Systematic studies of the South African Campanulaceae *sensu stricto* with an emphasis on generic delimitations

Christopher Nelson Cupido

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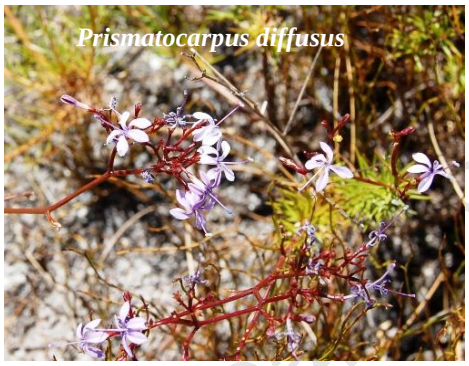

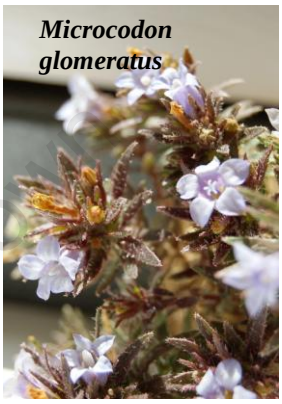

Representatives of Campanulaceae diversity in South Africa

Dedicated to Ursula, Denroy, Danielle and my parents

DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Christopher N Cupido Cape Town, September 2009

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ABSTRACT

The South African Campanulaceae *sensu stricto*, comprising 10 genera, represent the most diverse lineage of the family in the southern hemisphere. In this study two phylogenies are reconstructed using parsimony and Bayesian methods. A family-level phylogeny was estimated to test the monophyly and time of divergence of the South African lineage. This analysis, based on a published ITS phylogeny and an additional ten South African taxa, showed a strongly supported South African clade sister to the campanuloids. Assessment of divergence times using a secondary calibration point suggests that this clade started to diversify during the Oligocene (28 mya), which coincided with global climatic changes from hot wet to cold dry conditions. A phylogenetic analysis of the South African lineage was undertaken based on morphological and DNA sequence data from the chloroplast *trn*L-F and the nuclear ITS regions. These data sets were analyzed separately and in combination. The phylogenetic hypothesis was used to re-assess the questionable generic boundaries in the family. The ITS data produced poor resolution under parsimony and poor support under Bayesian methods. The resulting phylogenies show five species assemblages that contradict traditional generic circumscriptions, which have primarily been based on the mode of capsule dehiscence. The date estimated for the South African clade was used as calibration point to estimate the age of the clades revealed by the molecular data. Radiation of the Campanulaceae in southern Africa seems to correlate with dramatic climatic and topographical changes such as aridification and continental uplift on the subcontinent that started during the Oligocene. The phylogenetic hypothesis was also used to trace the evolution of nine characters considered important in the circumscription of genera. An uncontradicted synapomorphy was found for the *Rhigiophyllum*-*Siphocodon* clade. The fruit character was found to be taxonomically unreliable at the generic level.

The phylogeny of the South African clade was further used to focus on the closely related genera, *Roella*, *Merciera* and *Prismatocarpus* – a group forming a well supported clade in most analyses. The total evidence analysis was used to evaluate the status of each of these genera. Several options were explored to translate the phylogeny into a classification. This process was guided by the primary criterion of monophyly followed by stability in nomenclature, strong statistical support for the taxon, maximum phylogenetic information and ease of identification of the taxon. The results favour retaining of *Roella*, *Prismatocarpus* and *Merciera* as separate genera. A synopsis of these three is provided.

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CHAPTER 1

GENERAL INTRODUCTION

The Campanulaceae *sensu stricto* (the bellflower family) provide an excellent opportunity to explore the philosophy and practice of the four concerns of modern systematics: diversity, phylogeny, biogeography and classification (Cracraft 2002). Its distribution is nearly cosmopolitan (Figure 1.1), taxonomic treatments of the family, including generic circumscriptions and intrafamilial classification, vary largely according to author and often lack agreement (Table 1.1), and phylogenetic work has only recently been attempted (Eddie *et al.* 2003, Cosner *et al.* 2004).

The taxonomic history of the Campanulaceae reflects the lack of consensus on its taxonomy since it was first erected. The debate concerning the family circumscription seems to have been largely settled, but major disagreement still exists regarding generic circumscriptions. This is discussed in the next sections with particular emphasis on developments in South Africa. To this end this thesis focuses mainly on re-evaluating the generic circumscriptions in the South African members of the family and it is envisaged that a robust phylogenetic framework will stimulate further systematic research in the family.

1.1. Campanulaceae

The Campanulaceae are classified in the asterid order Asterales, which includes among other families the Asteraceae, Stylidaceae, Goodeniaceae and Menyanthaceae (APG 2003, Bremer *et al.* 2003). Relationships among the families of the Asterales are unclear (APG 2003), but the monophyly of the order is strongly supported.

Fifty five to 60 genera and about 950 species (Takhtajan 1997) of annuals, perennial herbs, and shrubs are recognized worldwide. The family is characterized by the presence of latex and predominantly epigynous flowers with actinomorphic,

sympetalous corollas (Morin 1983). The fruit is a capsule or, very rarely, a berry. This narrow circumscription results in a family that is more homogenous and possibly monophyletic (Kovanda 1978, Lammers 1992), as opposed to a broader circumscription including the lobeliad and cyphiad members.

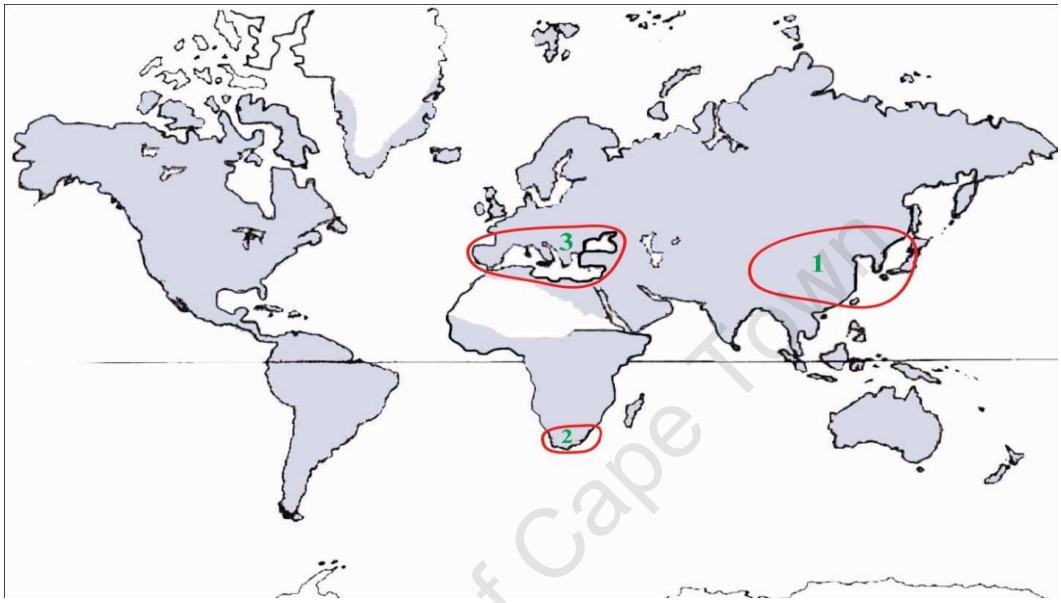


Figure 1.1. Global distribution of the Campanulaceae (modified from Kovanda 1978) and centres of diversity after Hong (1995). 1= Eastern Asiatic Region, 2= Cape Region, 3= Mediterranean Region.

1.2. Systematics of Campanulaceae

1.2.1. Family circumscription

The broad circumscription of the Campanulaceae Juss. sensu Schönland (1889) and Cronquist (1981) has always been disputed. Most disagreements concern the designation of taxonomic rank to the campanulad and lobeliad members of the family. The campanulads are characterized by actinomorphic flowers and free anthers, and the lobeliads by zygomorphic flowers and fused anthers. A group with zygomorphic flowers and free anthers, the cyphiads, is considered intermediate to the campanulads

and lobeliads. Traditionally, three taxa have been recognized and Bentham (1876) followed the classification of Sonder (1865) recognizing the three taxa as tribes of the Campanulaceae: Lobelieae, Cyphieae, and Campanuleae. Dahlgren (1980, 1983), De Candolle (1830), Fedorov (1972), Kovanda (1978), Lammers (1992), and Takhtajan (1987) preferred to recognize them as families, whereas Cronquist (1988), Schönland (1889), Thorne (1992), Wagenitz (1964), and Wimmer (1968) relegated the families to subfamilial rank. New evidence from morphology and *rbc*L DNA sequence data have shown that the cyphiads as traditionally circumscribed are not monophyletic. They comprise three morphologically and geographically distinct groups: *Cyphia* P.J.Bergius in tropical and southern Africa; *Cyphocarpus* Miers in northern Chile; and *Nemacladus* Nuttal, *Parishella* A.Gray, and *Pseudonemacladus* McVaugh in western North America. This contributed to the current recognition of five taxa: Campanuloideae, Cyphioideae, Lobelioideae, Cyphocarpoideae, and Nemacladiodeae (Lammers 1998, 2007 a, b). Authors such as Gustaffsson and Bremer (1995) and Takhtajan (1997) recognize these taxa as families: Campanulaceae, Cyphiaceae, Lobeliaceae, Cyphocarpaceae, and Nemacladaceae. The Angiosperm Phylogeny Group is undecided on the family circumscription, but provided the option of recognizing subfamilies or families (APG 2003).

1.2.2. Classification of the Campanulaceae

The first groupings within Campanulaceae *sensu stricto* (Table 1.1) were proposed by De Candolle in1830 who divided the family into two subtribes, the Campanuleae and the Wahlenbergeae, based on the mode of capsule dehiscence. A few years later in 1839 he divided the family into three tribes, segregating *Merciera* A.DC. into the tribe Mercieae on the basis of its unique ovary structure. In Schönland's 1889 treatment of the Campanulaceae *sensu lato,* the Campanuloideae were separated into three tribes, Campanuleae, Sphenocleae, and Pentaphragmeae. Sphenocleae and Pentaphragmeae are now treated as the family Sphenocleaceae and Pentaphragmataceae, respectively (Kovanda 1978, Cronquist 1981, Takhtajan1997). Both these monogeneric families lack the invaginating stylar hairs typical of the Campanulaceae. *Sphenoclea* Gaertner,

is a genus of two species, one pantropical and the other one occurring in West Africa. *Pentaphragma* Wallich ex G.Don comprises about 30 species endemic to south-eastern Asia, the Malay Archipelago (excluding Java and Nusa Tenggara) and New Guinea (Lammers 1992). The remaining tribe Campanuleae is equivalent to the Campanulaceae in the strict sense.

Schönland (1889) subdivided the Campanuleae into 3 subtribes, the Campanulinae, the Wahlenberginae, and the Platycodinae, using differences in fruit dehiscence and morphology of the calyx as a basis. Since Schönland, various authors have proposed classifications for the Campanulaceae. Kovanda (1978) subdivided the family into 3 subtribes, the Campanulinae, the Wahlenberginae, and the Platycodinae, ignoring the rank of tribe. In response to the anomaly of recognizing subtribes but not tribes, Yeo (1993) elevated the subtribe Platycodoninae of Schönland, which has never before been treated as a tribe, to the tribe Platycodoneae. Kolakovsky (1987, 1994) recognized 4 subfamilies and 18 tribes: Prismatocarpoideae, Canarinoideae, Wahlenbergioideae, and Campanuloideae. In his classification, the South African genera are classified in the Prismatocarpoideae (*Prismatocarpus*, *Roella*, *Craterocapsa*, *Treichelia*) and the Wahlenbergioideae (*Wahlenbergia*, *Theilera*, *Microcodon*) whilst *Siphocodon*, *Merciera*, and *Rhigiophyllum* are omitted from his classification. In the most recent classification, Takhtajan (1997) followed Kolakovsky, recognizing 4 subfamilies, but only differing in the number of tribes: Cyanthoideae, Ostrowkioideae, Canarinoideae, and Campanuloideae. The southern African genera are classified in the Campanuloideae in his treatment.

