; ‘South Central African centre’ including: F Nyika Plateau and southern Tanzanian highlands and G Mount Mulanje; ‘Zimbabwe overlap region’ including: H Chimanimani Mountains and Nyanga Plateau; the ‘Drakensberg range’ I; and the Greater Cape Floristic Region J. Areas north of the Limpopo from White (1978). Map adapted from (Linder 1990).

number of migration events by the number of (sampled) species in the source area.

### 3. RESULTS

The ancestral node of each of the four clades (*Disa*, Irideae p.p., the *Pentaschistis* clade and Restionaceae) optimizes to the Cape and to no other area. Approximately 94% of the nodes in the four clades can be optimized unambiguously. Most of these optimize to single areas (see electronic supplementary material Fig. 1, 2 and 3) and in two clades (Irideae p.p. and *Disa*) there are four nodes which optimize unambiguously to multiple areas. A minimum of 31 dispersal events have been documented (see table 1). Where range expansion has occurred without speciation, the precise route of migration cannot be known from a species-level study. Only unambiguous migrations are considered further, but detailed descriptions of the optimizations and migrations inferred for each clade are given in the electronic supplementary material. In all clades, most dispersal events out of the Cape are to the Drakensberg range (five events in *Disa*, three events in Irideae p.p., five events in the *Pentaschistis* clade and five or six events in Restionaceae), although there are two

dispersals (in Irideae p.p.) directly to areas north of the Limpopo River. From the Drakensberg range there are 12 events to north of the Limpopo River, meaning that the predominant source of the flora north of the Limpopo River is the Drakensberg range, rather than the Cape (see figure 3). There are only two dispersals into the Cape from the Drakensberg range (in *Disa*), demonstrating that most of the species diversity in the Cape is derived from a single lineage for each clade. In *Disa* and Irideae p.p., there has been diversification in the Drakensberg range but in the *Pentaschistis* clade and the Restionaceae all species in the Drakensberg range have their sister species in the Cape, indicating that they have migrated into the region. *Disa* and Irideae p.p. have species sampled in the Zimbabwe overlap region. With one exception in the Irideae p.p., all of these species have their sister species in other areas, indicating that the rate of local diversification has also been very low there. The migration events from the Cape to the Drakensberg range are more frequent than in the opposite direction, even if the number of taxa in the source area is taken into account (table 2, Wilcoxon sign ranks test: Cape to Drakensberg range versus Drakensberg range to Cape:  $p=0.068$ ).

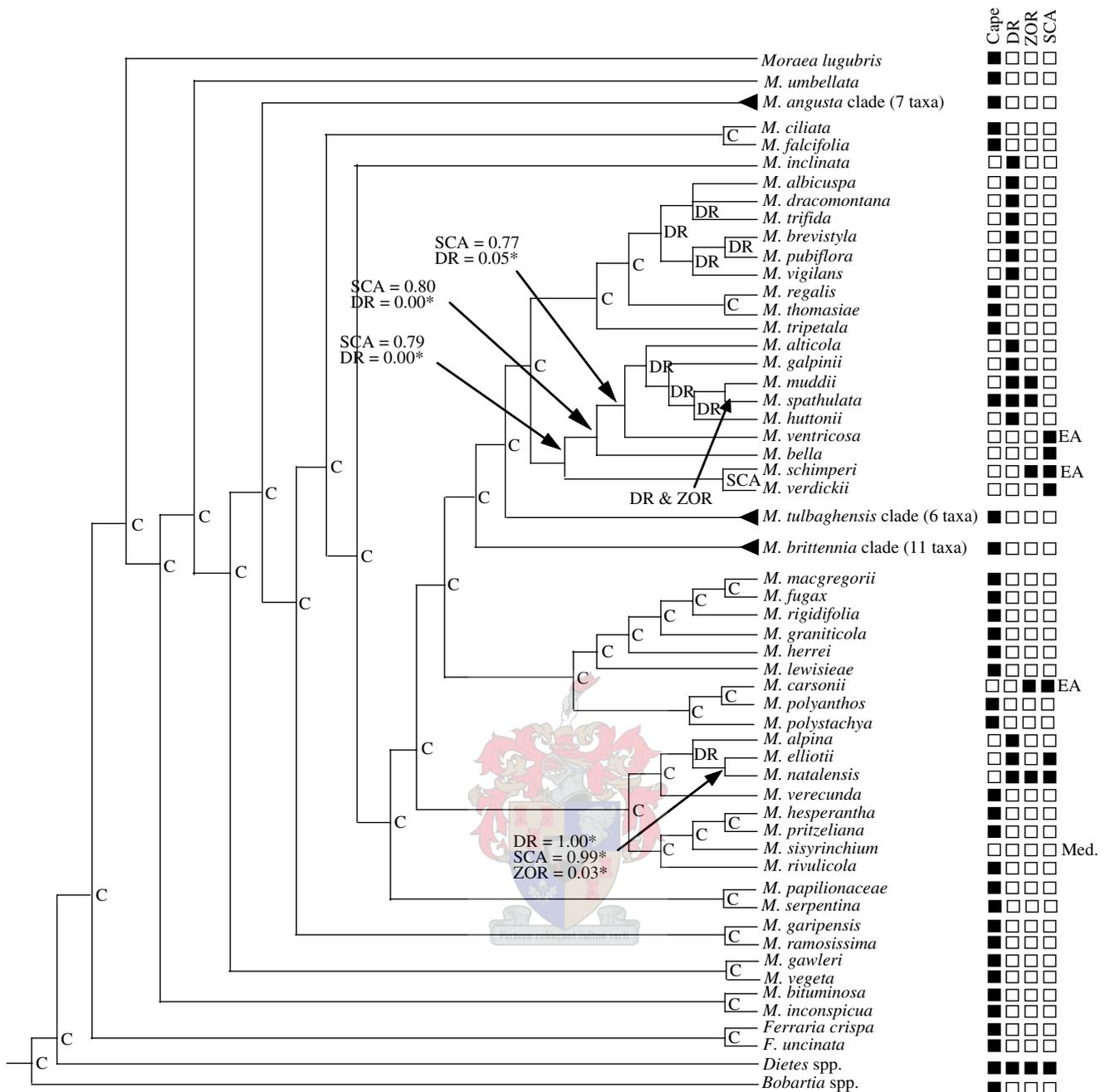


Figure 2. Optimization of ancestral node distribution for the Irideae p.p. including proportional likelihoods of areas for nodes that do not optimize unambiguously. Area: C, Cape; DR, Drakensberg range; ZOR, Zimbabwe overlap region; SCA, South Central Africa; EA, East Africa and Med, Mediterranean.

The age estimates for the migration events are shown in table 1 (in a few cases the source area is uncertain but we know a migration event took place and this is indicated by '?' in table 1). Where a migration could have occurred on either of the two adjacent branches (because an intermediate node could not be unambiguously optimized), we report the range of the age estimates and the Credibility Interval (CrI). In the Irideae p.p. and *Disa* especially, there have been frequent range expansion without lineage diversification and the ages given are therefore maximum estimates (represented by '\*' in table 1).

#### 4. DISCUSSION

##### (a) Methods

To reconstruct historical biogeography and biome assembly, it is essential to know the source and the

direction of migrations (Crisp 2006). These can only be deduced if the ancestral areas of distribution are known. Dispersal–Vicariance Analysis (DIVA; Ronquist 1997), a parsimony-based method, has commonly been used to infer ancestral areas, but we have instead used a likelihood-based method due to several advantages it has over DIVA. Perhaps the most important one is that it uses branch length information to calculate the probability of character state change, which on an ultrametric tree is directly related to time. Since older species will have had more time to disperse than younger species, this is an important parameter to take into account. A second advantage is that likelihood optimization allows for quantification of the level of uncertainty in optimization (Schluter *et al.* 1997). This is particularly important where there has been a lot of change in a short time

Table 1. Summary table of migrations inferred from ancestral state reconstruction, showing the age estimation of migration events. (\*, migration without speciation, therefore the event may be between the date range given and the present. Where a range of nodes and thus age estimates is used, the credibility interval (CrI) shown represents the upper and lower extremes.)

taxon	from where	to where	age estimate (mean)	95% CrI
<i>Moraea albicuspa</i> clade	Cape	DR	9.11	4.06, 16.37
<i>M. inclinata</i>	Cape	DR	25.65	17.25, 34.80
<i>M. alpina</i> clade	Cape	DR	18.33	11.06, 26.84
<i>M. verdickii</i> grade	Cape	SCA	14.98	8.19, 23.32
<i>M. sisyrinchium</i>	Cape	Mediterranean	13.56	7.04, 21.80
<i>M. alticola</i> clade	SCA	DR	8.26	3.68, 14.80
<i>M. carsonii</i>	Cape	ZOR, SCA, EA	7.69	2.87, 15.20
<i>M. spathulata</i> / <i>M. muddii</i> clade	DR	ZOR	4.68	1.50, 9.69
<i>M. spathulata</i>	DR, ZOR	Cape	2.73*	0.28, 6.95
<i>Dietes</i>	Cape	EA, SCA, ZOR, DR	51.45*	46.05, 54.78
<i>M. schimperi</i>	SCA	ZOR, EA	10.07*	4.59, 17.31
<i>M. natalensis</i> / <i>M. elliotii</i> clade	DR	SCA	12.03*	5.78, 20.15
<i>M. natalensis</i>	DR, SCA	ZOR	4.28*	0.58, 10.43
<i>Disa tripetaloides</i>	Cape	DR	1.37*	0.14, 3.50
<i>D. bracteata</i>	Cape	DR	2.71*	1.16, 5.01
<i>D. sagittalis</i> and <i>D. zimbabweensis</i>	Cape	DR, ZOR	15.19	11.30, 19.44
<i>D. lugens</i> var. <i>lugens</i>	Cape	DR	1.7*	0.56, 3.65
<i>D. cephalotes</i> clade	Cape	DR	8.87	3.53, 12.92
clade 'z'	Cape	DR	17.88–15.39	21.41, 11.91
<i>D. baurii</i>	Cape	DR, ZOR, SCA	5.67	2.91, 9.30
<i>D. brevicornis</i>	Cape	DR, ZOR, SCA	0.82*	0.08, 1.67
<i>D. cornuta</i>	Cape	DR, ZOR	6.52*	3.84, 10.07
<i>D. borbonica</i>	Cape	Reunion Island	1.57	0.82, 1.99
<i>D. patula</i> var. <i>transvaalensis</i>	DR	ZOR	0.60*	0.03, 1.88
<i>D. woodii</i>	DR	ZOR	1.32*	0.18, 3.19
<i>D. rhodantha</i>	DR	ZOR	1.32*	0.18, 3.19
<i>D. versicolor</i>	DR	ZOR, SCA	0.61*	0.03, 1.91
<i>D. saxicola</i>	DR	ZOR, SCA	5.43*	2.69, 9.05
<i>D. fragrans</i> ssp. <i>fragrans</i>	DR	ZOR, SCA	4.87*	2.49, 8.18
<i>D. miniata</i> clade	DR	SCA	10.50–5.79	14.20, 3.00
<i>D. perplexa</i> clade	DR	SCA	10.61*	7.38, 14.28
<i>D. chrysostachya</i>	DR	Cape	2.67*	1.08, 5.14
<i>D. buchenaviana</i>	DR	Madagascar	8.23	5.36, 13.11
<i>D. caffra</i>	DR, SCA	Madagascar	2.38*	0.84, 4.73
<i>D. aconitoides</i> ssp. <i>aconitoides</i>	DR	Cape	9.05*	6.07, 12.67
<i>D. aconitoides</i> ssp. <i>goetzeana</i>	DR	EA	8.18	5.29, 11.70
<i>D. similis</i>	DR	SCA	8.18*	5.29, 11.70
<i>D. hircicornis</i>	DR, SCA	ZOR, EA, WA	2.38*	0.84, 4.73
<i>D. perplexa</i>	DR, SCA	ZOR, EA, WA	5.97*	3.38, 9.49
<i>D. zombica</i>	SCA	ZOR	2.45*	0.94, 4.83
<i>D. miniata</i>	SCA	ZOR	2.35*	0.78, 4.73
<i>D. ornithantha</i>	SCA	ZOR	2.30*	0.79, 4.61
<i>D. erubescens</i> ssp. <i>erubescens</i>	SCA	ZOR, EA	0.54*	0.03, 1.70
<i>D. ochrostachya</i>	SCA	EA, WA	2.35*	0.78, 4.73
<i>D. stairsii</i>	?	EA	5.79	3.00, 9.54
<i>Pentaschistis tysonii</i>	Cape	DR	9.95	7.75, 11.67
<i>P. basatorum</i>	Cape	DR	9.13	7.35, 11.37
<i>P. aurea</i> subsp. <i>pilosogluma</i>	Cape and/or DR	DR	1.75	0.25, 3.79
<i>P. exserta</i>	Cape	DR	5.35	3.76, 7.67
<i>P. chippindalliae</i>	Cape	DR	3.77	2.44, 5.47
<i>P. andringitrensis</i>	Cape	Madagascar	8.03	6.24, 10.27
the summer rainfall clade	Cape	?	5.29–6.14	3.66, 8.32
<i>P. insularis</i>	?	Amsterdam Islands	0.44	0.02, 1.29
<i>P. pictigluma</i> clade	?	EA	1.04	0.47, 1.88
<i>P. natalensis</i>	?	SCA/Madagascar	1.25–3.11*	0.20, 4.61
<i>Restio zuluensis</i>	Cape	DR	5.87	2.28, 11.89
<i>Ischyrolepis schoenoides</i>	Cape	DR	4.66	1.39, 10.30
<i>Calopsis paniculata</i>	Cape	DR	17.31	9.95, 29.44
<i>Re. sejunctus</i>	Cape	DR	5.35*	1.65, 11.59
<i>Rhodocoma fruticosa</i>	Cape	DR	1.25*	0.04, 4.45
<i>Re. galpinii</i>	?	DR	16.64	8.61, 29.22
<i>Re. mahonii</i> and <i>Re. mlanjiensis</i>	?	EA and SCA, SCA	16.64	8.61, 29.22

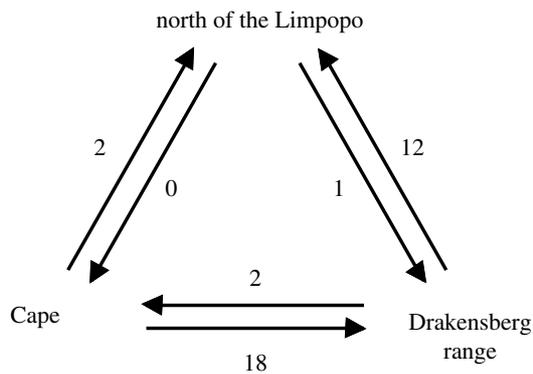


Figure 3. Diagram showing the number of migration events between the Greater Cape Floristic Region, the Drakensberg range and the north of the Limpopo River. Unambiguous migrations only were considered.