Table 1.1. Classification of the Campanulaceae. Only genera sampled in this study are included.

De Candolle 1830	De Candolle 1839	Schönland 1889	Kovanda 1978	Kolakovsky 1987 and 1994	Takhtajan 1997
Subtribus I (Wahlenbergeae) *Microcodon* *Prismatocarpus* *Roella* *Lightfootia* *Wahlenbergia* **Subtribus II (Campanuleae)** **incertae sedis** *Merciera*	**Wahlenbergieae** *Lightfootia* *Microcodon* *Wahlenbergia* *Prismatocarpus* *Roella* **Campanuleae** **Mercieae** *Merciera*	**-Lobelioideae** **-Cyphioideae** **-Campanuloideae** **--Pentaphragmeae** **--Sphenocleae** **--Campanuleae** **Campanulinae** **Wahlenberginae** *Lightfootia* *Merciera* *Prismatocarpus* *Rhigiophyllum* *Roella* *Siphocodon* *Treichelia* *Wahlenbergia* **Platycodinae** *Microcodon*	**Campanulinae** **Wahlenberginae** *Wahlenbergia* *Roella* *Lightfootia* **Platycodinae**	**-Prismatocarpoideae** *Craterocapsa* *Prismatocarpus* *Roella* *Treichelia* **-Canarinoideae** **-Wahlenbergioideae** **--Wahlenbergieae** *Microcodon* *Theilera* *Wahlenbergia* **--Azorineae** **--Musschieae** **--Echinocodoneae** **--Annaeae** **--Muehlbergelleae** **--Theodorovieae** **--Gadellieae** **--Ostrowskieae** **-Campanuloideae** **--Campanuleae** **--Phyteumateae** **--Peracarpeae** **--Sergieae** **--Michauxieae** **--Neocodoneae** **--Edraiantheae** **--Sachokieleae** **--Mzymteleae**	**-Cyanthoideae** **--Cyanantheae** **--Codonopsideae** **--Platycodoneae** **-Ostrowkioideae** **-Canarinoideae** **-Campanuloideae** **--Wahlenbergieae** *Wahlenbergia* (including *Lightfootia* nom. illeg.) *Theilera* *Microcodon* **--Azorineae** **--Musschieae** **--Echinocodoneae** **--Campanuleae** **--Peracarpeae** **--Michauxieae** **--Phyteumateae** **--Edraiantheae** **--Jasioneae** **--Prismatocarpeae** *Prismatocarpus* *Roella* *Craterocapsa* *Treichelia* **--Siphocodoneae** *Siphocodon* *Rhigiophyllum* **--Mercieae** *Merciera*

1.3. Campanulaceae in southern Africa

The floristic region referred to as southern Africa (Botswana, Lesotho, Namibia, South Africa and Swaziland) (Figure 1.2) occupies an area of approximately 2 674 000 km^2 in which about 20 400 plant species are found (Goldblatt 1997). Within South Africa most Campanulaceae species are concentrated in the Western Cape Province, particularly an area referred to as the Cape Floristic Region (CFR). The CFR stretches from the Bokkeveld escarpment in the north to Port Elizabeth in the east, covering an area of about 90 000 km^2, less than 5% of the total area of the southern African subcontinent (Goldblatt 1997). An estimated 9030 species occur in the CFR, which amounts to 44% of the species found in southern Africa.

Figure 1.2. The southern African subcontinent showing the Cape Floristic Region (after Goldblatt 1978).

In southern Africa approximately 250 species (Wellman and Cupido 2003), assigned to 12 genera, belong to the Campanulaceae (Table 1.2). Of these 12 genera, 8 are endemic to South Africa and one to Namibia, whilst the remaining three occur in other countries within or outside the southern African region. Although the family is otherwise poorly represented in the Southern Hemisphere, South Africa shows great

diversity with 10 genera. *Wahlenbergia* Schrad. ex Roth (including *Lightfootia* L'Hér.), the largest and most widely distributed of the South African genera, consists of 170 species (Cupido and Conrad 1999) that occur in the south-western Cape, KwaZulu-Natal, Eastern Cape, Mpumalanga and Limpopo. This genus is mixed, containing annuals, perennial herbs and sometimes shrubs. The 30 species of small shrubs, perennial herb and two annual species that belong to the genus *Prismatocarpus* L'Hér. occur in the south-western Cape and Eastern Cape. *Roella* L. is a genus of small shrubs and herbs that are found mainly in the south-western Cape, and one of the 24 known species extends into the Eastern Cape and KwaZulu-Natal. The genus *Microcodon* A.DC. is small and is found only in the south-western Cape. It consists of four species, all of which are annuals. *Merciera* A.DC. is a genus of six species that is also restricted to the south-western Cape. All species are perennials and look very similar to *Roella ciliata*. *Craterocapsa* Hilliard and Burtt is the only genus in South Africa that has no members in the southwestern Cape. It occurs in KwaZulu-Natal, Eastern Cape, Free State, Northern Province, and Gauteng Province, and consists of five species of perennial herbs. The plants grow prostrate and are often mat-forming. *Siphocodon* Turcz. is a genus of only two species restricted to the southwestern Cape. These slender wiry perennials are often entangled with itself and with other plants. *Rhigiophyllum* Hochst. consists of one species that is found only in the southwestern Cape. This rigid, erect shrublet is easily recognised by its egg-shaped leaves, densely arranged on the stems and by the deep blue flowers that are borne in terminal heads. Like *Rhigiophyllum*, *Treichelia* Vakte is a monotypic genus from the south-western Cape. These dwarf coarse herbs bear their flowers in dense terminal heads with long

narrow bracts in between the flowers. *Theilera* Phillips comprises two species that occur in the south-western Cape as well as in the Eastern Cape. They are erect shrublets with slender branches and are found mainly inland. Hong (1995) described South Africa as one of three centers of diversity of Campanulaceae (Figure 1.1). Five of the eight South African endemic wahlenbergioid genera (*Treichelia, Siphocodon, Rhigiophyllum, Microcodon and Merciera*) are endemic to the Cape Floristic Region (Goldblatt 1978). In addition to the high number of endemic genera, 63% of the world's *Wahlenbergia* species occur in South Africa. Many species in the family have great horticultural potential, but only a few species of *Wahlenbergia* are presently in cultivation.

Table 1.2. Genera and number of species occurring in each of the southern African countries.

Country	**Genus**	**Number of species**
Botswana	*Gunillaea*	1
	Wahlenbergia	5
Lesotho	*Wahlenbergia*	18
	Craterocapsa	2
Namibia	*Gunillaea*	1
	Wahlenbergia	15
	Namacodon	1
South Africa		
	Wahlenbergia (including *Lightfootia*)	170
	Microcodon	4
	Roella	24
	Theilera	2
	Prismatocarpus	30
	Treichelia	1
	Siphocodon	2
	Rhigiophyllum	1
	Merciera	6
	Craterocapsa	5
Swaziland	*Wahlenbergia*	11
	Craterocapsa	1

1.3.1. Taxonomic history of the South African Campanulaceae

The earliest family treatment for South Africa was published by Buek (1837) who described several new species, based on the collections of Christian Ecklon and Karl Zeyher, in all six genera known at the time. One species erroneously assigned to *Merciera*, has since been transferred to the Rubiaceae (Sonder 1865).

Sonder, in Flora Capensis (1865), wrote the most comprehensive account to date of the South African Campanulaceae. Apart from describing new species and providing the first keys to *Lightfootia* and *Wahlenbergia*, he also erected a new genus *Leptocodon* to accommodate a species of *Microcodon* described by Buek (1837). Unfortunately the generic name was illegitimate having been published after *Leptocodon* (Hook.f.) Lemaire. Sonder (1865) also considered *Rhigiophyllum* a doubtful genus of the Campanulaceae.

In the recent work by Goldblatt and Manning (2000), only species from the winter rainfall area were considered and several taxonomic changes were proposed. The most significant was the transfer of the monotypic genus *Theilera* to *Wahlenbergia.* Wellman and Cupido (2003) expanded the work of Goldblatt and Manning, providing an updated annotated checklist for the family of southern Africa. Not all taxonomic changes proposed by Goldblatt and Manning (2000) were accepted in this treatment.

Various other authors have published species level treatments or described new taxa. Vatke (1874) erected the genus *Treichelia* for a *Microcodon* species described by Buek (1837), which Sonder (1865) transferred to the illegitimate *Leptocodon.* Adamson (1950) placed *W. depressa* Wolley-Dod, a later homonym of *W. depressa* Wood and Evans, in synonymy under *Treichelia*.

Towards the end of the nineteenth century Schlechter (1897) added a new species to the monotypic genus *Siphocodon*, which was erected by Turczaninow (1852). More than a decade later, von Brehmer (1915) revised *Wahlenbergia* for almost the entire

African continent. He provided subgeneric classifications, and keys to all species, of *Lightfootia* and *Wahlenbergia*. More than 50% of the species accepted by von Brehmer were based on single collections, casting doubt on the validity of many of them (Thulin 1975).

Adamson revived Campanulaceae research in South African by publishing accounts on *Roella* and *Prismatocarpus* in 1952, *Merciera* in 1954 and *Lightfootia* in 1955a. *Lightfootia* has since been placed in *Wahlenbergia* for nomenclatural and taxonomic reasons (Lammers 1995). Since the major accounts of Adamson, Hilliard and Burtt (1973) described a new genus *Craterocapsa* with four species, two previously assigned to *Wahlenbergia* and one to *Roella*, and one new species. More recently, new species were added to *Craterocapsa* and the monotypic genus *Theilera* by Hong (2002). *Theilera* was established by Phillips (1927) to accommodate *Wahlenbergia guthriei* L.Bolus, a species with an unusually long, tubular corolla. In the same year, Cupido (2002) described a new species of *Merciera* and later published a synopsis of the genus (Cupido 2006). Recently the *Theilera* species described by Hong (2002) was renamed by Cupido (2009).

1.3.2. Generic delimitation

Genera are erected when novel plants that do not fit comfortably into existing genera are discovered, or as segregates from larger genera. In the latter category, the most noteworthy examples from South African Campanulaceae are *Theilera*, *Microcodon* and *Craterocapsa* p.p. from *Wahlenbergia*, *Treichelia* from *Microcodon* and *Merciera* from *Trachelium* and *Roella*. Ultimately species of many genera can be traced back to *Campanula*. Despite the removal of small genera the monophyly of the larger genera such as *Wahlenbergia* remains questionable. The criteria used to establish segregate genera are not always explicit. In the Campanulaceae, genera have often been proposed because of the exaggerated importance attached to a single character, and maintained because of tradition (McVaugh 1945).

The diversity in capsule structure, and particularly the mode of dehiscence (Figure 1.3), has been used to separate genera in the Campanulaceae (Hilliard and Burtt 1973; Thulin 1975). This character is not always homogenous within the existing South African genera. For example, Hilliard and Burtt (1973) showed that not all capsules of *Roella* species dehisce by an apical hole as stated by Adamson (1952); in a few species the dehiscence takes place by vertical splits as seen characteristically in *Prismatocarpus*.