Table 2. The migration rate for each clade, calculated as the number of migrations per sampled taxon in the source area.

	Cape to DR	DR to Cape
<i>Disa</i>	0.0732	0.0435
Irideae p.p.	0.0526	0
the <i>Pentaschistis</i> clade	0.0806	0
Restionaceae	0.0174	0

(Schluter *et al.* 1997) or when optimizing more basal nodes (Mooers & Schluter 1999).

We used presence-only coding and binary optimization, rather than multistate coding and optimization, since currently available likelihood optimization software does not allow polymorphic character states at internal nodes, even if polymorphic states at the tips are accepted. Many of the species we investigated occur in more than one area (e.g. 19% of the *Disa* species occur in multiple areas) and we therefore assume that some internal nodes might also have had wider distributions. Such a scenario can only be reconstructed using binary optimization (Hardy & Linder 2005), since internal nodes can be optimized as polymorphic with statistical significance.

Topological uncertainty was not dealt with directly, except in the case of Irideae p.p., where the phylogenetic hypothesis contained polytomies (Goldblatt *et al.* 2002). In *Disa* and the *Pentaschistis* clade, there were several poorly supported nodes in areas of the topology where several distributional changes probably took place. However, there was also ambiguity in the optimization of these nodes (due both to this frequent change and to the short branch lengths involved). These ambiguous nodes were omitted from further analysis.

### (b) Directionality and the sourcing of the Afrotemperate flora in tropical Africa

The most recent common ancestor of all the four clades was unambiguously traced to the Cape. In total, 18 migrations from the Cape to the Drakensberg range and 12 from the Drakensberg to the rest of the Afrotemperate region north of the Limpopo River are documented. There are two migrations from the Cape to the north of the Limpopo River. Migration events in the opposite directions are rare (figure 3). This pattern refutes the hypothesis that north to south has been the prevailing

direction of migration for taxa shared between the Cape and the Afrotemperate floras (Levyns 1938, 1952, 1964; Axelrod & Raven 1978). Although neither the *Pentaschistis* clade nor *Disa* nor Irideae p.p. were mentioned explicitly, Levyns (1964) discussed many lineages that are similarly distributed within the Cape. Although she favoured a southern origin for a few taxa, such as Restionaceae and *Phyllica*, the distribution of other lineages was postulated to be the result of north to south migration. This 'southward migratory stream' was based mainly on the widespread but scattered distribution of more 'primitive' relatives or members of the lineages in tropical Africa. These were seen as relics of a once more continuous vegetation. In contrast, more 'advanced' members of the lineages were found in the southwestern part of the Cape where, as 'youthful endemics' they usually have a narrower distribution.

Although the 95% CIs of the age estimates are large, we date the migration of this flora to the tropical Afrotemperate regions to between 0.54 (0.02–1.80 CrI) and 10.0 (4.59–17.31 CrI) Myr ago (see table 1). Such recent migrations into these areas are congruent with the recent formation of the uplands of tropical Africa, which dates to the Miocene, with further uplift in the Pliocene and the Pleistocene (Grove 1983; Partridge *et al.* 1985). Palynological evidence suggests that *Podocarpus* and *Juniperus* were not in the Turkana Basin, northern Kenya, before 25 Myr ago (Vincens *et al.* 2006), and that they were present in Fort Ternan, Kenya, at ca 14 Myr ago (Bonnieffille *et al.* 2004). In contrast, the Cape Fold Mountains and the Lesotho highlands (within the Drakensberg range) precede the evolution of the Angiosperms and were partially preserved through the early African erosion cycle (King 1963; Grove 1983; Partridge & Maud 1987; Partridge 1998). There was important rejuvenation with two major periods of uplift in the early Miocene and the Pliocene especially in the Drakensberg (Partridge & Maud 1987; Partridge 1998; Partridge & Maud 2000). Stem lineages of members of the Cape clades date to the late Cretaceous and throughout the Tertiary (Galley & Linder 2006), consistent with the ancient Cape mountains. Migrations into the Drakensberg date to as early as 25.65 (17.25–34.80 CrI) Myr ago, and there is an increase in the number of events in the last 9 Myr, consistent with recent uplift in the area.

### (c) The Drakensberg range in the spread of the Afrotemperate flora

The Drakensberg range plays an important role as a 'stepping-stone' for plants between the Cape and the tropical Afrotemperate region (figure 3). The close floristic affinity between the Cape and the Drakensberg range is well known (Weimarck 1941; Killick 1963, 1978; Hilliard & Burtt 1987; Carbutt & Edwards 2002). We demonstrate it to be the result of many migration events occurring over a wide time span and largely in one direction. This unidirectional migration cannot be explained simply by the number of taxa in the source area.

The Drakensberg range has been proposed to be the source of many of the Cape elements in the mountains of tropical Africa (Weimarck 1941). This has been demonstrated for *Aloe* (Holland 1978) and Coryciinae s.s. (Linder 1994). This stepping-stone role of the Drakensberg range in the spread of species through the Afrotemperate

region is well supported by our data (figure 3). Although there are a few exceptions (*Moraea carsonii* and the *Moraea verdickii* grade, plus potential additional cases in *Restio* and *Pentaschistis*), direct migration from the Cape to the areas north of the Limpopo is not the norm. Furthermore, any extinction in the Drakensberg range would mask an indirect route. The Drakensberg range could also be the source of other Austral Afrotropical taxa also represented in the Cape, such as *Satyrium* (Orchidaceae), *Kniphofia* and *Aloe* (Asphodelaceae).

#### (d) Speciation outside of the Greater Cape Floristic Region

The Cape is known for its very high species richness (Levyns 1964; Goldblatt 1978; Goldblatt & Manning 2002; Linder 2003) concentrated in a relatively small number of clades (Levyns 1964; Goldblatt 1978; Linder 2003). It is unclear whether the high richness of the Cape relative to the other regions is the result of a more rapid diversification rate or simply of accumulation of species over a longer time period.

The wind-pollinated Restionaceae and *Pentaschistis* clades are represented by singleton species in the Drakensberg range, meaning that there has been no local diversification (i.e. speciation has not exceeded extinction). This cannot be explained by a lack of time to speciate since some of these migration events are very old (table 1), and in the *Pentaschistis* clade, for example, one Drakensberg species (*Pentaschistis basutorum*) is sister to a clade of at least 48 Cape species. Since all but one Drakensberg species from both clades have been sampled, we would not expect the pattern to change much with increased sampling. The biotically pollinated *Disa* and Irideae p.p. show a different pattern. *Disa* has reached the Drakensberg range at least 10 times and includes two clades that have subsequently radiated in the region, resulting in 12 and 26 taxa, respectively. Likewise Irideae p.p. has reached the Drakensberg at least six times and has speciated *in situ* resulting in three clades of three, five and seven taxa. The relative influence of pollinators and habitat diversity in the Cape and the Drakensberg range may have played an important role in the origin of differences between these two sets of clades and should be investigated.

While the Drakensberg range represents a source area for the more northerly part of the Afrotropical region, the Zimbabwe overlap region acts more like a sink. *Disa* and Irideae p.p. reached the Zimbabwe overlap region at least 15 and 4 times, respectively, but we document only one instance of local speciation. However, unlike *Pentaschistis* and Restionaceae in the Drakensberg range, these migrations are on average among the youngest events (table 1). Weimarck (1941) viewed the Inyangani subcentre (=Zimbabwe overlap region) as a 'relic', but although the habitats in these areas may be old, many of the species are clearly relatively recent additions. It is possible that the small surface area of the uplands in this region (approx. 1600 km<sup>2</sup>, Timberlake & Muller 1994) may be linked to a higher probability of local extinction (Gaston 2003). A consequence would be that the contemporary taxa are relative newcomers to the area. *Disa* and Irideae p.p. in the Zimbabwe overlap region have been sourced from both the north (South Central Africa) and the south (the Cape or the Drakensberg range), and this mixed sourcing of the flora is consistent with the

suggestions of Weimarck (1941) and Van Wyk & Smith (2001). Four species of Irideae p.p., one of *Disa* and one of Restionaceae are endemic to the Zimbabwe overlap region but were not sampled here. Although this makes our figure an underestimation, including these would not alter our conclusions that this region has a low diversification rate and that there are multiple sources to its flora.

All the four lineages have species in South Central and Eastern Africa. For three of these (Restionaceae, *Pentaschistis* clade and *Disa*), we know that *in situ* speciation has contributed at least half of the species. One of the two species of Restionaceae evolved in South Central Africa. The five *Pentaschistis* taxa in Eastern Africa form a clade, showing a radiation from a single immigration to the area. *Disa* is represented by 45 species in South Central and Eastern Africa, of which 22 are included in our analysis. This indicates two radiations, one with two species and the other with 11 or 12 taxa. However, morphological data indicate that the first radiation includes 8, and the second includes 20 species. There are possibly more radiations but species sampling would need to be extended to test these. Unfortunately our sampling of the Irideae p.p. of South Central and Eastern Africa is not adequate for a conclusive biogeographical optimization. The *in situ* diversification in South Central and Eastern Africa contrasts with the situation in the Zimbabwe overlap zone, where there has been almost no diversification. Furthermore, in tropical Africa, the Restionaceae and the *Pentaschistis* clade also speciated unlike in the Drakensberg range. Overall, it seems that there has been more speciation in the geographically much more extensive and fragmented Afrotropical flora of South Central and Eastern Africa than in the Drakensberg range.

#### 5. CONCLUSION

Biota comprise independent lineages that react differently to barriers, changes in climate, vegetation and pollinators. The biogeographical histories of their components are therefore not necessarily the same even if they occur in the same area. Using well-sampled phylogenies, the source areas of biota can be identified and the use of molecular clocks further allows us to put these events into a temporal framework. The Cape elements that we investigated occur in the tropical African mountains as a result of migration from the Cape or Drakensberg and also as a result of *in situ* speciation. This is similar to the situation in the Andes, where for the clades investigated the diversity is largely the result of recent and rapid *in situ* speciation (Hughes & Eastwood 2006). We also demonstrate a unidirectional migration in the Afrotropical flora. In contrast, migration across the Tasman Sea between New Zealand and Australia is bidirectional (Winkworth *et al.* 2002).

We do not attempt to provide a hypothesis for the origin of the complete Afrotropical flora, but rather for what is referred to as the Cape element, which nonetheless forms a substantial part of this flora. There has been a lot of migration throughout the region. In some areas, this immigration is the only source of diversity, whereas for other areas *in situ* diversification has been very important. These findings are however lineage dependant. We present overwhelming support for south to north migration for all clades and show that the Cape element in the Afrotropical flora is, at least in part, Cape derived. The Drakensberg

range has played an important role as a stepping-stone in the spread of the flora through this region. Clades such as *Stoebe*, *Oxalis*, *Cineraria*, *Felicia*, *Ursinia*, *Lobelia*, *Cyphia*, *Cliffortia*, *Pelargonium* and *Phylliceae* have, like our study groups, their greatest species richness in the Cape and probably show a similar pattern. In contrast, *Satyrium*, *Aloe* and *Kniphofia* have most species in the tropical mountains and may show a different pattern. A critical evaluation of the last set of genera would constitute an appropriate test of the generality of our Cape to Cairo hypothesis.

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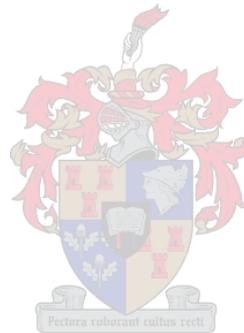
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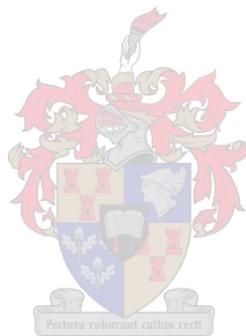
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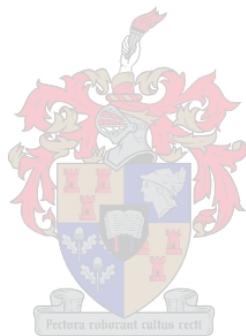
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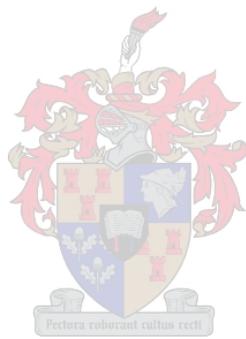
## Chapter 3

### The Cape element in the Afrotemperate flora: from Cape to Cairo?

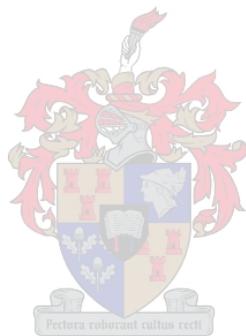


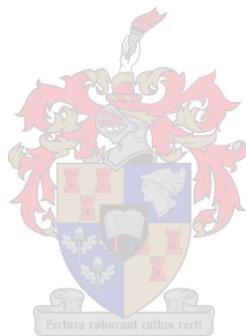


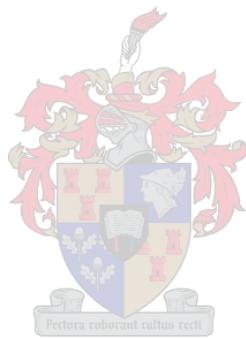


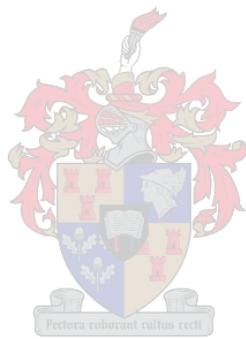


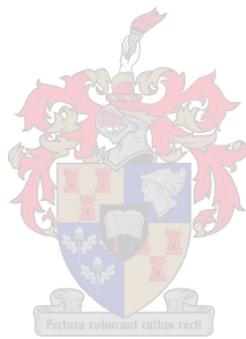












## Electronic Supplementary Material

### Material and methods

#### *Multidivtime analysis*

We followed the protocols described by Rutschmann (2004). The nucleotide frequencies, transition / transversion ratio and rate heterogeneity between the sites for each dataset were calculated with Baseml, implemented in PAML (Yang 1997). These values parameterize the F84 model, which is the most complex model implemented in Multidivtime (Thorne & Kishino 2002). Using this model we calculated the branch lengths using Estbranches, a component of Multidivtime, which were then rate corrected with Multidivtime. The Markov Chain Monte-Carlo was run for 10,000 generations, retaining every 100<sup>th</sup> sample, after discarding the first 100,000 generations. We repeated this twice, and compared the results to ensure that stationarity had been reached. The dating was calibrated to the mean dates obtained by the global analysis (described below). The standard deviations for rttm and rtrate were set as equivalent to rttm and rtrate. Rttm was set to the estimate age of the basal node, with each time unit equivalent to 10 Myr. Estbranches was used to obtain a tree with estimated branch lengths. From this, we estimated the median amount of evolution between the root and all the tips of the ingroup. Rtrate was set to this amount, divided by rttm (the number of time units from the base to the tip of the tree). Bigtime was set to approximately double the estimated age at the basal node.