Prismatocarpus schinzianus Markgraf was transferred to a new genus *Namacodon* (Thulin 1974) because it differs from *Prismatocarpus* in its unique mode of scepticidal dehiscence, 3-locular ovary and pollen grains released in tetrads. Similarly, Thulin erected the genus *Gunillea* for certain former species of *Prismatocarpus* and *Wahlenbergia* having indehiscent capsules that open slowly by irregular decomposition of the pericarp and that have hair-like projections on the testa.

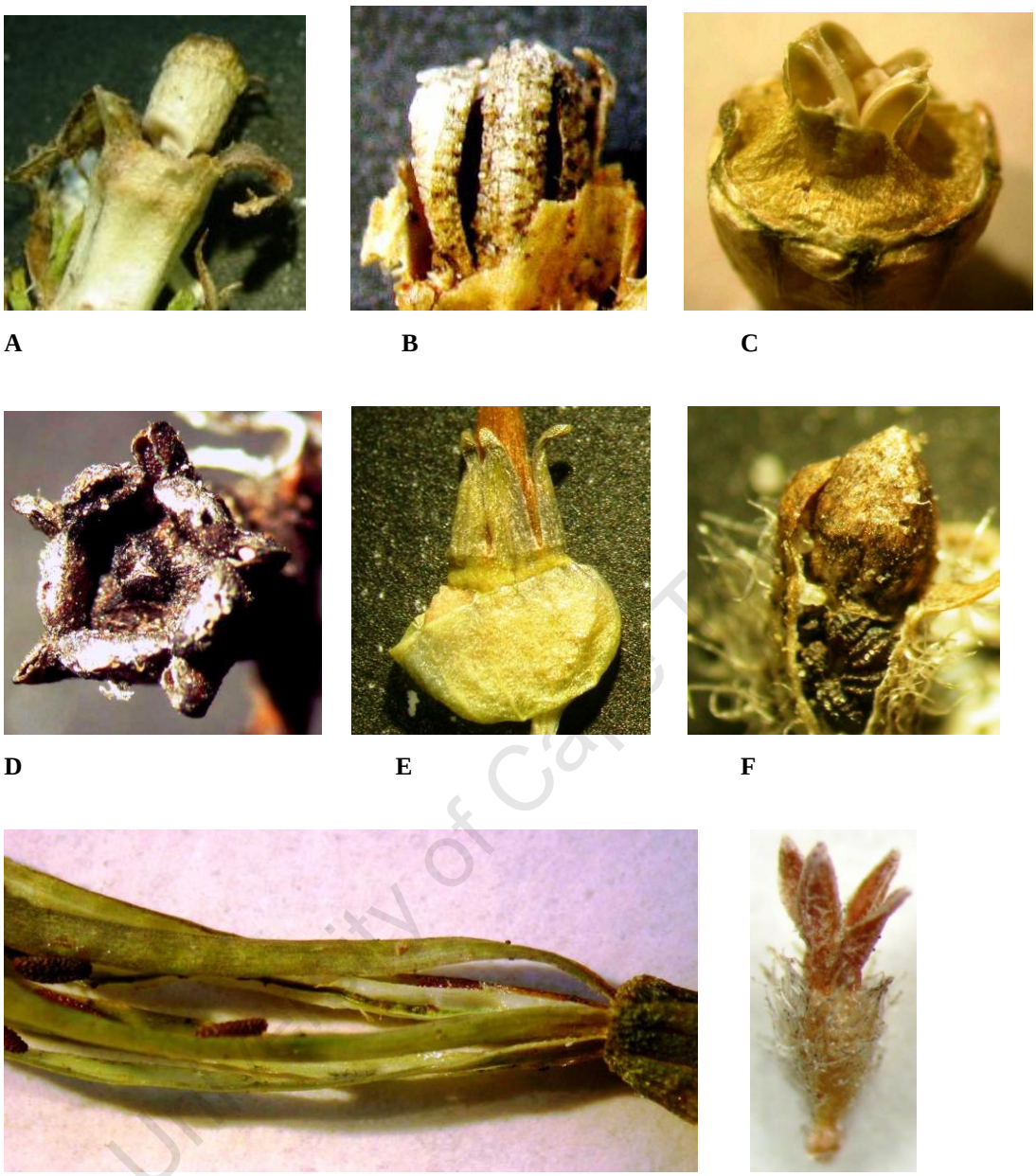
The genus *Theilera* is questionably distinct from *Wahlenbergia* (Thulin, 1975), mainly differing in its long cylindrical corolla tube. Marloth (1932) reported that the capsules dehisce by an apical orifice, whereas from Thulin's observations it opens by apical valves as in *Wahlenbergia*. Phillips (1927), who erected *Theilera,* gave no reasons for doing so. He may have attached great importance to the cylindrical corolla tube, which was unique in the *Wahlenbergia* from which it was segregated. The case of *Treichelia* is similar. Schönland (1889) stated that the capsule dehisces by a lid. In contrast, Adamson (1950) stated that the dehiscence takes place by slits between the ribs of the capsule.

Craterocapsa (Hilliard and Burtt 1973) was erected to accommodate species of *Wahlenbergia* and *Roella* in which the capsule dehisces via an apical operculum. With the exception of *Craterocapsa insizwae*, the ovary is consistently 3-locular. *C. insizwae* includes the 2-locular *Roella insizwae* Zahlbruckner, considered a doubtful species by Adamson (1952) due to the unavailability of sufficient study material, and

the 3-locular *Wahlenbergia ovalis* v. Brehm. The inclusion of *W. ovalis* in *Craterocapsa* was done with ‘only slight doubt’ (Hilliard and Burtt 1973).

In the course of fieldwork and routine plant identifications, it became apparent that it is not always possible to assign certain taxa to any of the currently known genera. A recent example is a *Microcodon*-like plant, collected in Malmesbury north of Cape Town and on Lion's Head in the Cape Peninsula, which has still not been identified. Thus, at the practical taxonomic level, the boundaries of some genera are questionable.

Apart from a few studies that could be described as merely incidental-for example Phillips’s (1927) treatment of *Theilera* and a few intuitive remarks by some taxonomists, no study has ever attempted to re-assess generic circumscriptions in South African Campanulaceae. Schönland's review, which is more than a 100 years old, remains the standard reference for the family in the region. More study material is currently available for the family and more localities known, albeit in a time of massive habitat destruction. Lowland species, some of which have high horticultural potential, are particularly under threat of extinction even before their biology is adequately understood. A convincing and robust generic framework for the South African representatives is crucial to resolve the numerous alpha taxonomic problems that exist in the family as well as for making informed conservation decisions.



A **B** **C**

D **E** **F**

G **H**

Figure 1.3. Representatives of modes of capsule dehiscence displayed in South African Campanulaceae. A; *Roella ciliata*, Cupido 103 (apical plug), B; *Roella spicata*, Barker 5289 (longitudinal slits, not corresponding with calyx lobes), C; *Wahlenbergia capensis*, Cupido 184 (apical valves), D; *Wahlenbergia acaulis*, Cupido 267 (protruding calyx lobes), E; *Siphocodon spartioides*, Cupido 133 (circumsessile), F; *Treichelia longibracteata*, Cupido 199 (operculum), G; *Prismatocarpus fruticosus* Cupido 127 (longitudinal slits, corresponding with calyx lobes), H, *Merciera tetraloba*, Cupido 117 (indehiscent). Mode of dehiscence is indicated in brackets.

1.3.3. Phylogenetic relationships within the Campanulaceae

Phylogenetic studies in the Campanulaceae have only recently been undertaken. Most notable molecular phylogenies of the Campanulaceae are those of Cosner *et al*. (1994), Eddie *et al.* (2003), Haberle *et al*. (in press) and Roquet *et al*. (2008, 2009). None of these included many South African taxa. No morphological phylogenetic studies of the Campanulaceae have ever been published.

Eddie *et al*. (2003) found congruence between their ITS phylogeny and De Candolle's (1830) classification of Campanulaceae. Although under-sampled for the wahlenbergioid genera (only three samples), strong support for the sister relationship between *Roella* and *Craterocapsa* was found. This provides corroboration for Hilliard and Burtt's (1973) suggestion that these two genera are closely related. Furthermore, the classification of Kolakovsky (1987, 1994), who placed *Roella* and *Craterocapsa* in the subfamily Prismatocarpoideae, and that of Takhtajan (1997), who placed these two genera in the tribe Prismatocarpeae of the subfamily Campanuloideae, is also upheld by the ITS phylogeny. The third wahlenbergioid taxon, the European *Wahlenbergia hederacea*, grouped with the campanuloid genera although it is considered to be typically wahlenbergioid. This is contrary to the classification of Kolakovsky (1987, 1994) and Takhtajan (1997) who placed *Wahlenbergia* in the tribe Wahlenbergieae, which does not form part of the campanuloid group of Eddie *et al*. (2003). The placement of *W. hederacea* in the ITS phylogeny raises questions around the monophyly of the South African Campanulaceae as well the wahlenbergioids. This issue needs to be addressed.

1.3.4. Molecular dating in the Campanulaceae

The few molecular phylogenies for the Campanulaceae published so far have essentially been used to resolve relationships among taxa and to address taxonomic questions. The development of numerous statistical methods that can be used in conjunction with trees make the investigation into the historical patterns of evolution

of taxa possible (Barroclough and Nee 2001). One of the methods used to assess historical patterns of evolution includes the estimations of divergence time. Thus far, published age estimates for the Campanulaceae have focused almost exclusively on *Campanula* (Park *et al*. 2006, Cellinese 2009, Roquet *et al*. 2009). With the purpose of reconstructing the first phylogeny for the South African Campanulaceae, the question of phylogenetic dating to provide a historical context for the South African taxa comes into focus and is subsequently explored in this study.

1.4. Aims of this study

Modern systematists strive to erect natural classifications, reflecting the evolutionary history of organisms. Phylogeny reconstruction provides a framework of relationships among organisms on which a natural classification system can be based. Morphological and DNA sequence data have been widely used in many studies to reconstruct phylogenies. DNA sequence data have the advantage over morphological data that they provide a large number of characters for each taxon. Discrete character states can also be unambiguously scored in most cases, which is not always the situation with morphology.

This study will reconstruct the phylogenetic history of the South African genera using morphological and DNA sequence data. The resulting phylogenetic hypothesis will be employed to:

- Test the monophyly of the South African Campanulaceae and estimate the time of divergence of this clade from the rest of the Campanulaceae
- Address the questionable generic boundaries in the South African genera
- Review the proposed subfamilial classification for the family
- Provide a context for investigating the evolution of reproductive and vegetative character variation in the South African members of the family and their biogeographical origin.

Chapter 2 incorporates ITS sequences obtained from this study into the matrix of a published ITS phylogeny to test the monophyly and estimate the time of divergence of the South African Campanulaceae. The core of the present study is set out in Chapters 3 and 4, which present, respectively, molecular and morphological phylogenetic analyses of representatives of the South African Campanulaceae. The emphasis of these two chapters is on re-assessing the generic circumscriptions in light of the criterion of monophyly. Evidence for subfamilial classification is also evaluated as well as the value of the fruit and other characters for delimiting genera and their evolution within the family. Chapter 5 (Generic status of *Roella* L., *Prismatocarpus* L'Hér. and *Merciera* A.DC.) uses phylogenetic information from Chapters 3 and 4 to evaluate in more detail the status of each of these genera. Chapter 6 provides a taxonomic account based on the findings in Chapter 5. Chapter 7 presents a summary and conclusions of the findings of this study.

CHAPTER 2

SOUTH AFRICAN CAMPANULACEAE: A TEST OF MONOPHYLY AND AN ESTIMATE OF DIVERGENCE TIME

2.1. Introduction

The concept of monophyly applies to a group of taxa that includes a most recent common ancestor plus all and only its descendents (Kitching *et al*. 1998). Such a monophyletic group is defined by synapomorphies. According to Davis (1999) monophyly can be viewed from two distinct perspectives, cladistic relationship and phylogenetic relationship. In the case of cladistic relationship monophyly is determined by the placement of taxa on a cladogram. Monophyly in terms of phylogenetic relationship implies hypotheses about past events (history). Therefore defining a group of taxa as monophyletic is expressing a hypothesis of common ancestry. In practice cladistic structure is used as the basis of hypotheses of evolutionary relationships.

The order Asterales to which the Campanulaceae belongs is a well-supported monophyletic group characterized by the presence of inulin and secondary pollen presentation mechanisms. It comprises the Campanulaceae, Asteraceae and about ten other small families (Bremer *et al*. 2003). Within the Asterales, the clade forming the Campanulaceae comprises five taxa treated as separate families or subfamilies (See Chapter 1). Knowledge of a putative taxon's closest relatives is important for an effective test of monophyly. Within the Campanulaceae the lobeliad and cyphiad groups are closely related to the campanulad group (Cosner *et al*. 1994, 2004). It is therefore easy to select taxa as outgroups for phylogenetic studies in any of these groups. However, the relationships within the campanulad group are for the most part unclear, with very few phylogenetic studies undertaken so far and a lack of agreement as to what constitutes a genus in the group (Eddie *et al*. 2003). With the ultimate aim of re-assessing generic limits in the South African Campanulaceae it is important to establish whether this group of taxa is monophyletic or not. This will provide a

launching pad from which a detailed study on generic limits within southern Africa can be undertaken.

The Campanulaceae s.str. are concentrated in three distribution centers; the eastern Asiatic region, the Cape region, and the Mediterranean region (Hong 1995). Hong did not consider the western North American region in his study even though this region is marked on one of his maps. The eastern Asiatic region contains the genus *Cyananthu*s, which, with its superior, 5- loculed ovary and low chromosome base number, has been regarded by some authors (e.g. Hutchinson 1969, Carolin 1978, Cronquist 1988) as the most primitive in the family. Consequently this area has been proposed as the region of origin of Campanulaceae, with the other two regions considered secondary differentiation centers (Hong 1995). Establishing the number of independent diversification centers for the family is important for testing evolutionary hypotheses on such factors as the tempo and mode of evolution. A phylogenetic reconstruction of the family with representatives of the various distribution centers will provide an effective test of the monophyly of South African Campanulaceae, otherwise referred to as the Campanulaceae of the Cape region (Hong 1995).

Not many phylogenetic hypotheses have been proposed for the family. Of the few molecular phylogenetic studies attempted to date, the ITS phylogeny of Eddie *et al*. (2003) remains the most comprehensive. It included 93 taxa, representing 32 of about 55 genera from across a broad geographical range. Although this study provides a basis for understanding the overall relationships within the Campanulaceae, only two South African taxa were sampled. Therefore, an expanded sampling of this major lineage will contribute to a better understanding of relationships and geographical patterns within the family.

2.1.1. The ITS gene region

The internal transcribed spacer (ITS) region is situated between the 18S and 26S subunits of the 18S-26S nuclear ribosomal RNA cistron. The ITS region (Figure 2.1) comprises three components, the 5.8S subunit and two spacers ITS-1 and ITS-2. ITS-1 is found between 18S and 5.8S and ITS-2 between 5.8S and 26S. In flowering plants the ITS region is generally under 700 bp long (ITS-1: 187-298 bp, 5.8S: 163 or 164 bp, ITS-2: 187-252 bp) (Baldwin *et al*. 1995).

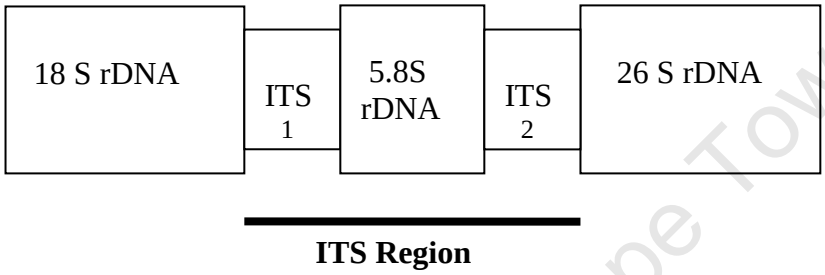


Figure 2.1. Repeat unit of 18-26S nuclear ribosomal DNA (after Baldwin 1992)

Sequence data obtained from nuclear ribosomal DNA, such as ITS, have proven to be useful in phylogenetic reconstruction (Suh *et al*. 1993), but is unfortunately not without problems. Amongst these is that in certain taxonomic groups the rDNA ITS region is not easily amplified and sequenced (Hershkovitz *et al*. 1999), most probably due to folding into helices or more complex structures of portions of ITS1 and ITS2 (Conn and Draper 1998). This folding of the DNA strands might impede polymerization steps during amplification and sequencing. Kimball and Crawford (2004) also pointed out that variation in the length of individual ITS regions and the presence of numerous indels can cause alignment difficulties. A consequence of alignment and sequencing problems is that homoplasy is increased, and this may be particularly evident when ITS is used as the molecular marker (Alvarez and Wendel 2003). A further concern is the presence of ITS polymorphism within a genome (Buckler *et al*. 1997). Through processes of unequal crossing over and/or gene conversion, concerted evolution is expected to homogenise the repeats. However if concerted evolution fails, non-homologous copies, which may represent different

evolutionary histories, may be present in a taxon. The presence of non-functional copies (pseudogenes) that may have evolved independently and at a different rate than the functional genes may also represent different evolutionary histories. Phylogenetic analysis of such divergent sequences may result in misinterpretation of phylogenetic patterns.

Despite these potential problems ITS has been widely used in a large number of studies (e.g. Kim and Jansen 1994, Campbell *et al*. 1997, Baldwin and Sanderson 1998, Barker *et al*. 2002, Hendrichs *et al*. 2004, Hidalgo *et al*. 2004, Kellermann *et al*. 2005, Martins and Hellwig 2005, Roalson 2005, Yukawa *et al*. 2005, Levin *et al*. 2006) as the preferred phylogenetic marker from the nuclear genome and its overall use as a molecular marker in plant systematics has overtaken *rbc*L (Hershkovitz *et al*. 1999, Bailey *et al*. 2003). ITS appears to be valuable for assessing relationships at lower taxonomic levels such as between genera or species, because the spacer regions often evolve more rapidly than coding regions (Suh *et al*. 1993). This property of ITS makes it suitable for assessing the monophyly of the South African Campanulaceae. Prior to the work of Eddie *et al*. (2003), ITS sequencing data proved useful for assessing relationships within and between genera of Campanulaceae (Ge *et al*. 1997; Kim *et al*. 1999), and between the families Campanulaceae, Cyphiaceae, Nemacladaceae, Cyphocarpaceae, and Lobeliaceae (Haberle 1998). More recently, ITS sequence data were used to assess phylogenetic and biogeographical relationships in *Campanula* (Park *et al*. 2006, Roquet *et al*. 2008). The availability of the extensive ITS phylogeny of Eddie *et al*. (2003) presents an ideal opportunity to use this region to test the monophyly of the South African Campanulaceae, and to estimate its time of divergence from the rest of the Campanulaceae.

2.1.2. Molecular dating of phylogenetic trees

The use of DNA sequences to estimate the timing of divergence events is based on the idea that the amount of difference between DNA sequences of two taxa is a function of the time since their evolutionary separation (Rutschmann 2006). If it is assumed that

nucleotide substitutions among taxa occur randomly over time, molecular distances reconstructed onto the phylogeny are expected to be proportional to the time elapsed (Hillis *et al*. 1996). However, variation in evolutionary rates among lineages is common in plants (Gaut 1998, Doyle and Gaut 2000), and we cannot assume a strict molecular clock in most cases. A variety of methods have been proposed to accommodate rate variation: local clocks (Yoder and Yang 2000), non-parametric rate smoothing (NPRS; Sanderson 1997), penalized likelihood (Sanderson 2002), and the Bayesian relaxed clock (Thorne *et al*. 1998). Each of these methods has advantages and disadvantages, and often age estimates derived from different methods can be in conflict (Bell and Donoghue 2005, Rutschmann 2006).

Molecular dating is also subject to errors from incomplete species sampling (Linder *et al*. 2005) or the use of secondary calibration points to date nodes on trees (Heads 2005). Regardless of these shortcomings, dating is useful for studies in evolutionary biology and historical biogeography (Vinnersten and Bremmer 2001). In this study the history of the South African Campanulaceae is interpreted in the light of paleoclimatic and geological events to assess whether they were responsible for the radiation of the family in southern Africa.

2.1.3. The genus *Wahlenbergia*

Wahlenbergia, with about 260 species (Lammers 2007a & b), is the largest genus of Campanulaceae in the Southern Hemisphere and is most abundant in South Africa. Other areas with significant *Wahlenbergia* species numbers are Australia and New Zealand. Europe has low diversity and the genus is represented there by two species, *W. hederacea* (L.) Rchb. and *W. lobelioides* (L.f.) Schrad. ex Link. *W. hederacea*, the only European species sampled in this study, is found in Belgium, Germany, Spain, France, Ireland, Holland, Portugal and the United Kingdom where it grows in moist grassy places on acid soils, usually along streams. *W. lobelioides*, a variable species divided into three subspecies (Thulin 1975), occurs in Madeira, the Canary Islands, the Cape Verde Islands, the western Mediterranean area from Morocco to Italy, and in

Egypt, Sudan, Ethiopia and Socotra. It occupies sandy or rocky places, riverbanks, roadsides and cultivated land. The vegetative morphology of *W. hederacea* is unlike that of the other wahlenbergioids (Eddie *et al*. 2003), which in combination with its unique distribution casts doubt on its position in *Wahlenbergia.* A further application of the ITS phylogeny is therefore to evaluate the relationship between wahlenbergioid genera in South Africa and the single European representative, *Wahlenbergia hederacea.*

2.2. Materials and methods

2.2.1. Data sampling

The ITS data matrix of Eddie *et al*. (2003) comprising 97 taxa was obtained from the Internet (http://www.biosci.utexas.edu/IB/faculty/jansen/lab/personnel/eddie/its.htm). A shortcoming of this data set from the perspective of the present study is the undersampling of southern hemisphere wahlenbergioid taxa. In addition to the two South African representatives (*Roella ciliata* and *Craterocapsa congesta*) already in this matrix I have added a further ten taxa (*Wahlenbergia krebsii, W. capensis, W. subulata, W. procumbens, Microcodon glomeratus, Merciera eckloniana, Prismatocarpus crispus, P. diffusus, Theilera guthriei, Rhigiophyllum squarrosum*), bringing the final number of species to 107. Groups formed by a *trn*L-F analysis of the South African taxa (See Chapter 3) served as a guide for the selection of taxa for this data set. At least one taxon from each group recovered in that analysis is included with eight of the 10 South African genera represented. The outgroup taxa were the same as used in Eddie *et al*. (2003). This represents a subset of the complete ITS data comprising 174 taxa.

2.2.2. Molecular techniques

The methods employed for DNA extraction, amplification, sequencing and alignment are set out in Chapter 3.