#### *Global analysis and calibration*

We built a phylogenetic tree for the Angiosperms, in which each study group was represented by two species, selected to span the basal node of the study group. Further species representing other groups as well as nodes for which fossils are available were also included. The topology of the tree was taken from the three-gene Angiosperm phylogeny (Soltis *et al.* 2000). The tree was calibrated by the first occurrence of tricolpate pollen 125 Myr ago (Anderson *et al.* 2005), the first occurrence of African Restionaceae in the 61 Myr old Banke deposits in South Africa (Linder *et al.* 2003), the genistoid legume fossils reported by Lavin *et al.* (2006) from 56 Myr ago, and the estimated age of the genus *Phyllica* to 12 Myr based on the age of St Helena

(Richardson *et al.* 2001a; Richardson *et al.* 2001b). The tricolpate pollen and the *Phyllica* node were used as absolute dates (upper and lower bounds), while the other two fossil deposits were used as lower bounds only. We used *rbcl* as “dating gene”, as sequences of this gene were available for all the study groups, and as its semi-clocklike behaviour has been well documented (Gaut *et al.* 1992). The sequences were largely downloaded from Genbank, some were obtained from various researchers working on the Cape flora (see EMS Table 1).

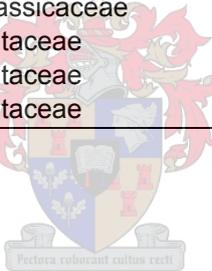
The *Disa* tree was additionally calibrated using *D. borbonica*, an endemic of Réunion. An upper age limit of 2 Myr was used, based on the age of Réunion (McDougall 1971). This assumes that speciation occurred as the result of dispersal to Réunion. An underestimation of the age of *D. borbonica* would occur with either of the following two scenarios: If the mainland sister species of *D. borbonica* went extinct; alternatively if *D. borbonica* originated elsewhere, migrated to Réunion and then went extinct in the source area. However, these latter two scenarios are less parsimonious, and unlikely considering the short time-scales.

EMS Table 1, showing the sources of the *rbcl* sequences used for the global analysis

Species	Family	Source
<i>Carpobrotus edulis</i>	Aizoaceae	F. Forest (unpubl. data)
<i>Psilocaulon parviflorum</i>	Aizoaceae	F. Forest (unpubl. data)
<i>Amaryllis belladonna</i>	Amaryllidaceae	Z69219
<i>Hessea zyheri</i>	Amaryllidaceae	AF116962
<i>Anginon rugosum</i>	Apiaceae	U50222
<i>Heteromorpha trifoliata</i>	Apiaceae	U50227
<i>Arctotheca calendula</i>	Asteraceae	F. Forest (unpubl. data)
<i>Didelta spinosa</i>	Asteraceae	F. Forest (unpubl. data)
<i>Linconia alopecuroides</i>	Bruniaceae	AY490993
<i>Lonchostoma monogynum</i>	Bruniaceae	AY490982
<i>Cotyledon orbiculare</i>	Crassulaceae	F. Forest (unpubl. data)
<i>Crassula perforata</i>	Crassulaceae	AF274594
<i>Acosmium dasycarpum</i>	Fabaceae	U74255
<i>Lotononis galpinii</i>	Fabaceae	Z95538
<i>Podalyria calyptrata</i>	Fabaceae	U74217
<i>Monsonia emarginata</i>	Geraniaceae	L14701
<i>Pelargonium capitatum</i>	Geraniaceae	L14702
<i>Ginkgo biloba</i>	Ginkgoaceae	DQ069500
<i>Spetaea lachenaliiflora</i>	Hyacinthaceae	J. Manning (unpubl. data)
<i>Veltheimia bracteata</i>	Hyacinthaceae	F. Forest (unpubl. data)
<i>Aristea glauca</i>	Iridaceae	AF206736
<i>Bobartia gladiata</i>	Iridaceae	AJ309699
<i>Moraea umbellata</i>	Iridaceae	AJ307149
<i>Watsonia angusta</i>	Iridaceae	AJ309666

<i>Disa spathulata</i>	Orchidaceae	AY368342
<i>Disa tripetaloides</i>	Orchidaceae	AF074151
<i>Endonema retzioides</i>	Penaeaceae	AJ605088
<i>Penaea mucronata</i>	Penaeaceae	AJ605090
<i>Pentameris thuarii</i>	Poaceae	N. Barker (unpubl. data)
<i>Prionanthium dentatum</i>	Poaceae	unpubl. data
<i>Leucodendron laureolum</i>	Proteaceae	U79180
<i>Spatalla curvifolia</i>	Proteaceae	F. Forest (unpubl. data)
<i>Protea repens</i>	Proteaceae	U79182
<i>Ranunculus acris</i>	Ranunculaceae	AY395557
<i>Baloskion tetraphyllum</i>	Restionaceae	AF148761
<i>Elegia macrocarpa</i>	Restionaceae	AY881424
<i>Willdenowia arescens</i>	Restionaceae	unpubl. data
<i>Phylica pubescens</i>	Rhamnaceae	Y16769
<i>Trichocephalus stipularis</i>	Rhamnaceae	F. Forest (unpubl. data)
<i>Hemimeris sabulosa</i>	Scrophulariaceae	AF123668
<i>Zaluzianskya katherinae</i>	Scrophulariaceae	AF123662
<i>Halleria lucida</i>	Stilbaceae	AF026828
<i>Retzia capensis</i>	Stilbaceae	Z29669
<i>Stilbe vestita</i>	Stilbaceae	Z68827
<i>Gnidia kraussiana</i>	Thymelaeaceae	AJ295267
<i>Lachnaea villosa</i>	Thymelaeaceae	AJ697804
<i>Heliophila dregeana</i>	Brassicaceae	B. Warren (unpubl. data)
<i>Heliophila digitata</i>	Brassicaceae	B. Warren (unpubl. data)
<i>Heliophila rigidiuscula</i>	Brassicaceae	B. Warren (unpubl. data)
<i>Agathosma ovata</i>	Rutaceae	F. Forest (unpubl. data)
<i>Adenandra uniflora</i>	Rutaceae	AF066803
<i>Calodendron capense</i>	Rutaceae	AF066805

## Results



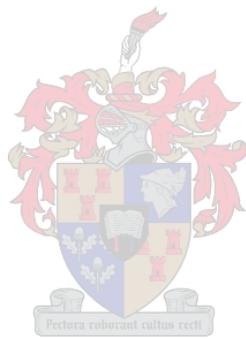
### Disa

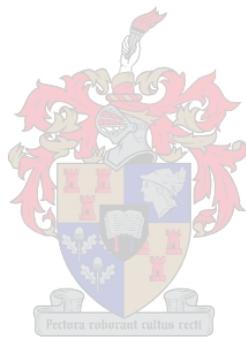
The MRCA of *Disa* is unambiguously traced to the Cape (ESM Figs. 1a,b). We identify at least five dispersal events out of the Cape (see Table 1): *D. baurii*, into the DR, SCA and ZOR; *Disa borbonica* onto Réunion; the *D. cephalotes* clade into the DR and one, possibly two, dispersal events into the DR represented by the clade comprising sections *Spirales*, *Aconitoideae*, *Micranthae*, and *Emarginatae* plus *D. tysonii*, further referred to as clade ‘z’. Although the MRCA of clade z cannot be optimised to any area, the subtending node optimises unambiguously to the Cape. Lastly, *D. zimbabweensis* occurs in the DR and ZOR and is sister to *D. sagittalis* (Cape and DR). The node subtending these taxa optimises significantly to Cape but also (with moderate support; 0.6) to the DR. It is therefore unclear whether *D. zimbabweensis* represents migration from the Cape or the DR into ZOR, but this species pair represents at least one migration out of the Cape.

Within clade ‘z’ one or two dispersal events from the DR northwards can be inferred: the *D. miniata* clade migrated to SCA, and most probably *D. aconitoides* ssp. *goetzeana* migrated to EA. The source area of the Madagascan *D. buchavianiana* (section *Emarginatae*) cannot be resolved; the sub-tending node has low support (0.54) for the DR. There has possibly also been westward dispersal into the Cape by section *Spirales*, but the node joining this clade with *Aconitoideae* and *Micranthae* does not optimise to any area. *D. stairsii* dispersed into EA but the source area is not known. Many *Disa* species occur in more than one area. Several species have expanded their ranges from the Cape (Table 1): *D. tripetaloides*, *D. sagittalis*, *D. lugens* and *D. bracteata* into the DR; *D. brevicornis* into DR, SCA and ZOR; and *D. cornuta* into the DR and ZOR. Within the part of section *Stenocarpa* that occurs in the DR there has been range expansion into ZOR and SCA (*D. saxicola*). The vast majority of range expansions, however, occurred within clade ‘z’. From the DR there has been range expansion across the Limpopo River into the ZOR (*D. patula* var. *transvaalensis*, *D. woodii*, *D. fragrans*, *D. rhodantha*, *D. versicolor*), SCA (*D. fragrans*, *D. versicolor*, the *D. perplexa* clade) and westwards back into the Cape (*D. chrysostachya*). The node subtending *D. caffra* (Madagascar, DR and SCA) and *D. hircicornis* (DR, SCA, ZOR and EA) optimises significantly to both DR and SCA, so we cannot establish from where this range expansion occurred. The source areas for *D. aconitoides* ssp. *aconitoides* (Cape and DR), *D. aconitoides* ssp. *goetzeana* (EA) and *D. similis* are probably DR, as optimisations at the subtending nodes receive moderate, but not significant, support (0.85, 0.84 and 0.84, respectively) for this area only. Within the South-Central African clade of the *Micranthae* there has also been range expansion into EA (*D. erubescens* ssp. *erubescens*, *D. perplexa* and *D. ochrostachya*), WA (*D. ochrostachya* and *D. perplexa*) and the ZOR (*D. zombica*, *D. erubescens* ssp. *erubescens*, *D. ornithantha*, *D. miniata* and *D. perplexa*). For many of these taxa the precise route of migration cannot be inferred because migration into more than one area has occurred without accompanying speciation.

#### *Irideae p.p.*

The root node unambiguously optimises to the Cape (Fig. 1). Internal nodes were optimised with statistical significance to Cape only, unless otherwise shown. Despite widespread distributions of many of the taxa, only two internal nodes were polymorphic, namely the most recent common ancestor (MRCA) of *Moraea muddii*





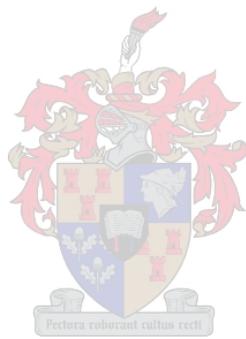
and *M. spathulata*, and of *M. natalensis* and *M. elliotii*. We identify three dispersal events from the Cape to the DR, one dispersal event to SCA and one to the Mediterranean. From one SCA group (the *M. verdickii* grade) there is range expansion into EA (*M. ventricosa* and *M. schimperi*) and the ZOR (*M. schimperi*) and dispersal southwards into the DR (*M. alticola* clade). From the DR this clade expanded northwards into the ZOR (*M. muddii* and *M. spathulata*) and southwards into the Cape (*M. spathulata*). From the second clade of the DR (*M. alpina* clade) there is range expansion into SCA (*M. natalensis* and *M. elliotii*) and into the ZOR (*M. natalensis*). Both *Dietes* and *M. carsonii* represent migration events for which the route cannot be inferred, except to say that they originated in the Cape. Speciation outside of the Cape has occurred in the DR and SCA and in a single case in the ZOR.

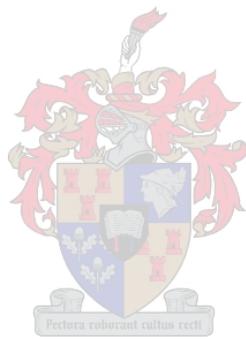
#### *The Pentaschistis clade*

The root node is unambiguously optimised to the Cape. All internal nodes of the tree are optimised to the Cape, unless otherwise shown in ESM Fig. 2. We identify eight migration events out of the Cape. A minimum of five of these represent dispersals to the DR (*P. tysonii*, *P. basutorum*, *P. exserta*, *P. chippindalliae* and an event in *P. aurea*) and one a migration to Madagascar (*P. andringitrensis*). The node subtending *P. aurea* subsp. *aurea* has proportional likelihoods of 0.78 (Cape) 0.18 (DR) and represents either a vicariance event leading to the two sub-species or (more likely) a single migration into the DR. The node subtending *P. natalensis* from SCA and Madagascar receives no likelihood support, therefore the source area for these cannot be identified. There are several more nodes within the summer rainfall clade that do not optimise to any area. From within this clade migration to EA (the *P. pictigluma* clade) and dispersal to Amsterdam Island (*P. insularis*) occurred, but due to the ambiguous optimisation of the internal nodes, the source area(s) cannot be determined.

#### *Restionaceae*

The root node and all internal nodes were unambiguously optimised to the Cape with the exception the *Restio galpinii* clade (ESM Fig. 3). Six or seven migration events out of the CFR are identified: into the DR there are two range expansions (*Rhodocoma fruticosa* and *Restio sejunctus*) and three dispersal events (*Ischyrolepis schoenoides*, *Restio zuluensis*, *Calopsis paniculata*). There is further migration out of





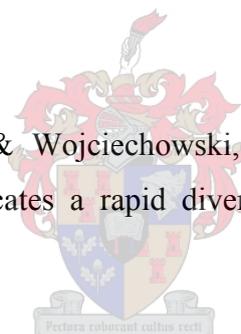
the Cape represented by the *Restio galpinii* clade. The MCRA of this clade optimises as absent for all areas, and the node subtending the two species from SCA optimises to SCA. This clade represents one or two migration events out of the Cape. This clade has undergone speciation outside of the Cape, representing one, possibly two speciation events in SCA, or possibly one in the DR.