2.2.3. Phylogenetic Analyses

2.2.3.1. **Maximum Parsimony analyses**

Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2003) with all characters treated as unordered with equal weighting (Fitch parsimony; Fitch 1971). A first run was performed using the heuristic search option and tree-bisection reconnection (TBR) branch-swapping with 1000 random addition replicates, saving five trees per replicate to minimize the time spent searching through large numbers of trees, steepest descent off and MULTREES in effect. Branches were collapsed if their maximum length equaled zero. All the trees obtained were then used as starting trees in a second analysis with same parameters as above, saving all optimal trees with a limit of 10 000 trees. Trees were rooted with outgroups comprising members of the Lobeliaceae.

Support for each clade retrieved by the analysis was assessed using bootstrap analyses (Felsenstein 1985). For the bootstrap analysis, a heuristic search with 1000 replicates, simple taxon addition and TBR branch-swapping was employed. Only bootstrap values over 50% are reported. Bootstrap values were interpreted as follows: 50-74 % weakly supported, 75-89% moderately supported, 90-100% strongly supported.

The consistency index (CI) (Kluge and Farris, 1969) was calculated to give an indication of the measure of fit between the data and the tree topologies. Values approaching one indicate a low level of homoplasy in the data set. The retention index (RI) (Farris, 1989), which measures the amount of similarity that can be interpreted as synapomorphy, was also calculated.

2.2.3.2. **Bayesian Analysis**

Bayesian analysis was performed using MrBayes 3.1 (Huelsenbeck and Ronquist 2001). The best model of DNA substitution for this data set was determined from a comparison of 56 models using the Akaike information criterion (Akaike 1974) as implemented in Modeltest (version 3.06; Posada and Crandall 1998). The general time

reversible (GTR) model of DNA substitution (Tavaré 1986) was chosen with among-site variation in rate heterogeneity approximated by a discrete gamma distribution (Yang 1993) with four rate classes. Five million generations were run with four independent chains (Markov chain Monte Carlo) and were sampled every hundred generations, resulting in an overall sampling of 50 000 trees.

Stationarity was established visually by plotting the negative log-likelihood (-LnL) values against generation time in Microsoft Excel to determine the burn-in period. All trees were transferred to PAUP* and trees visited prior to reaching stationarity were discarded. The remaining trees were used to generate a 50% majority-rule consensus tree with posterior probability values (PP- values) shown as percentages above the branches. PP-values of ≥95% are considered evidence of significant support for a group (Miller *et al*. 2004).

2.2.4. Dating of the South African lineage

The age of the most recent common ancestor of the South African lineage was estimated using Bayesian inference as implemented by the program BEAST (Drummond *et al*. 2002, Drummond and Rambaut 2006a). The date estimates were made under a general times reversible model of nucleotide substitution (Tavaré 1986) with a discrete gamma distribution model of evolution (Yang 1993) with four rate categories. The posterior distribution of the date being estimated was approximated by sampling parameter values every 1000th cycle over 25 000 000 MCMC steps, after discarding 2 500 000 burn-in steps. The molecular clock assumption was relaxed by allowing the rate to vary throughout the tree in an autocorrelated manner. A Yule prior on branching rates was employed, which assumes a constant speciation rate per lineage (Drummond *et al*. 2007). Convergence of the sampled parameters was checked using the program Tracer (Rambaut and Drummond 2004). This application evaluates posterior samples of continuous parameters from Bayesian MCMCs, and allows visual inspection of the chain behaviour, estimation of the effective sample size of parameters and the plotting of marginal posterior densities. The effective sample size is the

number of independent samples that would be the equivalent to the autocorrelated samples produced by the MCMC. This provides a measure of whether the chain has been run for an adequate length (for example, if the effective sample sizes of all continuous parameters are greater than 200) (Drummond *et al*. 2006b). The program TreeAnnotator (Rambaut and Drummond 2007) was used to summarise the information from a sample of trees produced by BEAST onto a single 'target' tree. The output file was then analysed in FigTree (Rambaut 2006).

Because the fossil record of Campanulaceae is poor (Muller 1981) no calibration point could be obtained for the group under investigation in this study. The tree was consequently calibrated using the ages calculated by Wikström *et al*. (2001) for the node linking *Campanula* with *Codonopsis*. Wikström *et al*. (2001) obtained an estimated age of 41 mya using ML, with a standard deviation of 3 mya. Accordingly, upper and lower bounds were set at 38 mya and 44 mya for the calibration point on the node that includes the most recent common ancestor of the Campanulaceae (ingroup). Monophyly was also enforced for the ingroup.

2.2.5. Estimation of per-lineage diversification rate

The per-lineage rate of diversification per million years for the South African clade was estimated as $(\ln N - \ln N_0)/T$ (Baldwin and Sanderson 1998), where initial diversity $N_0 = 1$, N is existing diversity and T is estimated clade age. The upper and lower HPD of age estimates were used as T.

2.3. Results

The reduced matrix used to test the monophyly of the South African Campanulaceae comprised 353 characters, of which 88 were constant, 61 variable but parsimony uninformative and 204 (58%) parsimony informative.

Across the range of taxa included, numerous insertion/deletion events are evident (see matrix 'ITS global-chapter 2'in Appendix A for details of indel positions). Overall the longest is a 17 basepair deletion (position 142 – 158 relative to the other taxa) in the outgroup species, *Lobelia tupa* and *L. tenera*. In contrast the ingroup has a longest deletion of 13 basepairs (position 171 – 183) which is shared by all *Jasione* species.

2.3.1. Maximum Parsimony

Under parsimony inference 10 000 equally parsimonious trees were retained of 470 steps, with a CI of 0.536 and a RI of 0.752.

In the strict consensus tree (Figure 2.2) the topology is poorly supported and the terminal nodes are poorly resolved. The platycodonoid taxon, *Leptocodon gracilis* resolved as sister to the rest of the Campanulaceae. The large Campanulaceae clade comprises four subclades, the largest of which is a campanuloid clade with no bootstrap support. An unsupported South African (wahlenbergioid) clade is sister to this large campanuloid clade. Sister to the combined clades is a strongly supported (100%) second campanuloid clade comprising *Githopsis diffusa* and *Heterocodon rariflorum*. A weakly supported clade (50%) comprises the remaining platycodonoid genera *Codonopsis*, *Platycodon*, *Campanumoea*, *Cyananthus* and *Canarina* is sister to the other three subclades.

Similar to the findings of Eddie *et al*. (2003), *W. hederacea* falls within the campanuloid group, the large clade sister to the South African group.

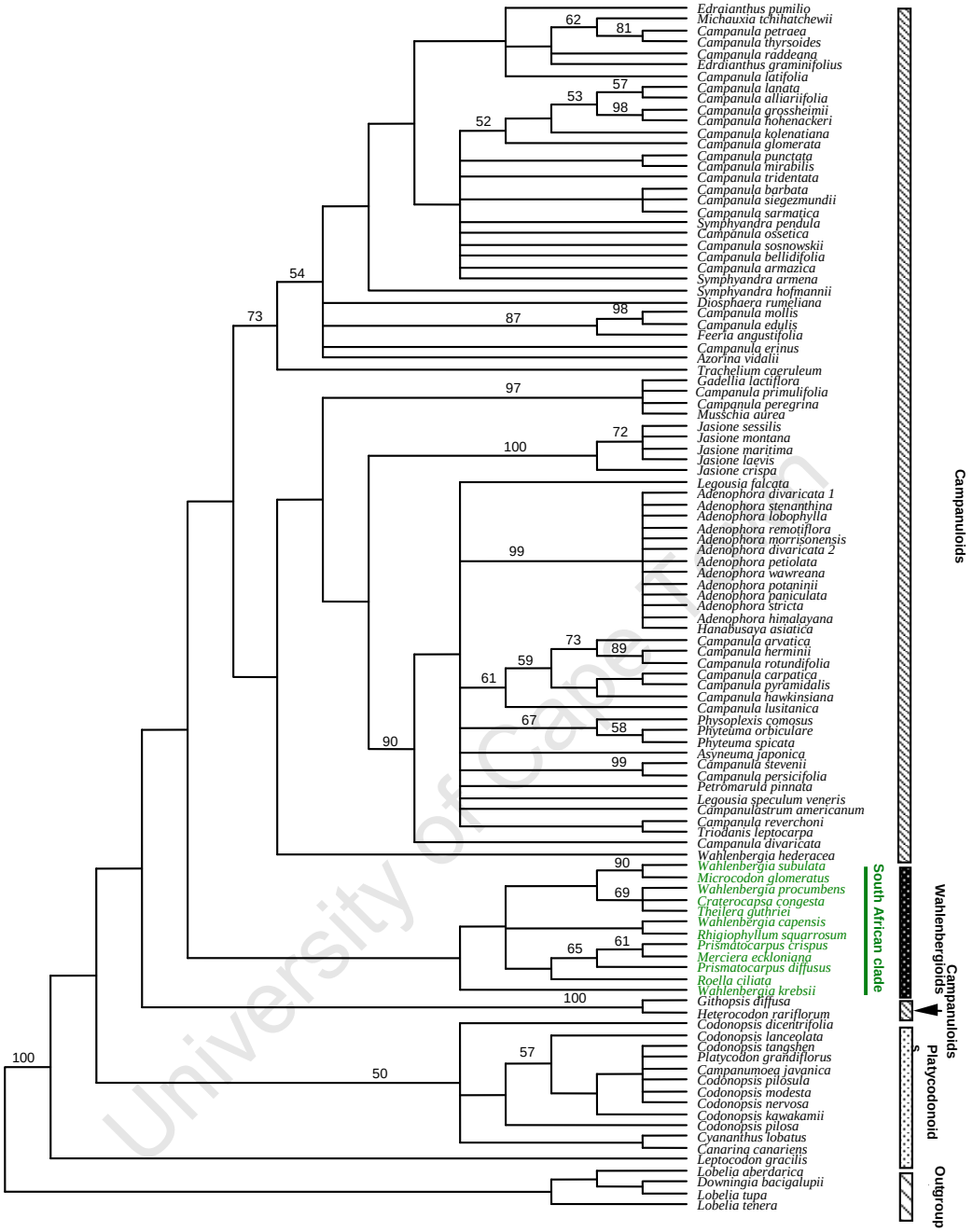


Figure 2.2. Strict consensus of 10 000 equally parsimonious trees (length=470, CI=0.536, RI=0.752) retained after heuristic search of the comprehensive ITS data set for 107 taxa of the Campanulaceae and four Lobeliaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

2.3.2. Bayesian Analysis

The likelihood scores of the Bayesian analysis reached stationarity after 58 640 generations. The burnin trees were discarded and the 50% majority rule consensus tree was then constructed from the trees obtained during the last 4 941 360 generations. The nucleotide substitution model parameter estimates, and their 95% credible intervals, are summarized in Table 2.1.

Table 2.1. Parameter values of the nucleotide substitution model as estimated from the Bayesian analysis of the ITS data set for 107 taxa of the Campanulaceae. TL= total tree length, r(A↔C), r(A↔G), etc.= the six reversible substitution rates, pi(A), pi(C),etc.= the four stationary nucleotide frequencies, alpha= the shape parameter of the gamma distribution of rate variation across sites.