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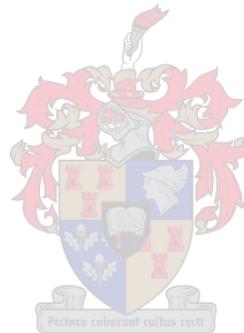
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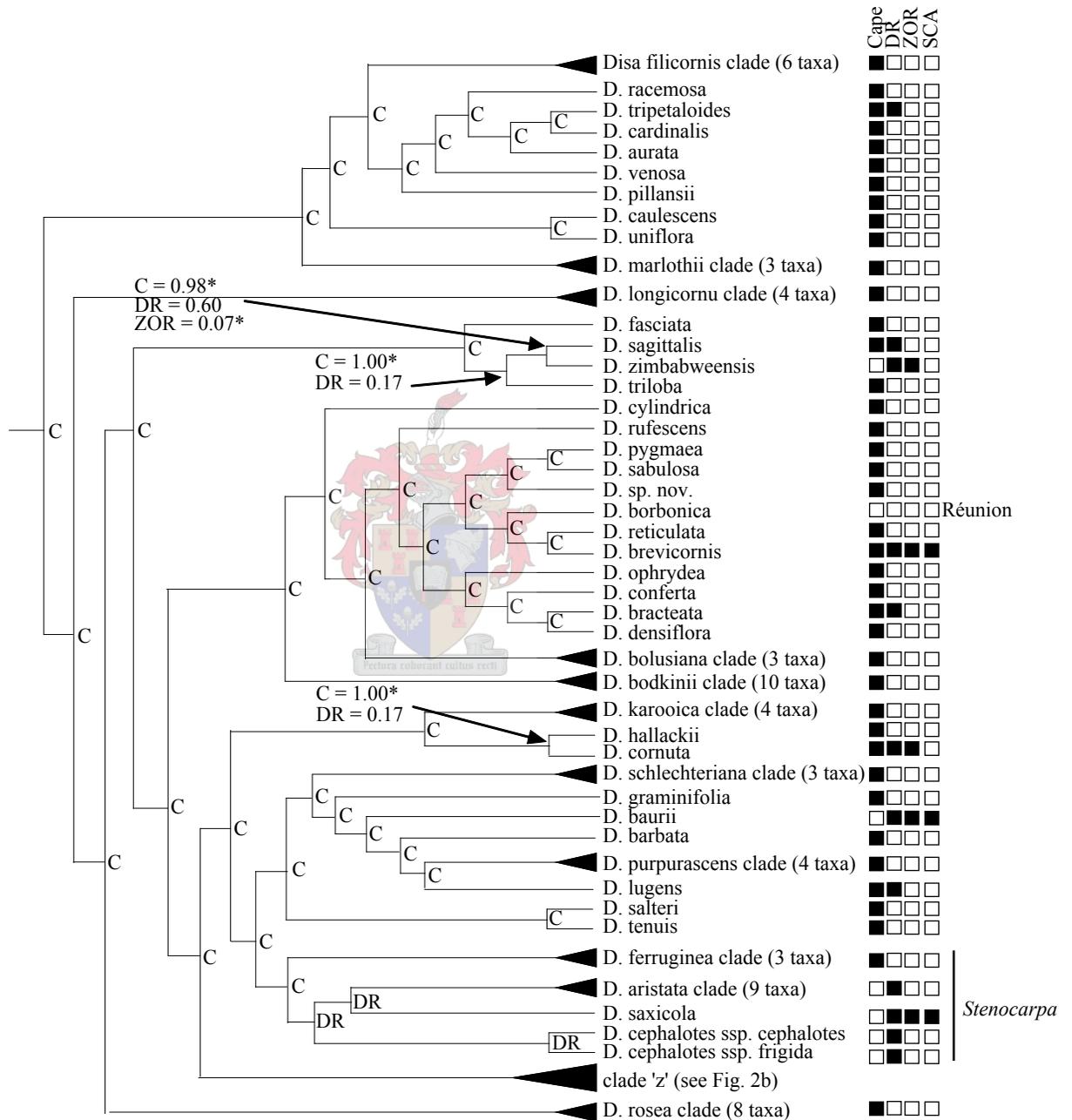
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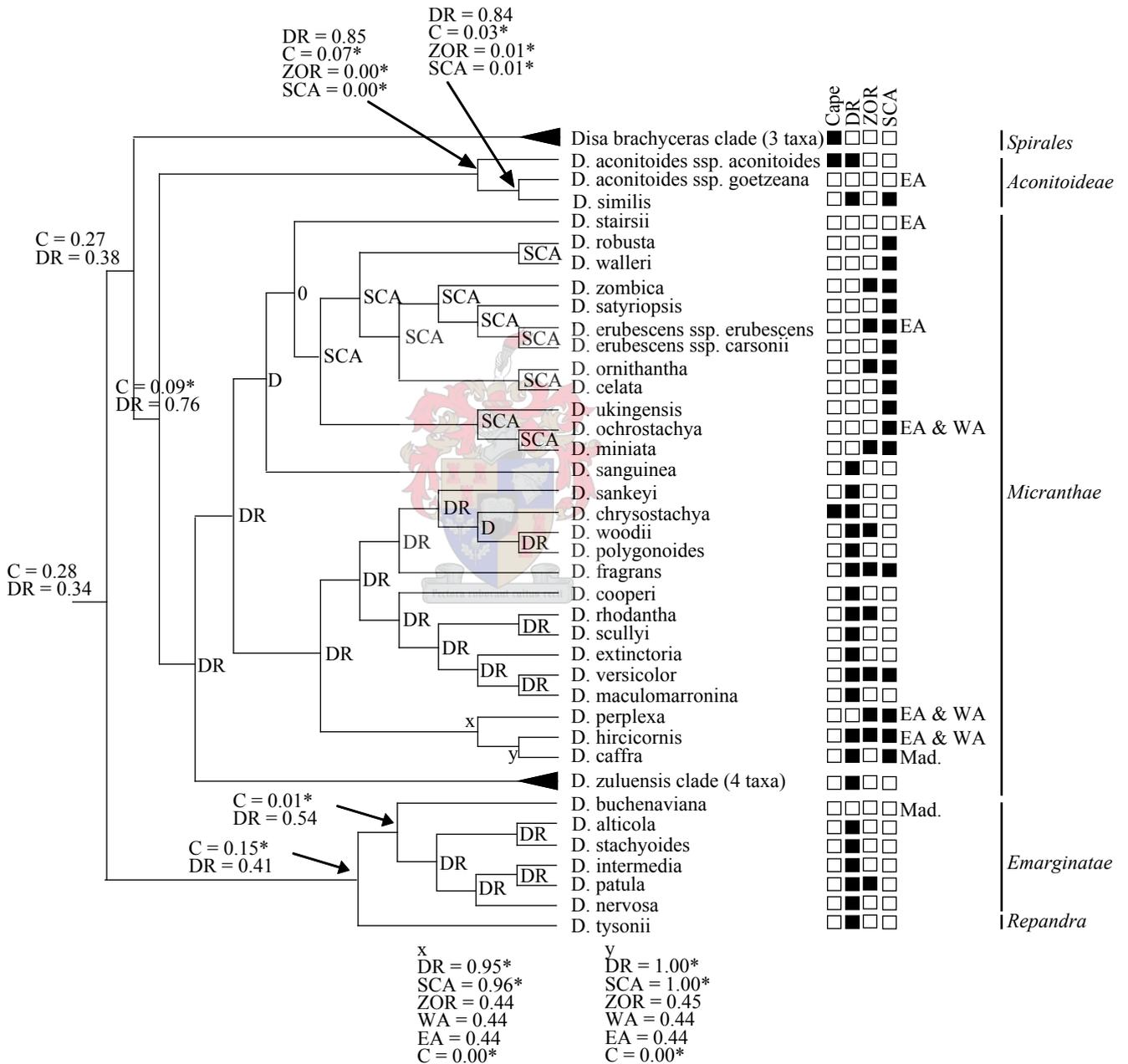
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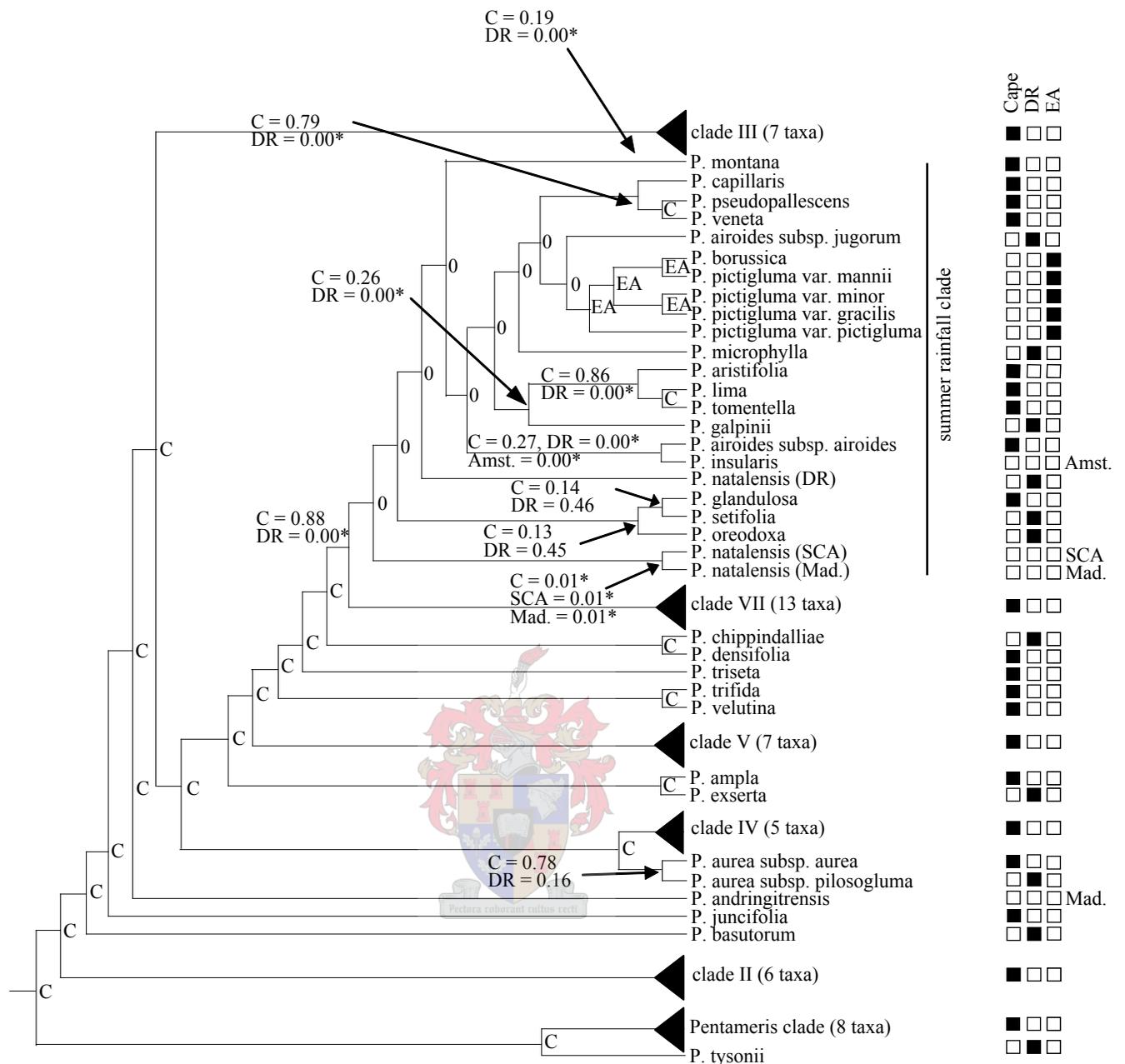
ESM\_Figure 1a

Optimisation of ancestral node distribution for *Disa* including proportional likelihoods of areas for nodes that do not optimise unambiguously. a) *Disa* except clade 'z' b) clade 'z'. Areas as follows: C, Cape; 0, optimises as absent for all areas.



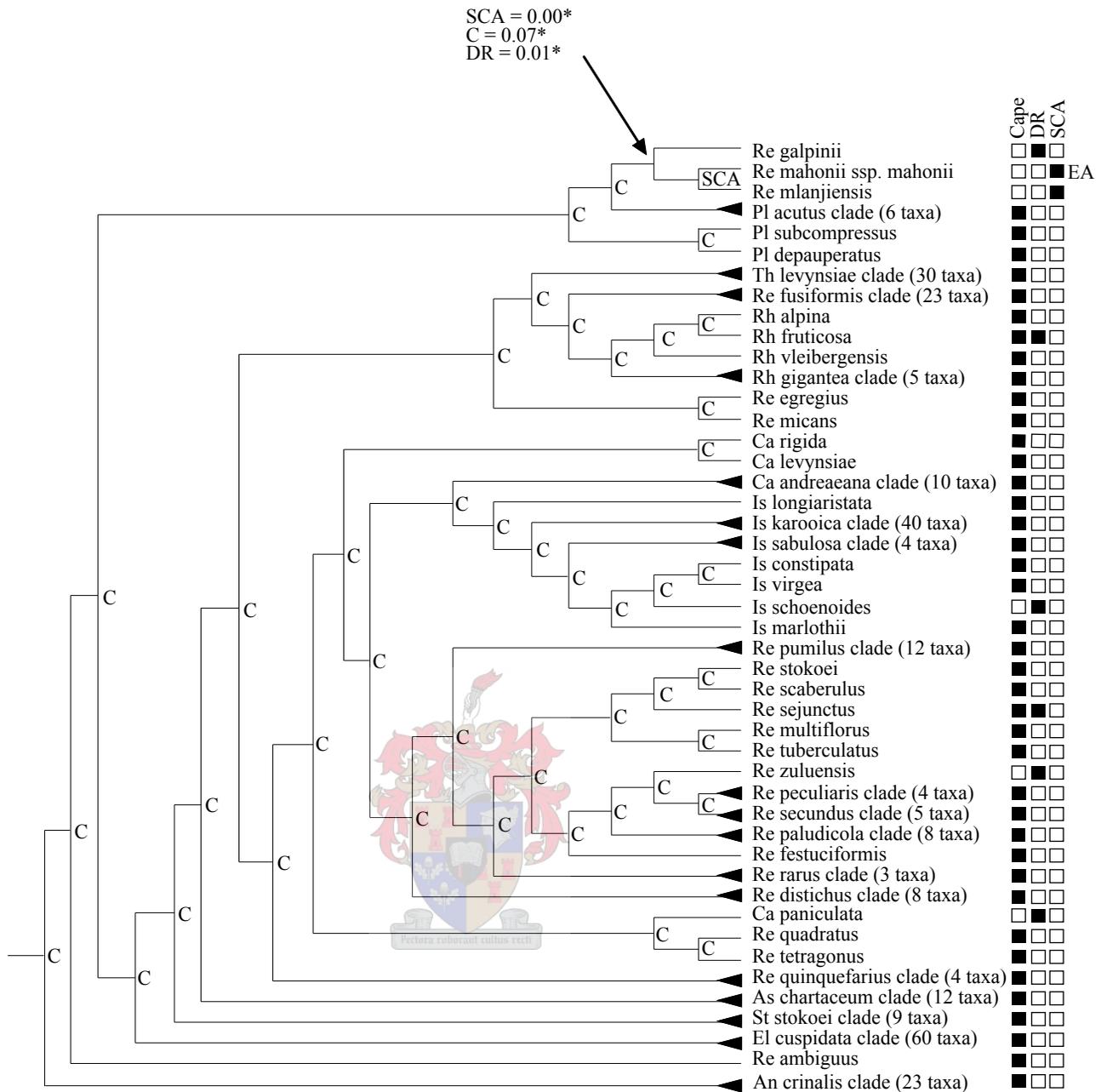
ESM\_Figure 1b

Optimisation of ancestral node distribution for *Disa* including proportional likelihoods of areas for nodes that do not optimise unambiguously. a) *Disa* except clade 'z' b) clade 'z'. Areas as follows: C, Cape; 0, optimises as absent for all areas.