	95 % Credible Intervals		
Parameter	Lower	Upper	Median
TL	9.525000	15.665000	12.475000
r(A↔C)	0.078382	0.127799	0.101086
r(A↔G)	0.131430	0.205282	0.165148
r(A↔T)	0.131497	0.204287	0.165385
r(C↔G)	0.042543	0.072481	0.055960
r(C↔T)	0.371753	0.481820	0.426825
r(G↔T)	0.063205	0.105750	0.082609
pi(A)	0.186520	0.250777	0.217505
pi(C)	0.265376	0.332988	0.298616
pi(G)	0.236563	0.306241	0.270350
pi(T)	0.187878	0.240326	0.212236
alpha	0.476046	0.895040	0.617256

In the 50% majority rule consensus (Figure 2.3) the terminal nodes are better resolved than those of the parsimony analysis. The campanuloids form a weakly supported single clade (PP=51) as opposed to two separate clades under the parsimony criterion. Of special interest to the present study is the South African wahlenbergioids. They form a strongly support clade (PP=100) sister to the campanuloids. Similar to the parsimony analysis the platycodonoids are not monophyletic, instead resolving into three clades. *Codonopsis dicentrifolia* is sister to the campanuloids and

wahlenbergioids while the *Cyananthus* - *Canarina* clade is unsupported as sister to a group comprising these three clades. The largest of the platycodonoid clades comprising *Campanumoea, Platycodon*, *Leptocodon* and the remaining *Codonopsis* species is unsupported as sister to rest of the Campanulaceae.

2.3.3. Estimates of divergence time

The divergence times for all major Campanulaceae clades (wahlenbergioids, platycodonoids, campanuloids) and the outgroup (Lobeliaceae) are shown in figure 2.4. The split between the South African clade (wahlenbergioids) and the campanuloids occurred about 35 mya (HPD=28-42). Onset of diversification in the SA clade is estimated at about 28 mya (HPD= 21-35), whilst diversification of the campanuloids began at 28 mya (HPD=20-37) and that of the platycodonoids at about 17 mya (HPD=10-29).

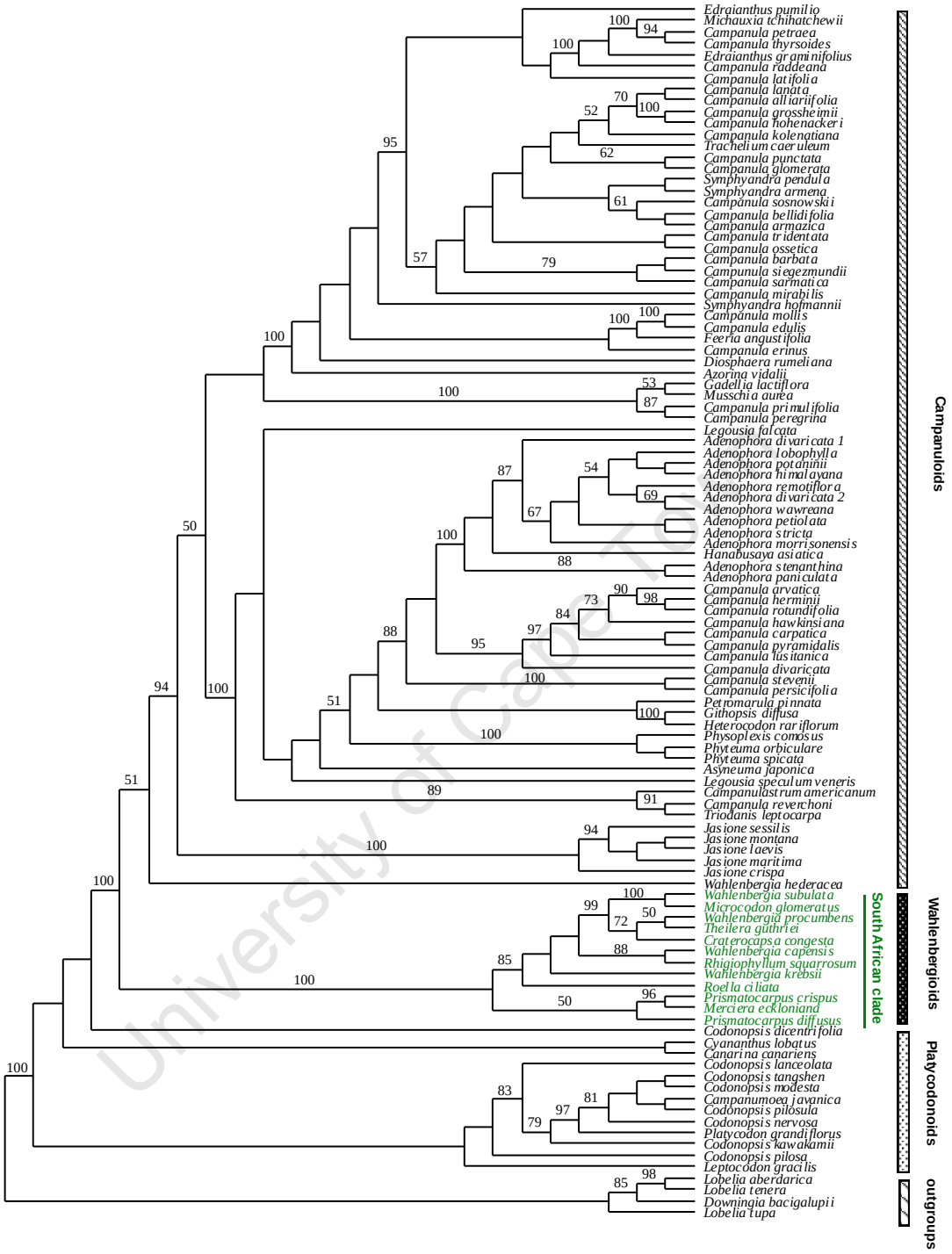


Figure 2.3. 50% majority rule consensus of trees retained in the Bayesian analysis of the ITS data set for 107 taxa of the Campanulaceae and four Lobeliaceae (outgroup). Numbers above branches indicate posterior probability values expressed as percentages.

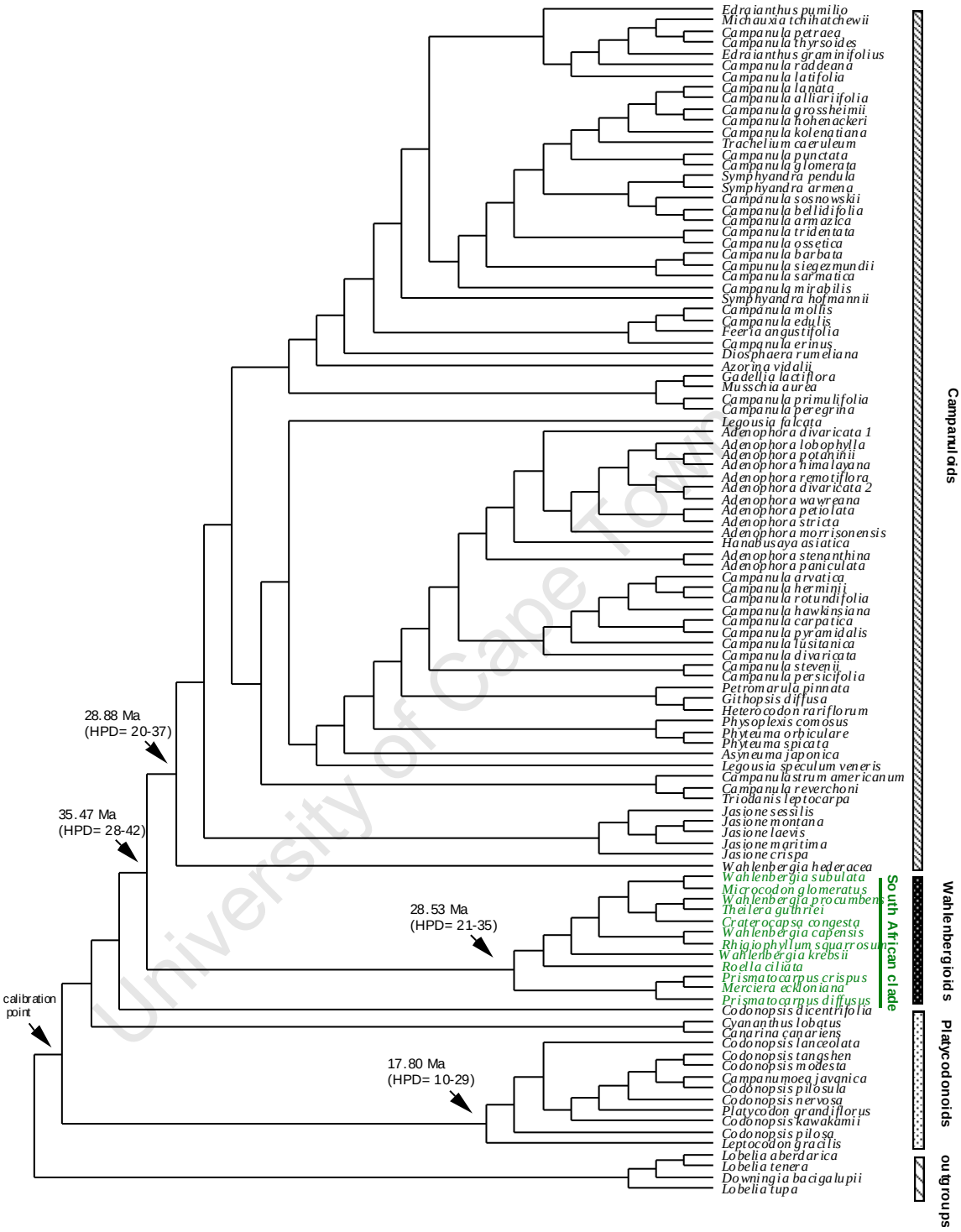


Figure 2.4. A summary of the divergence times for the major clades of Campanulaceae estimated by Bayesian inference as implemented by the program BEAST (Drummond *et al*. 2002, Drummond and Rambaut 2006a). HPD= 95% highest posterior density.

2.4. Discussion

2.4.1. Monophyly of the South African Campanulaceae

The ITS data provide evidence for the monophyly of the South African Campanulaceae, and this is underscored (at least under Bayesian approaches) by strong statistical support. Thus all extant members of the family in South Africa appear to be derived from a common ancestor. The diversification may have been a response to selection pressures present in the diverse new environment. The diversification may also simply be as a response to time and isolation – i.e. the number of species present is not different from that expected given average speciation rates and the amount of time elapsed. Distinguishing between these alternatives would require better sampling of the South African clade. However, the per-lineage diversification rate per million years lies between 0.14-0.23. This estimated tempo of radiation in the South African Campanulaceae is lower than the mainly southern African, Ruschioideae-Aizoaceae (0.77-1.77 species per million years, Klak *et al*. 2004), but compares well with the overall rate for angiosperm families (median of 0.12 and maximum of 0.39 species per million years, Magallón and Sanderson 2001).

The relationship between tropical African and southern African taxa will help to explain the pattern of Campanulaceae radiation on the continent. The two regions have *Wahlenbergia* species such as *W. androsacea*, *W. denticulata* and *W. krebsii* in common. This suggests a biogeographical connection between the regions. Whether there was southward migration of Campanulaceae elements from tropical African to southern Africa and eventually to South Africa or *vice versa* has to be demonstrated. These migration events may have occurred during the Tertiary as a result of progressive aridification of the African continent (Coetzee 1980). Again, better sampling coupled with dating estimates would provide a test of this hypothesis.

Relationships within the South African clade are not easy to explain because morphologically diverse taxa form sister relationships with each other. Although this

clade is better sampled here than in Eddie *et al*. (2003). The effects of incomplete taxon sampling on relationships are unknown at this stage. In Chapter 3 a more comprehensive sampling is undertaken that deal with these issues. However, the sister relationship between *Merciera* and *Prismatocarpus* is expected. These genera together with *Roella* are considered closely related (Adamson 1952, 1955b). The sister relationship between *Roella* and *Craterocapsa* found in the Eddie *et al*. (2003) study, is not maintained in this topology. Hilliard and Burtt (1973) and Hong (1995) considered these two genera as closely related. In the Eddie *et al*. (2003) study their sister relationship is surely an effect of sampling.

The status of South Africa as one of the centres of diversity of the Campanulaceae is corroborated by these data. Furthermore, its strongly-supported (at least under Bayesian approaches) sister relationship to the campanuloids indicates a geographical affinity with Europe. The other major centre of diversity, Asia, is represented by the platycodonoids, comprising *Platycodon*, *Codonopsis*, *Leptocodon*, *Campanumoea* and *Cyananthus*, which with the non-Asian genus, *Canarina* form sister relationships to the rest of the Campanulaceae. *Canarina* is found in the Canary Islands and eastern Africa. The platycodonoids have been described by some authors as being the most primitive members in the family and this is then use as an indicator to support an Asiatic origin of the family as opposed to an origin in Africa, which is often suggested as the alternative centre of origin. Among those are Hong and Ma (1991) and Hong (1995) who used results of character analysis to suggest that these genera are all relatively primitive in the family, in the sense that they retain many plesiomorphic states. However no extant taxa can be regarded as primitive simply because they have retained more plesiomorphic states than others. Overall in their scheme, *Cyananthus* emerged as the most 'primitive' genus. Although this genus displays the greatest number of primitive characters, it also has specialized features associated with adaptation to high altitudes (Eddie *et al*. 2003). (Cosner *et al*. 2004) interpret the platycodonoid clade as the basal clade and argues that this basal position suggests an Asiatic origin. Firstly, placement of genera on a cladogram cannot by itself be use as evidence of primitiveness. Secondly, as stated above, the platycodonoid clades are

sister to and not basal to rest of the Campanulaceae as described by Cosner *et al*. (2004). The evidence presented here is unclear whether the Campanulaceae has an African or Asiatic origin due to the non-monophyly of the platycodonoid genera.

A perceived advantage of applying the criterion of monophyly to classification is that is compels one to discover morphological synapomorphies to diagnose the clades. Unfortunately the defining synapomorphies for the South African clade remains undiscovered at this stage. Possibly a combination of characters could be used to define it or it is only detectable in conjunction with samples from the rest of Africa.

Although the results suggest a positive test for the monophyly of the South African Campanulaceae it should be viewed with caution due to the absence of wider sampling of wahlenbergioid species from Australia and New Zealand. These countries represent the remaining centres of diversity for wahlenbergioids, especially the genus *Wahlenbergia*. To this end, collaboration with researchers in New Zealand has been established to place this study in a broader context before publication. A recent study by Harberle *et al*. (in press) based on three chloroplast gene regions suggests that the South African Campanulaceae are not monophyletic, however this study lacks samples of South African *Wahlenbergia* species and *Treichelia longibracteata*. The present study does provide a test for the monophyly of the wahlenbergioids and a starting point for investigations into the intrafamilial relationships between the taxa comprising this clade. One can view the monophyletic group as the raw material from which further natural units (genera) can be discovered through phylogenetic studies. This will ultimately provides a framework for a stable predictive classification system. In Chapters 3 and 4 the current, unsatisfactory generic limits within this South African clade, are re-assessed in an attempt to improve the classification.

2.4.2. Age of the South African clade

Various molecular dating methods (local clocks, nonparametric rate smoothing, penalized likelihood, and Bayesian relaxed clock) have been developed, but there is no

single ‘best’ method (Rutschmann 2006). In this study only the Bayesian relaxed clock method has been employed due to time constraints. However, the Bayesian approach, like the penalized likelihood method, is useful to correct for rate heterogeneity and is less influenced by incomplete taxon sampling (Linder *et al*. 2005). The results obtained from these two methods were also favoured in the molecular dating of the Dipsacales (Bell and Donoghue 2005). A common error introduced in molecular dating is calibration error (Heads 2005). The most frequently used method of calibrating divergence times of taxa is equating their age with the oldest known fossil. In the case of the Campanulaceae, fossil evidence is poor and for this study group secondary calibration points published by Wikström *et al*. (2001) were used. One has to bear in mind that the ages estimated by Wikström *et al*. (2001) for the angiosperms were based on a single calibration point (Fagales-Cucurbitales split) which could contain error. This error is potentially compounded in this study.

Despite the limitations in the techniques used, these data indicate that the South African Campanulaceae and campanuloids shared a common ancestor that lived 35 million years ago. After the initial split there were two surviving lineages during the subsequent seven million years. The extant South African diversity of Campanulaceae traces back to a common ancestral species that lived 28 million years ago. This split between the campanuloids and wahlenbergioids correlates with a north-south migration or vicariance of the respective groups, with the campanuloids predominantly inhabiting the northern hemisphere while the wahlenbergioids inhabited the southern hemisphere, where they are represented in Africa, South America, Australia, New Zealand, and other smaller islands. The presence of campanuloid and wahlenbergioid species in tropical Africa suggest that this region can be seen as a zone of overlap that was formed by north- and southward migration of species. A southward migration of tropical African species into the Cape flora as first suggested by Levyns (1964) was probably influenced by the development of high volcanic mountains in Ethiopia and East Africa during the Tertiary (Axelrod and Raven 1978) accompanied by global climatic changes (Kennet 1980), such as the glaciation of Antarctica, a drop in the sea levels, and the start of a dry cold phase (Zachos *et al*. 2001). It is assumed that these

conditions formed the setting for the ancestral wahlenbergioid elements that by way of adaptive responses to the changes in climate and topography triggered their early diversification. The timing of diversification of each major clade within the South African Campanulaceae is further explored in Chapter 3. However there is virtually no evidence of southward migration of Cape lineages.

In contrast, a northward migration of Cape lineages with tropical representatives (e.g. *Disa*, Restionaceae, Irideae p.p and the *Pentaschitis* clade) into tropical Africa was suggested by Galley *et al*. (2006), and Galley and Linder (2006). They demonstrated that the migration of at least some of these lineages to tropical Africa occurred via the Drakensberg in the last 17 myr.

2.4.3. The position of *W. hederacea*

These data are not strongly contradictory of *W. hederacea* being sister to the wahlenbergioid clade since bootstrap support for the campanuloid group is lacking. However, the distant relationship between this species and the rest of the wahlenbergioid genera suggests strongly that, at least, it should be excluded from *Wahlenbergia*. It obviously is not closely related to the wahlenbergioid genera of the southern hemisphere sampled in this study.

The taxonomic history of *W. hederacea* is proof of its uncertain position. It has been treated as a separate genus several times, e.g. as *Schultesia* Roth, *Valvinterlobus* Dulac, *Aikinia* Salisb. ex A.DC. or as a species of *Roucela*. In a separate study using cpDNA, Cosner *et al*. (2004) found that the three South African genera sampled, grouped with *W. gloriosa* Lothian, an Australian species. Recent cpDNA results of Haberle *et al*. (in press) confirm this affinity between the wahlenbergioid genera of South Africa and Australia. In the study presented here, no Australian representatives where sampled and their absence could potentially change relationships among the wahlenbergioids. However, the cpDNA results point to a well-defined wahlenbergioid group that is non-European and mainly distributed in the southern hemisphere. This

means that *W. hederacea* should be removed from *Wahlenbergia* and classified elsewhere as was done by earlier researchers.

CHAPTER 3

MOLECULAR PHYLOGENETICS OF THE SOUTHERN AFRICAN CLADE

3.1. Introduction

Advances in technology have led to an increase and improvement in the techniques available to all scientific disciplines. For systematics, new biochemical techniques have made sub-cellular molecules accessible as a source of taxonomic information. The use of molecular data for reconstructing phylogenetic hypotheses has often resulted in the establishment of new classification systems (e.g. in the African Restionaceae; Eldenäs and Linder 2000, Colchicaceae; Vinnersten and Reeves 2003, *Stapeliopsis*; Bruyns *et al*. 2005, Rubiaceae; Alejandro *et al*. 2005, Asteraceae; Martins and Hellwig 2005, Ebenaceae; Duangjai *et al*. 2006). For plant systematists, molecular data are available from three genomes - the chloroplast, the mitochondrion and the nucleus. The unique properties of each of the three genomes are important considerations in determining their utility in phylogenetic reconstruction. The chloroplast and mitochondrial genomes are typically inherited uniparentally, usually maternally in angiosperms (Birky 1995), but biparental chloroplast inheritance has been reported at low frequencies, for example in *Iris* (Cruzan *et al*. 1993), *Turnera* (Shore *et al*. 1994), and in *Passiflora* (Hansen *et al*. 2007). The nuclear genome is inherited biparentally. The size of the genomes differs considerably with the nucleus being the largest, followed by the mitochondrion and then the chloroplast. Because of frequent genome rearrangements in the mitochondrion of individual plants its usefulness in inferring relationships is limited (Palmer 1992) and until recently was not generally employed in plant studies. However this is changing as an increasing number of phylogenies based on mitochondrial markers are published (e.g. Bakker *et al*. 2000, 2004; Davis *et al*. 2004; Merckx *et al*. 2006, Nyffeler 2007). Most genes in the chloroplast genome are single copy (Olmstead and Palmer 1994), are structurally conservative and genome rearrangement is rare. In contrast many nuclear genes belong to multigene families, which can reduce their phylogenetic usefulness (Soltis and Soltis 1998). Given these differences in properties it is advisable to use evidence from

both the chloroplast and nuclear genomes to reconstruct phylogeny (Rieseberg and Soltis 1991). In this study the chloroplast gene region, *trn*L-F and the nuclear gene region, ITS (described in Chapter 2) were sampled for DNA sequence data.

3.1.1. The *trn*L-F gene region

The *trn*L-F gene region (Figure 3.1) comprises two non-coding chloroplast DNA sequences, the *trn*L intron and *trn*L/*trn*F intergenic spacer (Taberlet *et al*. 1991). The *trn*L intron is situated between the two *trn*L exons, and the spacer region between the *trn*L exon and *trn*F gene (Taberlet *et al*. 1991). This region has been widely used in studies of phylogenetic relationships at the generic and family level (e.g. Mes *et al*. 1997, Eldenäs and Linder 2000, Reeves *et al*. 2001, Klak *et al*. 2003, Albach *et al*. 2004, Caputo *et al*. 2004, Kocyan *et al*. 2004, Pardo *et al*. 2004, Plunkett *et al*. 2004, Alejandro *et al*. 2005, Wang *et al*. 2005) because it is relatively small, easy to amplify and sequence, and generally exhibits a high rate of evolution and great variation (Bakker *et al*. 2000; Fukuda *et al*. 2001, 2003).

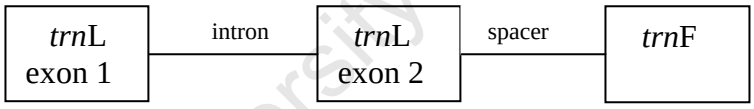


Figure 3.1. Structure of the *trn*LF gene region (after Taberlet *et al*. 1991)

3.1.2. Combining data in phylogenetic analysis

The process of combining different data sets in phylogenetic studies, for example sequences from several gene regions or molecular and morphological data sets is well documented (e.g. Eldenäs and Linder 2000, Reeves *et al*. 2001, Klak *et al*. 2003, Rivadavia *et al*. 2003, Albach *et al*. 2004, Caputo *et al*. 2004, Hidalgo *et al*. 2004, Kocyan *et al*. 2004, , Pardo *et al*. 2004, Plunkett *et al*. 2004, Alejandro *et al*. 2005, Martins and Hellwig 2005, Wang *et al*. 2005,Yukawa *et al*. 2005) . However, there is

continued debate whether or not data sets should be analyzed separately or in combination (Swofford 1991, Chippindale and Wiens, 1994, Huelsenbeck *et al*. 1996). The controversy exists because independent data partitions collected from the same taxa often produce conflicting phylogenies (Kluge 1989, Bull *et al*. 1993, Rodrigo *et al*. 1993, de Queiroz *et al*. 1995, Miyamoto and Fitch 1995). Possible reasons for this incongruence are sampling error, the use of inappropriate phylogenetic models (Hipp *et al*. 2004), lineage sorting (Maddison 1997, Avise 2000), hybridization (Rieseberg 1997, Avise 2000), gene duplication and different rates of evolution.