ESM\_Figure 2

Optimisation of ancestral node distribution for the *Pentaschistis* clade including proportional likelihoods of areas for nodes that do not optimise unambiguously. Areas as follows: C, Cape; Amst., Amsterdam Island and St. Paul's Island; 0, optimises as absent for all areas. Genus name P is *Pentaschistis*.



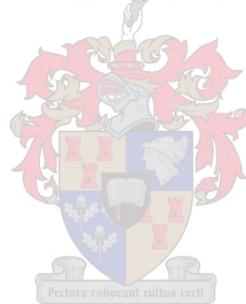
ESM\_Figure 3

Optimisation of ancestral node distribution for Restionaceae including proportional likelihoods of areas for nodes that do not optimise unambiguously. Area: C, Cape.

Genera names as follows: An, *Anthochortus*; As, *Askidiosperma*; Ca, *Calopsis*; El, *Elegia*; Is, *Ischyrolepis*; Pl, *Platycaulos*; Re, *Restio*; Rh, *Rhodocoma*; St, *Staberoha*; Th, *Thamnocortus*.

## Chapter 4

# The evolutionary history of the orchid genus *Disa* and its implications for southern African paleoclimate reconstruction



# The evolutionary history of the orchid genus *Disa* and its implications for southern African paleoclimate reconstruction

Benny Bytebier<sup>1,\*</sup>, Dirk U. Bellstedt<sup>1</sup> and H. Peter Linder<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Stellenbosch University, Private Bag X1, 7602 Matieland, South Africa

<sup>2</sup>Institute for Systematic Botany, University of Zurich, Zollikerstrasse 107, CH 8008, Zurich, Switzerland

\*Author for correspondence (bytebier@sun.ac.za)



Key words: Cape clades, Cape flora, likelihood optimisation, Orchidaceae, paleoecology

Running title: Evolutionary history of *Disa*

(prepared for Evolution)

## Abstract

A robust, well-sampled and dated molecular phylogeny, combined with likelihood optimisation techniques, have allowed us to reconstruct the temporal occurrence of ancestral ecological attributes of the African orchid genus *Disa*. The first appearance of species in the grassland and savanna biomes, as well as in the subalpine habitat, are in agreement with the existing, reliable geological and paleontological information. This suggests that phylogenies can indeed be used to date events for which other information is lacking or inconclusive, such as the age of the fynbos biome and the start of the winter rainfall regime in southern Africa. Our results postdict that these are much older than what is currently accepted and date back to at least the Oligocene.

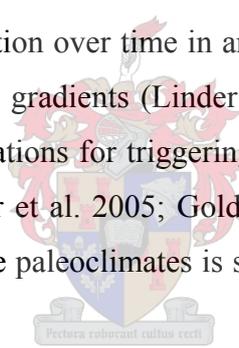
## Introduction

Paleoclimatological and paleoecological reconstructions are mostly based on plant and animal fossil data (van der Hammen and Hooghiemstra 2000; Jacobs 2004; Jacobs and Herendeen 2004). Although geochemical and geomorphological information is also used (Diester-Haass et al. 1992; Zachos et al. 2001), this comes mainly from marine basins and the link between marine and terrestrial realms is not always clear (Bobe, 2006). For southern Africa the Neogene fossil record is remarkably poor and does not allow for confident reconstruction of the climate (Linder 2003). Other methodologies and independent lines of evidence would thus be welcome to augment our patchy understanding of past ecosystems. Here, we draw on neontological data to develop hypotheses on past environments and address the question of what the evolutionary history of a taxon can tell us about past climates and landscapes.

We use the large African orchid genus *Disa* as our study group. This charismatic group of orchids is well known, and *Disa uniflora*, the “Pride of Table Mountain”, is often used as an emblem (e.g. of the Mountain Club of South Africa). Of 178 species, 99 occur in the Cape Floral Region (CFR) and 85 are restricted to it (Goldblatt and Manning 2002). The various species can be found from the coastal sands to the summits of the highest mountains, yet they are never common (Linder and Kurzweil 1999). Most are geophytes, well-adapted to a fire prone environment. Indeed, many

species will only flower in the first year after fire. Outside of the CFR, the diversity of species decreases eastwards and northwards and, although the genus can be found throughout Africa, it is mostly restricted to Afromontane grasslands. Recently, Bytebier et al. (in press) presented a robust phylogenetic analysis of the genus based on 70% sampling of all species, representing all sections, the full geographical range of the genus and all major habitats it occurs in.

The CFR is more species rich than can be expected from its surface area or latitude (Linder 2003). About 9,000 species can be found in 90,000 km<sup>2</sup> of which 67 % are endemic (Goldblatt and Manning 2002). Almost half of this species richness is accounted for by only 33 “Cape floral clades”(Linder 2003), of which the orchid genus *Disa* (including *Schizodium*) is one. The radiation of these Cape floral clades was not triggered by one single event (Linder 2005), but is more likely the outcome of the recruitment of diverse lineages over the entire Cenozoic into the flora (Galley and Linder 2006) and the diversification over time in an area with a heterogenous habitat distribution and steep ecological gradients (Linder 2005). Most authors have sought paleoclimatic changes as explanations for triggering (Klak et al. 2004; Richardson et al. 2001) and sustaining (Bakker et al. 2005; Goldblatt et al. 2002) these radiations. Yet our current knowledge of the paleoclimates is still poor, and consequently can be fitted to almost any explanation.



In this paper we (i) use a molecular phylogenetic hypothesis and likelihood optimisation to reconstruct the historical ecology of the genus; (ii) use a molecular clock to date the various changes in ecology; (iii) test if ecological traits are constrained by the phylogeny; (iv) use these to elaborate on the Neogene paleoclimates of southern Africa; (v) discuss the possible connections between these climate changes (or the absence thereof) and the modern diversity of the flora.

## Material and methods

### *Phylogenetic hypothesis and age estimation*

Phylogenetic relationships were inferred for 7 outgroup and 136 ingroup taxa, representing 70% of all recognised *Disa* taxa. One nuclear and two plastid gene regions were sequenced and compiled in a matrix with 4094 characters, 1096 (26.8%) of which were parsimony informative. In a parsimony analysis, 87 nodes of 142 (61%) were supported with a bootstrap support values of 75% or higher, while the topology resulting from a Bayesian inference analysis had 101 (71%) nodes with a posterior probability of 0.95 or above. The phylogenetic analysis is discussed in detail in Bytebier et al. (in press). As starting tree for this study we used the tree with the highest likelihood score from a 2 times 2.5 million generations Bayesian inference analysis. A constant molecular clock for this tree was rejected and it was made ultrametric using a Bayesian relaxed clock (Renner 2005; Rutschmann 2006), as implemented in Multidivtime (Thorne and Kishino 2002). We followed the protocols described by Rutschmann (2004) and the details and parameters can be found in Galley et al. (accepted). Two calibration points were used: the node subtending *Disa borbonica*, an endemic of Réunion, was set to maximum 2 Myr, which is the age of this volcanic island (McDougall, 1971). The age of the genus was set to 28.5 Myr, which was the date derived from a separate, more extensive analysis based on the *rbcL* gene region (Galley et al., accepted).

### *Coding of biomes*

*Disa* species occurring in southern Africa were coded for occurrence in biome by Linder et al. (2005a). The biomes were as defined by Rutherford and Westfall (1986) and summarize climatic, edaphic and biotic information into broad descriptive units (Table 1). The areas occupied by the biomes in southern Africa was taken from Rutherford (1997). Orchid distribution is mainly based on specimens deposited in the BOL and PRE herbaria as compiled in Linder & Kurzweil (1999). For coding of the species occurring outside of southern Africa, we relied on Linder (1981a-d), Flora of Tropical East Africa (Summerhayes 1968), Flora Zambesiaca (la Croix and Cribb 1995), Flore d'Afrique Centrale (Geerinck 1984) and Orchids of Malawi (la Croix et al. 1991) and on the personal field experience of the authors. All *Disa* species were eventually assigned to five biomes: Fynbos, Succulent Karoo, Grassland, Savanna and

Forest. We used binary coding (absence/presence) so that more than one one biome could be assigned to a taxon and which also allowed for polymorphic states at internal nodes.

Table 1: Main ecological characteristics of the biomes (summarised from Rutherford and Westfall 1986).

<b>Biome</b>	<b>Rainfall</b>	<b>Vegetation</b>
Fynbos	Summer dry, 300-3000 mm	Slow growing heathland, fire cycle 5-20 years
Succulent Karoo	Summer dry, 50-300 mm	Dwarf shrubland with succulents, no fire
Grassland	Winter dry	Grassland, fire cycle 1-4 years
Savanna	Winter dry	Woodland and thicket, fire cycle 1-4 years
Forest	All Year Rain, min 800 mm	Closed evergreen forest, no fire

#### *Coding of rainfall seasonality*

For *Disa* species occurring in southern Africa, the distribution maps from Linder & Kurweil (1999) were superimposed on the rainfall seasonality map of Schulze (1997). For species occurring outside of southern Africa, rainfall seasonality data was extracted from Linder (1981a-d). The seasonality of the rainfall was then coded for every species as presence/absence in areas with Winter rainfall, All Year rainfall, Summer rainfall and/or Bimodal rainfall. Although this might seem as an crude and simplistic way of coding a complex ecological parameter, we believe that it is adequate given the geographical and phylogenetic scale of our analysis.

#### *Coding of habitats*

*Disa* species occurring in southern Africa were earlier coded for Habitat by Linder et al. (2005a), using the following categories: Grassland, Woodland, Subalpine, Marsh, Scrub, Mature Heath, Postfire, Streambank and Epilythic (Table 2). To this, we added Southeast Cloud Zone habitat. The importance of southeast clouds as a source of water during the dry summers in the CFR was documented by Marloth (1904). Although we could not trace information on the distribution of these clouds, our field experience indicates that this a climatological event restricted to the top ridges of the Cape Fold Mountains within sight of the Indian Ocean. The habitats for the species occurring outside of southern Africa were coded with the help of the monographic and

floristic treatments mentioned above and with the personal field experience of the authors.

Table 2: Main ecological characteristics of the habitats (summarised from Linder et al. 2005)

<b>Habitat</b>	<b>Description</b>
Grassland	Grassland at all altitudes, both well-drained and marshy
Woodland	Savanna or "miombo" woodlands; sparse overstorey of trees and regularly burnt grassy understorey
Subalpine	Above 2500 m; severe frost in winter; temperate grassland with heathy patches
Marsh	Seasonally or perennially waterlogged; mostly grassy but may be in heathland, woodland or forest
Scrub	Thicket vegetation or short stunted trees; no shady areas under trees
Mature Heath	Mature fynbos; species that appear after fire are not included here
Postfire	Fynbos in the first year after fire; category restricted to those orchids that flower only in the first year after a fire before the fynbos has been re-established
Epilithic	Growing on rock, in shallow soil over bedrock or in very rocky soil
Streambanks	Along stream margins, often on bedrock or hanging over the water
Southeast Cloud	Restricted to the top ridges of the Cape Fold Mountains within sight of the Indian Ocean which are covered with clouds when the southeast winds blow from spring to autumn

### *Optimisation of discrete characters*

Reconstruction of ancestral states was done in the programme Mesquite version 1.1 (Maddison and Maddison 2006) using a likelihood reconstruction method with an “asymmetric Markov k-state 2 parameter model” and “root state frequencies same as equilibrium”. This model has one parameter for the rate of change from state 0 to 1 (the "forward" rate) and another for the rate of change from 1 to 0 (the "backward" rate) and thus, allows a bias in gains versus losses. We compared the lnL scores of a two-rate (forward and backward rates independent) and a one-rate (forward and backward rates constrained to be equal) model for each character. The accuracy of parameter estimation depends on the amount of data available as well as model complexity (Mooers and Schluter 1999). For several characters (but not all) the two-rate model resulted in a significantly improved fit and we therefore preferred this model since it makes fewer assumptions (i.e. it does not assume the forward and

backward rates of characters change to be equal). We are aware, however, that the one-rate model handles trees with few transitions and an imbalance of character states better than the two-rate model (Mooers and Schluter 1999) and we therefore also checked the optimisations with this model. In most cases this did not give a significantly different result and if it did, we report these differences explicitly. Each node was optimised for each ecological character and a threshold value of 2 log likelihood (lnL) units was used to infer statistical significance (Mooers and Schluter 1999; Maddison and Maddison 2006). To establish the number of times an attribute evolved, we used statistically significant nodes. In addition, we also took the nodes, which were not significant but had a proportional likelihood above 0.75, into account. These are listed as the lower figure in the "No of times evolved column" of Table 3

#### *Randomness of the distribution*

Randomness of the distribution of the character states on the tree was assessed as follows. Terminals on the phylogenetic tree were replaced with the character state (0 or 1) for one particular character at a time. The character history was then traced using parsimony reconstruction with the states unordered as implemented in Ancestral States Reconstruction Module of Mesquite 1.1 (Maddison and Maddison 2006). This gave the minimum number of steps to explain the current character distribution on the tree. The character states were then reshuffled 1000 times, each time keeping the frequencies of the character states fixed. The number of steps to explain the character distribution on the 1000 randomised trees was plotted and the 95 % confidence interval was calculated and compared with the number of steps on the real tree. If the number of steps was outside the 95% confidence interval, then the Null hypothesis that the character states were randomly distributed on the phylogenetic trees was rejected.

## **Results**

#### *Optimisation for biomes*

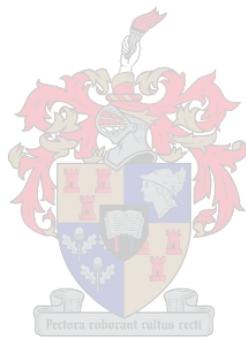
The generic node could be unambiguously and significantly optimised to the Fynbos biome (proportional likelihood = 0.999) (Fig. 1). At least six, and maybe up to eight, independent migrations occurred from Fynbos to Grassland, with possibly two reversals. *D. zimbabweensis*, *D. bauri* and the *D. cephalotes* clade of section

*Stenocarpa* represent unambiguous migrations. The clade ((*Emarginatae*, *D. tysonii*) (*Spirales*, (*Aconitoideae*, *Micranthae*))) (hereafter called clade "z"), represent either one transition event with a potential reversal of section *Spirales* to Fynbos or two transition events from Fynbos to Grassland, depending on the optimisation method. To add to the uncertainty, the basal nodes in clade "z" have no phylogenetic support (Bytebier et al., in press), and an alternative phylogenetic hypothesis with a basal split between *Spirales* and the rest would result in a single transition from Fynbos to Grassland with no reversal. A similar situation is seen in the *D. borbonica* / *D. reticulata* / *D. brevicornis* clade, which represents either one transition event with a reversal of *D. reticulata* to Fynbos or two transition events. *D. cornuta* extended its biome occupation from Fynbos into Grassland. Only one species, *D. karooica*, made the transition from Fynbos to Succulent Karoo. *D. stairsii* migrated from the Grassland biome into the Forest biome. Transition from Grassland to Savanna also occurred only once. The Most Recent Common Ancestor (MRCA) of the (*Aconitoideae*, *Micranthae*) clade can be optimised to Savanna (Fig. 1) and thus this species was polymorphic and occurred in Grassland as well as in Savanna.

The pattern of migration between biomes is directional from Fynbos to Grassland and Succulent Karoo, and then from Grassland to Savanna and Forest, with very few, if any, reversals. Species that occur in Fynbos do not occur in Grassland, with one notable exception, the widespread species, *Disa cornuta*.

The age of first occupation of each biome is given in Table 3. The MRCA of the genus *Disa* evolved 28.5 Mya in the Fynbos biome. The MRCA of clade "z" was the first to occupy Grassland around 17.88 Mya. Transition to Savanna dates back to around 15.39 Mya, whereas transition to Succulent Karoo and Forest is more recent and dates back to 6.7 and 5.79 Mya respectively.

The significance tests show that the biome character states are not randomly distributed across the tree. For Fynbos, Grassland and Savanna, the null hypothesis that these states were randomly distributed across the tree was rejected. Since only one species occurs in each of the Succulent Karoo and Forest biomes, a significance test was not relevant here.



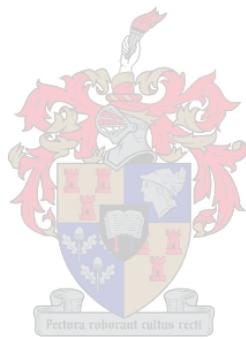


Table 3. Temporal evolution of ecological attributes.

Attribute	No. of species	No. of times evolved	Mean age of first occupation (Mya), with standard deviation	First occupant
Fynbos biome	78	1-3	28.5 ± 0.89	MRCA <i>Disa</i>
Grassland biome	54	6	17.88 ± 1.81	MRCA clade "z"
Savanna biome	11	1	15.39 ± 1.81	MRCA ( <i>Aconitoideae</i> , <i>Micranthae</i> )
Forest biome	1	1	5.79 ± 1.71	<i>D. stairsii</i>
Succulent Karoo biome	1	1	6.70 ± 1.90	<i>D. karooica</i>
Winter rainfall	68	3	28.5 ± 0.89	MRCA <i>Disa</i>
All Year rainfall	49	26-37	11.48 ± 1.77	<i>D. subtenuicornis</i>
Summer rainfall	59	8-11	15.39 ± 1.81	MRCA ( <i>Aconitoideae</i> , <i>Micranthae</i> )
Bimodal rainfall	6	6	5.79 ± 1.71	<i>D. stairsii</i>
Grassland habitat	53	8-11	15.39 ± 1.81	MRCA ( <i>Aconitoideae</i> , <i>Micranthae</i> )
Woodland habitat	4	2-3	8.18 ± 1.65	<i>D. aconitoides</i> ssp. <i>goetzeana</i>
Subalpine habitat	8	8	5.79 ± 1.71	<i>D. stairsii</i>
Marsh habitat	21	5-6	11.62 ± 1.78	MRCA <i>D. sanguinea</i> - <i>D. woodii</i> clade
Scrub habitat	1	1	1.75 ± 1.00	<i>D. crassicornis</i>
Mature Heath habitat	37	8-10	16.55 ± 1.83	MRCA ( <i>Repandra</i> , <i>Reticulibractea</i> )
Postfire habitat	30	7	18.05 ± 1.75	MRCA <i>Disella</i>
Streambank habitat	9	6	14.25 ± 1.86	<i>D. elegans</i>
Epilythic habitat	25	18-20	16.14 ± 2.27	MRCA <i>Phlebidia</i>
Southeast Cloud Zone habitat	18	17	16.14 ± 2.27	<i>D. longicornu</i>

Note: age of first occupation = age of the MRCA of a clade that optimises significantly to the attribute or, in case of biomes and rainfall zones, age of a species restricted to the attribute and in the case of habitat, age of a species occurring in it, whichever is earlier.

### *Optimisation for rainfall seasonality*

The generic node could be significantly optimised to Winter rainfall (proportional likelihood = 0.998), and it is also significantly not Summer rainfall (proportional likelihood = 0.001), but an All Year Rainfall origin, although unlikely could not be confidently rejected (proportional likelihood = 0.440, not significant) (Fig. 1).

Thirty taxa have extended their rainfall seasonality pattern from Winter to All Year, while another six species have dispersed from Winter to All Year rain. The node subtending *D. cornuta* can be significantly optimised to Winter rainfall. Thus this species extended its range from Winter into the All Year, and then further into the Summer rainfall zone. *D. tripetaloides*, the only other species that covers three rainfall zones, extended its range from All Year zone to both Summer and Winter rainfall zone. Only one other event from All Year to Winter rainfall can be documented and this concerns *D. reticulata*, although dispersal from the Summer Rainfall zone remains a possibility (proportional likelihood 0.813, not significant). The node subtending *D. baurii* optimises significantly to the Winter rainfall area, and thus this species dispersed most likely directly from the Winter to the Summer rainfall zone, even though an All Year rainfall origin cannot be excluded (proportional likelihood = 0.572, not significant). The MRCA of clade “z” does not optimise significantly to any area, but the parent node of this MRCA is significantly Winter rainfall. This leaves two scenarios: a direct dispersal of the MRCA from Winter to Summer rainfall and a return of section *Spirales*, or two independent dispersals. The node subtending the MRCA of the *D. cephalotes* clade of section *Stenocarpa* cannot be optimised significantly to any zone. This is most likely due to the fact that two species were not sampled. *D. porrecta*, morphologically the sister species to *D. ferruginea* (Linder 1981a), occurs in the All Year and Summer rainfall areas, while *D. arida*, most closely related to *D. gladioliflora* (Linder 1981a) is restricted to the All Year rainfall zones. Inclusion of these taxa in the tree would optimise the MRCA of section *Stenocarpa* to “All Year” (data not shown), which would indicate that the *D. cephalotes* clade of section *Stenocarpa* possibly used the All Year rainfall zone as a stepping stone towards the Summer rainfall areas. Three more events can also not be reconstructed. The MRCA of the clades (*D. sagittalis*, *D. zimbabweensis*), (*D. borbonica*, (*D. reticulata*, *D. brevicornis*)) and (*D. ferruginea*, *D. gladioliflora*) cannot be significantly assigned to any area. From the Summer rainfall zone, we can

document three range extensions into the All Year rainfall zone (*D. polygonoides*, *D. chrysostachya* and *D. aconitoides* ssp. *aconitoides*). Six species extended their ranges (*D. perplexa*, *D. hircicornis*, *D. ochrostachya*, *D. erubescens* ssp. *erubescens*, *D. aconitoides* ssp. *goetzeana*), while one (*D. stairsii*) dispersed from the Summer to the Bimodal rainfall zone.

A strong directionality from the Winter to the All year rainfall zone can be observed. Dispersal and particularly range extension from Winter rainfall area into the All Year rainfall zone to the east has occurred many times, and seems to be an easy process. The All Year rainfall zone seems to be a sink area rather than a source area and very few species have dispersed or extended their ranges from this zone. Direct dispersal from Winter to the Summer rainfall area is not common, but has happened at least twice and possibly more.

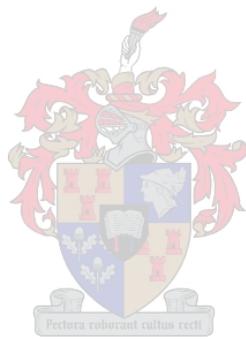
The age of first occupation of each rainfall zone is given in Table 3. The MRCA of the genus *Disa* evolved 28.5 Mya in the Winter Rainfall Zone. The MRCA of the clade (*Aconitoideae*, *Micranthae*) was present in the Summer Rainfall zone 15.39 Mya. Occupation of the All Year rainfall zone can be dated back to 11.48 Mya and the Bimodal Rainfall zone was first occupied 5.29 Mya by *D. stairsii*.

The significance test showed that the taxa occurring in Winter, All Year and Summer rainfall taxa are not randomly distributed across the tree, but are constrained by phylogeny. While this seems obvious for the distribution of taxa in the Winter and Summer rainfall zones, it seems less so for the All Year taxa. This could have been due to the presence of one large Summer rainfall clade which distorted the test. To investigate this further (and also to get more clarity of the state of generic node), we pruned all taxa occurring in the Summer and Bimodal rainfall areas from the tree and repeated the significance test. In this case the distribution of All Year rainfall taxa is not significantly different from random, while the distribution of those in the Winter Rainfall area remains significantly different from random. In this reduced tree, the proportional likelihood of the generic node with the 2-rate parameter model is 0.866 (not significant) for Winter rainfall and 0.552 (not significant) for All Year, while the 1-rate parameter model optimises the root to significantly Winter rainfall (0.999) and ambiguous (0.5) for All Year rainfall.

### *Optimisation for habitat*

The MRCA of the genus does not optimise significantly to any of the defined habitats. In fact, in most cases it optimises significantly to “not” the habitat (Fig. 2), except in three cases, where there is doubt. The likelihood ratio for the generic nodes is slightly in favour of a Epilythic habitat (0.541), but a Postfire habitat (0.460) or a Southeast Cloud Zone habitat (0.190) cannot be excluded.

Although 18 taxa occur in the Southeast Cloud Zone habitat, the significance test indicates that this attribute is randomly distributed along the phylogenetic tree. Nine taxa are typically associated with Streambanks. Two clades, both part of section *Disa*, could be significantly optimised for this character namely (*D. uniflora*, *D. caulescens*) and ((*D. tripetaloides*, *D. cardinalis*), *D. aurata*) (Fig. 2). The significance test indicates that this character is not randomly distributed, but is constrained by the phylogeny. Twenty five taxa are adapted to the Epilythic habitat. Although all taxa of four clades i.e. (*D. tenuifolia*, *D. filicornis*), (*D. vaginata*, *D. glandulosa*), (*Coryphaea*, *Vaginaria*), and section *Phlebidia* all share this habitat, only the MRCA of *Phlebidia* and *Coryphaea* could be significantly optimised to it (0.881 and 0.897 respectively) (Fig. 2). Although the proportional likelihood values for the others are high (0.803, 0.870, 0.730 respectively), they are not significant. The significance test indicates that this character is not randomly distributed. Thirty taxa occur in a Postfire habitat. Two large clades could be significantly optimised for this habitat (Fig. 2). The MRCA of sections (*Disella*, *Monadenia*) has a likelihood ratio value of 0.950 of being Postfire adapted, while section *Disa* with the exclusion of (*D. uniflora*, *D. caulescens*) has a ratio of 0.903 of having been Postfire adapted. The significance test also indicates that this character is not randomly distributed. Thirty seven taxa prefer to grow in a mature Heath habitat. The MRCA of four clades can be significantly optimised to Heath (Fig. 2). These are the MRCA of (*Reticulibractea*, (*D. cornuta*, *D. hallackii*)); (*Trichochila*, *Stenocarpa*); *Spirales* and *Schizodium*. *D. ovalifolia*, the sister species to section *Schizodium* is also a Heath adapted species, yet their MRCA cannot be significantly optimised to Heath (0.735). A similar situation is seen for the two clades (*Reticulibractea*, (*D. cornuta*, *D. hallackii*)) and (*Trichochila*, *Stenocarpa*). These are also sister clades and although their MRCA has a high proportional likelihood of being Heath (0.861), the values is not significant. It is interesting to note





that although many species occupy this habitat, the generic node is significantly not-Heath. The significance test again indicates that this character is not randomly distributed. Twenty one taxa are Marsh adapted. This character can be optimised to a small and a large clade (Fig. 2), namely (*D. atricapilla*, *D. bivalvata*) and section *Micranthae* excluding the *D. galpinii* clade. The attribute is not randomly distributed according to the significance test. Scrub habitat is represented once. Only *D. crassicornis* sometimes occurs in this habitat. Fifty three taxa prefer Grassland. This attribute can be optimised to three clades (Fig. 2): the MRCA of the *D. stricta* - *D. amoena* clade, (*Micranthae*, *Aconitoideae*) and *Emarginatae*. The latter two are closely related and together with *Spirales* form clade “z”, which has a high proportional likelihood (0.816), which, however, is not significant. The MRCA of (*D. borbonica*, (*D. brevicornis*, *D. reticulata*)) also has a high, but not significant, proportional likelihood (0.880) of being Grassland. The significance test again indicates that this character is not randomly distributed. Four species grow in Woodland. Two of them (*D. walleri*, *D. robusta*) are sister species and their MRCA optimises significantly to this habitat (Fig. 2). The attribute is not randomly distributed according to the significance test. Eight taxa are adapted to Subalpine environment. These taxa are scattered over the phylogenetic tree and no nodes can be optimised. This attribute is randomly distributed over the tree. The age of first occupation of each of the habitats is given in Table 3.

## Discussion

### *Reliability of data and methods*

We did not take phylogenetic uncertainty directly into account in these analyses. We used the tree with the highest likelihood score from a Bayesian inference analysis as a representative of the phylogenetic hypothesis and for calculating an ultrametric tree. However, since the phylogenetic hypothesis contains some poorly supported nodes (Bytebier et al. in press), care needs to be taken in the interpretation of events around these nodes. In particular, the current position of section *Spirales* in the single tree leads to a number of interpretations which do not seem to be parsimonious.

Although in recent years improved methods have become available (Renner 2005; Rutschmann 2006), molecular dating remains vulnerable to errors from several

sources (Bell and Donoghue 2005). First of all, dating requires a robust phylogenetic hypothesis. Most likely, ours fulfills this requirement as it is based on both nuclear and chloroplast data, which is devoid of incongruence and returned a well-supported topology (Bytebier et al. in press). It also requires adequate sampling, as undersampling may negatively affect node age estimation (Linder et al. 2005b). We sampled 70 % of the genus (Bytebier et al. in press) and used a relaxed Bayesian clock method, which introduces less distortion compared to other methods (Linder et al. 2005b). A major choice of error can be calibration points (Heads 2005). No reliable fossils are known for the Orchidaceae (Schmid and Schmid 1977). The family has been estimated to be 69 My old based on dating of all angiosperms (Wikström et al. 2001) and 111 My old based on dating of the monocots only (Janssen and Bremer 2004). Orchidaceae consist of five subfamilies (Cameron et al. 1999; Chase et al. 2003; Freudenstein et al. 2004) and Orchidoideae, the subfamily to which *Disa* belongs, is sister to the largest and most derived subfamily, Epidendroideae. Using the age of the family as a secondary date would thus result in considerable overestimation of age. Therefore, we chose to use a separate calculation of the age of the genus (see Galley et al., accepted), well aware that the use of secondary dates can result in accumulated error (Grauer and Martin 2005). As a second calibration point we used the age of the volcanic island of Réunion, which is estimated to be 2 My old (McDougall 1971). *Disa borbonica*, is endemic to Réunion and cannot be older than the island.

#### *Are ecological attributes conservative?*

Occurrence in a particular habitat is strongly determined by phylogeny. The MRCA of almost all phylogenetically delimited sections (Bytebier et al., in press) can be optimised to a particular habitat. Section *Phebidia*, *Vaginarina* and *Coryphaea* are lithophytic or grow in rocky soil; *Disella* and *Monadenia* flower in the first year after fire; *Reticulibractea*, *Trichochila*, *Stenocarpa*, *Spirales*, *Ovalifoliae* and *Schizodium* occur in mature heath vegetation, while *Aconitoideae* and *Micranthae* are grassland species. Section *Stenocarpa* can further be neatly divided into two clades, one which is fynbos adapted and the other which occurs in grassland. Almost the only exception is the (*Disa*, *Atromaculiferae*) clade, which is sister to the rest of the genus and which cannot be assigned to one particular habitat. The basal split within the genus seems to have resulted in one small clade that has occupied a variety of habitats and the rest of

the genus which progressively filled particular niches, with the extent of speciation depending on the ecological space available within the niche. For instance, section *Phlebidia* is mostly restricted to horizontal rock ledges. Due to the limited availability of this habitat, the potential for diversification was limited and thus speciation resulted in four species only. By comparison, adaptation to fire resulted in the opening of a spatially large niche and once this key innovation was acquired, occupation of the many subhabitats resulted in the major diversification of sections *Monadenia* and *Disella*. Similarly, adaptations to cope with a long period of drought such as hysteranthly allowed the *Trichochila* clade to diversify in the wide ecological space of the mature fynbos habitat. Of all the ecological attributes we tested, only two are not phylogenetically constrained: Southeast Cloud Zone and Subalpine habitats. The species occupying these habitats are not closely related.

Our data support the concept of niche conservatism (Harvey and Pagel 1991) or the hypotheses that beta-(or habitat) niches (Silvertown et al. 2006) evolve only slowly, which has been demonstrated in many (Peterson et al. 1999; Patterson and Givnish 2002; Ackerley 2003), but not all studies (Rice et al. 2003; Hardy and Linder 2006). This makes it possible to project habitat attributes to ancestral nodes. In addition, Svenning (2003) tested the climatic adaptability among cool-temperate tree genera by comparing congeneric values between North America and Europe or Asia and also found these to be strongly conservative. It allowed him to predict the present European status (extinct, relictual or widespread) of genera present during the Pliocene from their modern requirements.

#### *In which biome did Disa evolve?*

Our optimisations show fynbos to be the ancestral biome for *Disa*. This means that the fynbos biome is at least 28.5 My old. Although the age of the fynbos biome is not known, this is not contradicted by the fossil data. Cape floral elements belonging to Restionaceae, Proteaceae and Ericaceae are already present in the Arnot Pipe deposits of Banke, which are confidently dated back to between 64 and 71 Mya (Scholtz 1985). It is possible though that these three families could then have formed part of a forest vegetation. These three families are also present in the lignite deposits of the Knysna area and although the dating of these deposits has been problematical, estimates of their age vary from as old as the Oligocene to as young as the Miocene

(Thiergart et al. 1963; Coetzee et al. 1983; Thwaites and Jacobs 1987). However, biomes are composites, and although there are good indications from the fossil record that these can be stable for millions of years (DiMichele et al. 2004), we do not know whether the same set of biomes existed in the past. Thus, it might be more revealing to analyse the evolution of particular habitat attributes rather than whole biomes.

#### *Age of grassland and savanna biome*

Grasslands in southern Africa are at least 17.9 My old, as the MRCA of the oldest clade to have occupied the grassland biome (clade "z") dates back to  $17.88 \pm 1.81$  Mya. Grasslands are of relatively recent origin. Although the fossil record for Poaceae dates back to the Paleocene, grasslands only became more extensive from the middle Miocene, about 15 Mya (Retallack 1992; Jacobs et al. 1999; Stromberg 2002; Jacobs 2004; Linder and Rudall 2005). Our inferred date of first occupation is thus consistent with the timing of grassland expansion. Furthermore, it seems that *Disa* occupied the grassland biome almost as soon as it became available. This is not surprising, since ground orchids in general are almost pre-adapted to this type of habitat. The grassland lacks competing shrubs and trees and furthermore orchids, because of their tubers, would be able to survive the regular fires that shaped and maintain this vegetation. Occupation of the grassland biome was followed relatively quickly by dispersal to the savanna biome (15.4 Mya), which also began to expand around the middle Miocene (Retallack 1992; Jacobs 2004). The fact that many species co-occur in both biomes would indicate that the transition from grassland to savanna was easily accomplished.

#### *Age of the subalpine habitat*

The occupation of the subalpine habitat appears to be Plio-Pleistocene in age. Species occupying this habitat (with the exception of *D. fragrans* and *D. sankeyi*) are not closely related to each other, and all of them have occupied this habitat recently. The first occupation in East Africa by *D. stairsii* dates back to 5.8 Mya, while the first occupation in the Drakensberg (*D. fragrans*) is 4.9 Mya, both around the Miocene/Pliocene boundary. Most of the Drakensberg subalpine species are, however, of Pleistocene origin. Southern Africa experienced two major periods of uplift, the first around 20 Mya and the second around 5 to 3 Mya (Partridge et al. 1995; McCarthy & Rubidge 2005). Both uplifts were most pronounced in the eastern part of southern Africa and the second one in particular is estimated to have uplifted the

Drakensberg area by 900 m, and as a result created the subalpine habitat. In eastern Africa, this habitat is also of recent origin and was created mostly as a result of rifting and volcanic activity during the Pliocene/Pleistocene (Partridge et al. 1995). The lack of diversification of subalpine lineages of the *Disa*s reflects the relative recency of the habitat. This is consistent with the observation that two of the Drakensberg subalpine taxa (*D. oreophila* ssp. *erecta* and *D. cephalotes* ssp. *frigida*) are recognised only at the subspecific level.

#### *Age of winter rainfall*

The onset of the winter-rainfall climate was hypothesised to be the single most important factor for vegetation change on the subcontinent and possibly the trigger for the radiation of the Cape flora (Levyns 1964; Axelrod and Raven 1978; Linder et al. 1992; Goldblatt 1997; Richardson et al. 2001). This event was associated with the intensification of the Benguela cold water upwelling in the late Miocene between 10 and 14 Mya (Siesser 1978; Tankard and Rogers 1978; Siesser 1980) and was thought to have led to increased aridification of the Cape and to the inception, during the Pliocene, of a Mediterranean type climate with most rainfall concentrated in winter (Axelrod and Raven 1978).

However, evidence is accumulating that the unfolding of events was somewhat different. As more and more of the Cape clades are dated, their origins do not point to a single point in time, but are staggered over a long period (e.g. *Heliophila*: late Pliocene (Mummenhoff et al. 2005); Ruschiodeae: late Pliocene-early Miocene (Klak et al. 2004); *Indigofera*: middle Miocene (Schrire et al. 2003); Irideae and Ixioideae: late Miocene (Goldblatt et al. 2002), *Muraltia*: late Oligocene (Forest et al. in press); *Pelargonium*: mid Oligocene (Bakker et al. 2005); Restionaceae: middle to the late Eocene (Linder and Hardy 2004)). Thus, it seems more likely that the richness of the CFR flora was not triggered by a single event, but has been accumulating over the entire Cenozoic (Linder, 2005).

We optimised the MRCA of the genus *Disa* to be a winter rainfall-adapted species. Consequently, since we also inferred the origin of the genus to be 28.5 Mya, this would suggest that the winter rainfall regime dates back to at least the Oligocene.

Linder (2003) critically reviewed all fossil evidence and concluded that "there is (...) no direct evidence to suggest the establishment of the winter-rainfall regime at the Miocene-Pliocene boundary". Furthermore, the heightened productivity under the Benguela current, which was associated with an upwelling of cold water and the onset of aridity and a seasonal climate on the west coast of southern Africa (Siesser, 1980), is mirrored by similar increases in other oceans (Diester-Haass et al., 2002) and is now suggested to be part of global response to paleoceanographic changes rather than the result of an upwelling of cold water. It thus remains to be established when the onset of winter rainfall climate in the southwestern Cape was initiated. Our results would suggest that the CFR was experiencing a winter rainfall regime in the Oligocene already, although our rainfall seasonality optimisation is not completely conclusive on this matter and it might be difficult to distinguish all-year rain from winter rain.

#### *Age of summer drought and fire*

The distinction between all-year rain (e.g. rain in winter) and winter rain (e.g. dry summers) might thus be critical. All year rainfall would not lead to long periods of drought, whereas winter rainfall would lead to a more seasonal climate with a long, dry period that would be conducive to veld fires. The appearance of fire as part of the ecosystem might thus provide us with a convincing test to choose between the alternative rainfall scenarios. There is no fossil evidence as to how long fire has been part of the Cape ecosystem. Research in Australia suggests that fire was largely absent prior to the Miocene (Herring, 1985; Kershaw et al., 2002; Linder, 2003; Hill, 2004). Our results would suggest that fire was part of the CFR ecosystem from at least the early Miocene, since the MRCA of two clades, namely section *Disella* and *Monadenia*, can be optimised to a postfire habitat and date back to 18 Mya or the early Miocene. This would strengthen our argument that the winter rainfall regime of the CFR is substantially older than is generally accepted.

#### **Conclusion**

The dating on the first occurrence of *Disa* in the alpine, grassland and savanna habitat is congruent with independently-obtained and reliable paleoclimatological data. Indeed, here the fit of events is quite remarkable. The remaining optimisations are not

in conflict with independent data, largely because there is none. Here it provides new insights, namely that the Cape flora could have radiated in a mountainous region that largely had a modern climate, possibly with fluctuations over time. This suggests that the current high diversity might be more the result of a low level of extinction rather than of recent, rapid radiation.

### **Acknowledgments**

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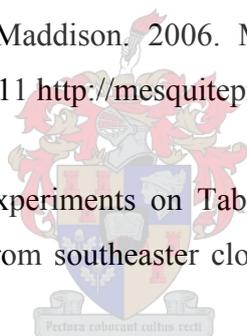
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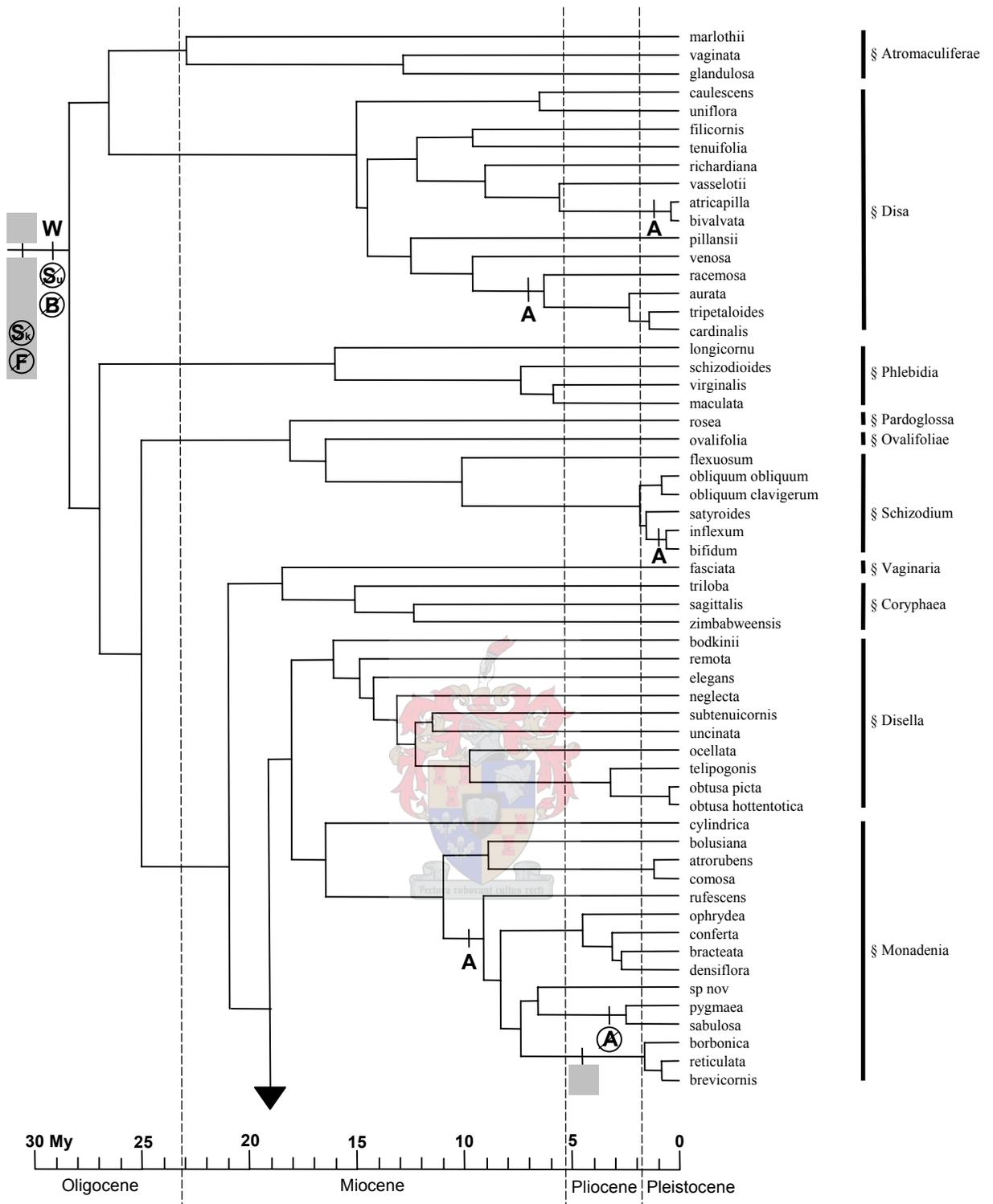


Fig. 1: Significant optimisations for Biome and Rainfall Season. F=Fynbos; Fo=Forest; G=Grassland; Sa= Savanna; Sk=Succulent Karoo; A=All Year Rainfall; B=Bimodal Rainfall; Su=Summer rainfall; W=Winter Rainfall. Biomes are shaded in grey; symbols in circle indicate that the optimisation was significantly not the indicated biome or rainfall attribute.

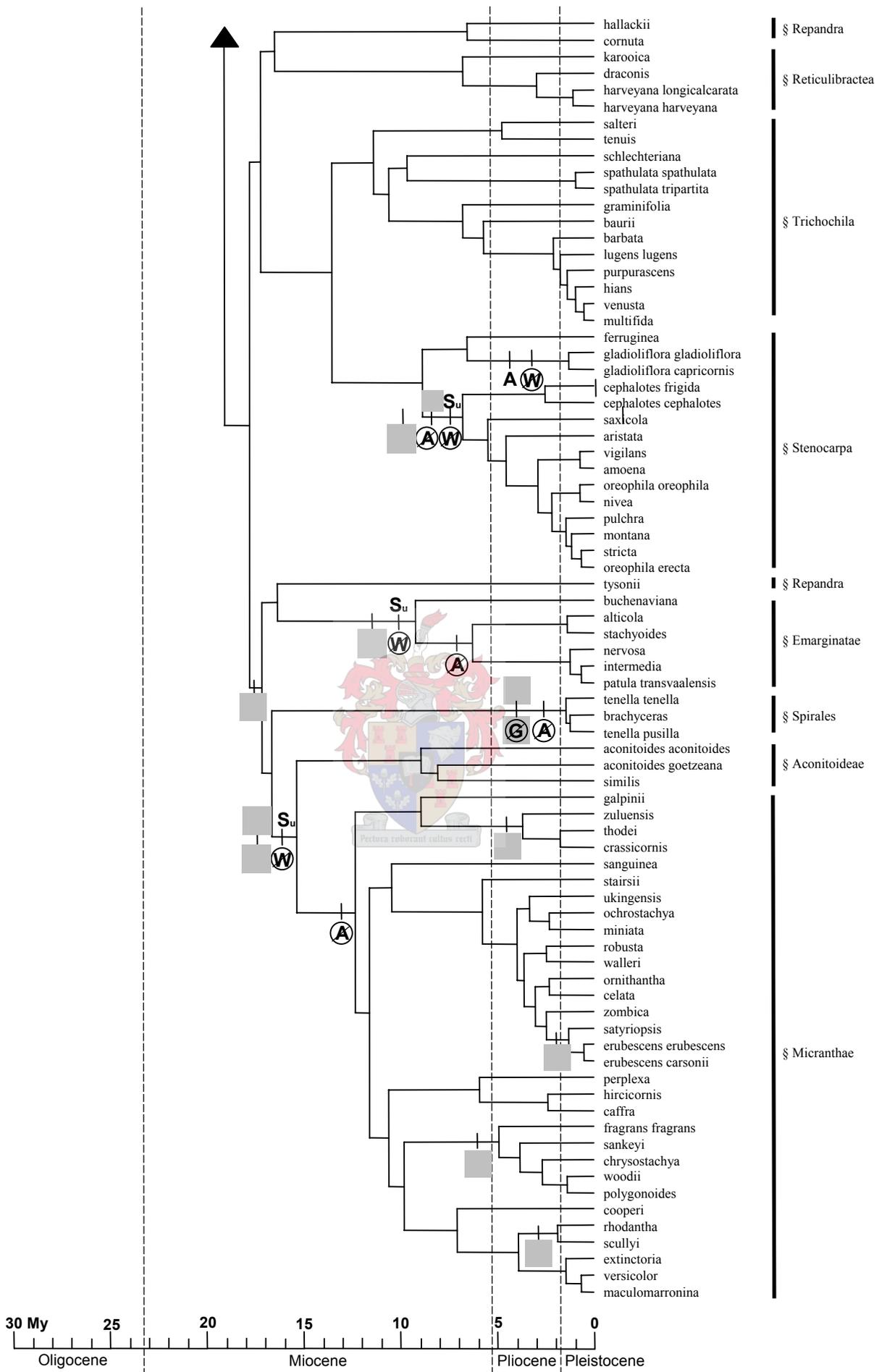


Fig. 1 (continued)

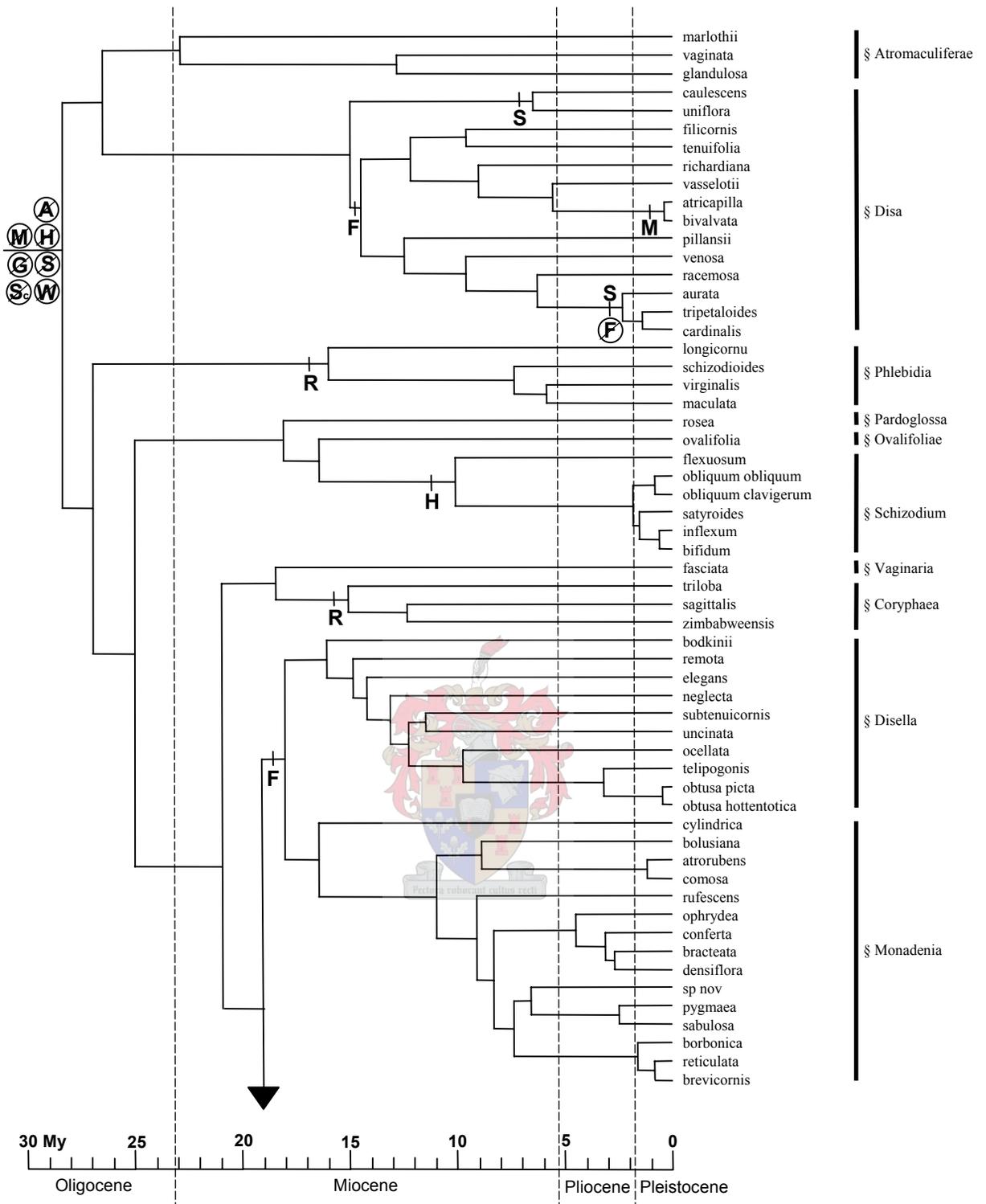


Fig. 2: Significant optimisations for Habitat. A=Subalpine; C=Southeast Clouds; F=Postfire; G=Grassland; H=Heath; M=Marsh; R=Epilythic; S=Streamside; Sc=Scrub; W=Woodland. Symbols in circle indicate that the optimisation was significantly not the indicated habitat attribute.

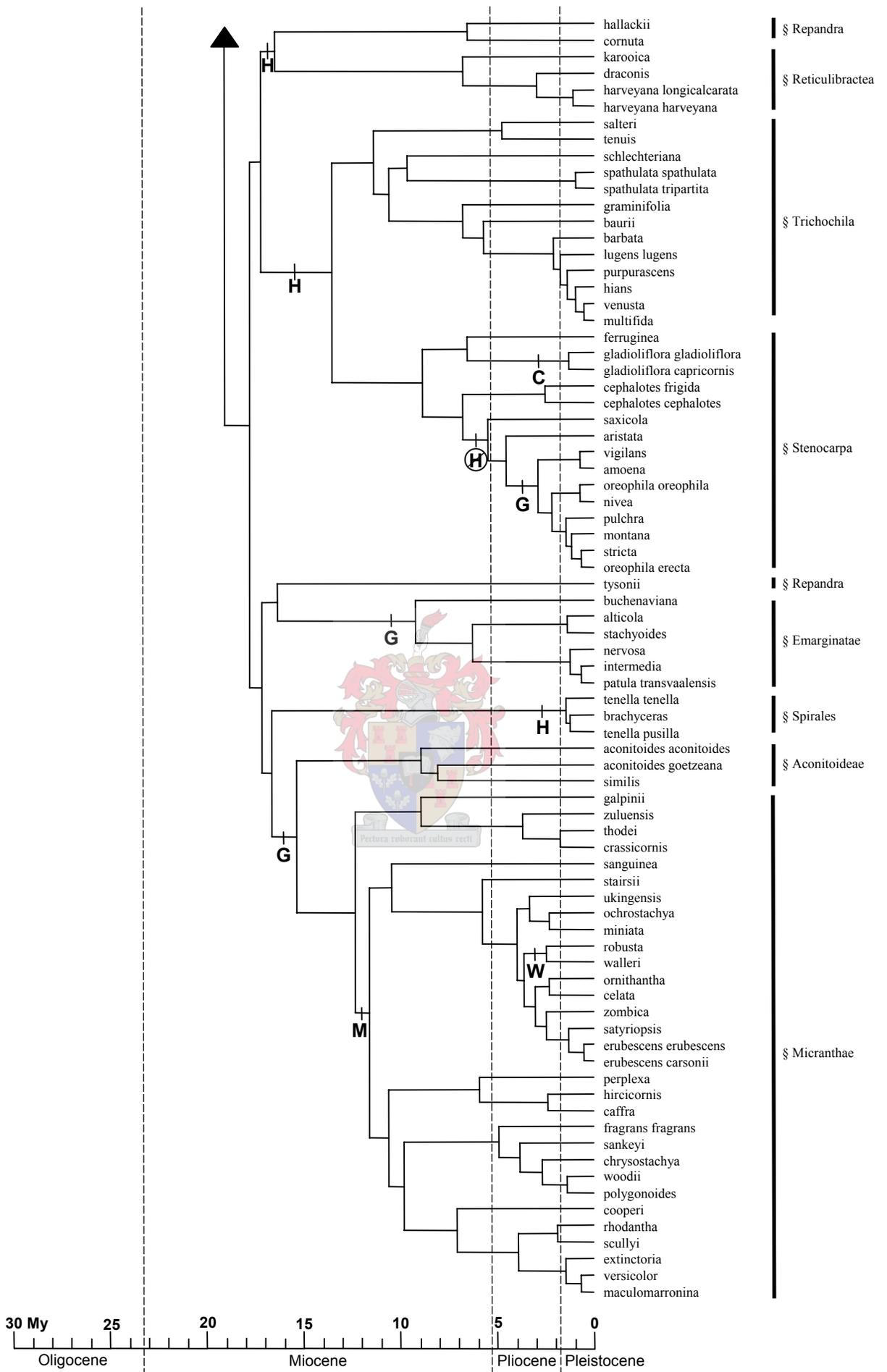


Fig. 2 (continued)