Three approaches, each with its own benefits and weaknesses have been developed to deal with partitioned data in phylogenetic analysis. In the first approach, Kluge (1989) and Nixon and Carpenter (1996) argued that all available data should always be combined in a simultaneous analysis. According to them the advantage of this so-called total evidence approach is that it maximizes the explanatory power of the data and a further advance is that as more data are added to the analysis the probability of estimating the correct phylogeny increases. Sometimes combining data sets provides resolution of relationships unresolved by separate analyses (Kluge and Wolf 1993, Nixon and Carpenter 1996), increases clade support, and reduces the number of most parsimonious trees (Chase and Cox 1998).

In direct contrast to the previous approach, Lanyon (1993) and Miyamoto and Fitch (1995) advocate analyzing data separately (partitioned analysis) and then using a consensus method to combine the results. They argued that different classes of data exist, which may reflect different evolutionary histories, and combining data may lead to misleading phylogenies. Unfortunately separate analysis does not discriminate between those cases in which combining partitions helps phylogenetic analysis, and those cases in which it hinders phylogenetic analysis (Huelsenbeck *et al*. 1996).

The third approach, the conditional combination or prior agreement approach is intermediate between the partitioned analysis and simultaneous analysis. It considers data partitions to be combinable if and only if they are not strongly incongruent with

one another (Bull *et al*. 1993, Rodrigo *et al*. 1993, Huelsenbeck *et al*. 1996, Baum *et al*. 1998, Thorton and DeSalle 2000, Yonder *et al*. 2001, Buckley *et al*. 2002). It is argued that combining strongly incongruent data partitions may reduce phylogenetic accuracy. In practice incongruence between multiple data sets is first assessed using the incongruence length difference (ILD test) (Farris *et al*. 1994, 1995) or other tests of taxonomic congruence (Templeton 1983, Larson 1994, Shimodaira and Hasegawa 1999) before deciding whether the partitions should be analyzed separately or in combination. The ILD test can be affected by several factors and it has been shown to be misleading under some circumstances (Wiens 1998, Dolphin *et al*. 2000, Reeves *et al*. 2001, Yoder *et al*. 2001). An alternative method to evaluate incongruence, is node-by-node comparison of patterns of internal support and levels of resolution between the results of the combined analysis and that of partitioned analysis (Eldenäs and Linder 2000, Reeves *et al*. 2001). According to this approach, if strongly supported and congruent clades are found, then these data matrices can be combined despite the negative results of partition homogeneity tests.

Sometimes, the type of data may influence the decision to combine data sets or not. A limitation of ITS sequences is the small number of characters available to reconstruct a phylogeny. Baldwin *et al*. (1995) suggested that it might be necessary to combine data from other sources, with ITS data, to obtain sufficient number of characters for well supported phylogenetic resolution. However, the chloroplast genome, although the most frequently and widely used in plant molecular systematics, is also not without disadvantages. Wolfe and Randle (2004) suggested that recombination of organellar genomes, heteroplasmy, haplotype polymorphism and paralogy may affect tree topology and the conclusions drawn from them. In this study, partitioned and simultaneous analyses were explored, in an attempt to obtain the best phylogenetic estimation for the available data.

3.1.3. Aims

In light of the problems outlined earlier for South African Campanulaceae (Chapter 1), the aim of this chapter is to use the molecular phylogenetic framework to:

1. explore the correspondence between genera based predominantly on fruit characters and the molecular evidence
2. clarify generic boundaries within the South African Campanulaceae
3. estimate the divergence times for the major South African clades and relate this to the diversification patterns in the clade

3.2. Materials and Methods

3.2.1. Taxon sampling

Taxa were selected to include at least one representative from each genus, maximum morphological and geographical diversity, and all life forms in the family. In the case of monotypic genera, only one sample was used. All species of genera with two or three species were investigated. In genera comprising more than three species, at least one species from each currently recognized infra-generic taxon was included in the study. For example, in the case of *Roella* one species per series and for *Prismatocarpus* one species per series of each subgenus were sampled. A voucher herbarium specimen for each collection was deposited at the Compton Herbarium (NBG), Kirstenbosch, Cape Town. Specimens were identified as far as possible to species with the aid of the most recent generic treatments, and the collections housed in BOL, NBG, PRE and SAM (abbreviations as in Holmgren *et al*. 1990). In cases where specimens could not be named with confidence, they were identified to genera or, as with the "Malmesbury plant" only to family. The unnamed specimens do not necessarily represent undescribed taxa but rather ambiguity in the current taxonomy.

DNA sequences from the *trn*L-F and ITS regions were obtained from 96 and 79 taxa, respectively (Table 3.1). Every attempt was made to have the same number of taxa for

each gene region, but for many taxa it was impossible to obtain ITS sequences. Most problems were experienced with amplification, despite the reported ease with which ITS amplifies because of its high copy number (Baldwin *et al*. 1995). All taxa were field collected and DNA was isolated from silica dried (Chase and Hills 1991) or fresh leaf material. In taxa with reduced leaves such as *Siphocodon spartioides* and *Wahlenbergia virgata*, the stem epidermis was also used in the isolation to ensure that a sufficient amount of isolated DNA was obtained.

3.2.2. Outgroup choice

The purpose of an outgroup is to establish by comparison with the ingroup, or study group, hypotheses on the transformation or polarity of character states. Character states are then hypothesized to be primitive (plesiomorphic) or derived (apomorphic). This method of outgroup comparison is different from the so-called outgroup rooting, in which the outgroup is used to root the tree to infer the cladogenic events responsible for the diversity in the study group. The latter procedure is relevant to this study.

Irrespective of the classification system followed, the close relationship between the campanulad, lobeliad and cyphiad components of the Campanulaceae is undisputed and well documented (Cronquist 1981; Lammers 1992; Gustafsson and Bremer 1995; APG 2003; Cosner *et al*. 2004). Consequently members of the Lobeliaceae and Cyphiaceae were used as outgroups in this study.

3.2.3. DNA Extraction and Amplification

Total DNA was extracted using a modification of the 2X CTAB method of Doyle and Doyle (1987). Plant material (0.5 – 0.1 g fresh or 0.2 g dried) was ground in mortars with pre-heated CTAB isolation buffer containing 10 µl of betamercaptoethanol, then transferred to 50 ml tubes, and incubated at 65°C for 10 minutes. After incubation, ground material was extracted with chloroform – isoamylalcohol (24:1) for 1 hour on a

horizontal shaker. Extracts were then centrifuged at 8000 rpm for 10 minutes to separate the aqueous phase containing DNA from the plant debris. The aqueous phase was transferred to 50 ml tubes. All DNA extracts were purified using the Qiaquick PCR kit (Qiagen) according to the instructions of the manufacturer. DNA quality was checked on agarose gels.

PCR amplifications for the *trn*L-F gene region and the entire ITS region (the two spacers, ITS1 and ITS2 and the intervening 5.8 S) were performed with *Taq* polymerase. Three to four μl of total DNA extract was used as template in the reaction. The 100 μl reactions contained 2.5 U *Taq* polymerase; magnesium-free thermophilic buffer (50 mM KCl, 10 mM tris-HCl, 0.1% Triton X-100); 3 mM $MgCl_2$; 0.004% bovine serum albumin (BSA, Savolainen *et al*. 1995); 0.2 mM pf dNTP and 100ng of each primer. For ITS, 5 μl of Dimethyl Sulfoxide (DMSO) was added to facilitate the separation of the double stranded DNA. Positive and negative controls were included to monitor the reaction. For the *trn*L-F region the primers 'c' and 'f' (Taberlet *et al*. 1991) were used to amplify the intron and intergene spacer region between the *trn*L and *trn*F exons. Where amplification of the 'c' to 'f' region failed, internal primers 'd' and 'e' (Taberlet *et al*. 1991) were used in conjunction with 'c' and 'f' to amplify the gene in two non-overlapping segments. The entire ITS region was amplified with primers AB101F and AB102R (Baldwin 1992).
PCR reactions were carried out in a GeneAmp® PCR System 9700 using the following PCR parameter: initial denaturation of double stranded DNA at 94 ºC for two minutes, followed by a number of cycles of 94 ºC denaturation for one minute, 48 ºC annealing for one minute (58 ºC for 30 seconds for ITS); 72 ºC extension for one minute, followed by a final extension 72 ºC for seven minutes. The *trn*L-F region was amplified in 30 cycles whereas ITS was amplified in 28 cycles. The PCR products were purified using QIAquick silica columns (Qiagen Inc.) or GFX™ PCR columns (Amersham Biosciences) according to the manufacturer's protocol.

3.2.4. Sequencing and Alignment

Sequencing of the PCR products was performed for 26 cycles in a GeneAmp® PCR System 9700 using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Each cycle consisted of 96 ºC denaturation for 10 seconds, 50 ºC annealing for five seconds and 60 ºC extension for four minutes. The same primers were used as for the original PCR. The samples were resolved on polyacrylamide electrophoresis gels on an Applied Biosystems 377 automated DNA sequencer.

For each taxon the complementary strands were assembled and edited using Sequencher 4.1 (Gene Codes Inc.). Sequences were aligned by eye. Gaps that result from the alignment of unequal sequences may contain useful phylogenetic information (Giribet and Wheeler 1999), but different methods of treating gaps may influence the resulting phylogenetic analysis (Eernisse and Kluge 1993, Simons and Mayden 1997). After evaluating various gap-coding methods, Simmons and Ochoterena (2000) proposed two methods, simple and complex, by which gaps coded as presence/absence characters can be implemented in phylogenetic analyses. The simple indel coding is easy to implement, but does not incorporate all available information whilst complex indel coding is more difficult to implement but allows all available information to be incorporated when retrieving phylogenetic information. In this study gaps were coded as missing data and not scored for inclusion in the analyses. The random appearances and overlapping of gaps in the matrices were not considered potentially phylogenetically informative at the generic level.

Aligning the ITS region, comprising 174 individuals representing 40 genera was problematic. This is not surprising as aligning non-coding sequences, like ITS, over large evolutionary distances is difficult (Kimball and Crawford 2004, Kemler *et al*. 2006). Two factors are involved here: the number of sequences and the degree of similarity between them. Hickson *et al*. 2000 found that the latter had the greatest influence on alignment accuracy. As a result, alignments with highly divergent

sequences will contain more error than less divergent sequences. The nature of this error is usually ignored (Rosenberg 2005) even though it may affect phylogenetic analysis. For this study, 77 ITS sequences were newly produced and 97 were obtained from GenBank. Boundaries of the ITS region were determined using sequences previously published for the Campanulaceae (Eddie *et al*. 2003). This resulted in the exclusion of the 5.8S subregion and part of ITS2. Sequences were aligned independently using a consistent alignment convention of moving characters to the left if alternate alignments were possible. Regions in the matrices that were difficult to align unambiguously were excluded. By doing this, otherwise alignable regions of the less divergent sequences of the South African genera relative to the campanuloids and platycodonoids became unavailable. This issue is contentious because removing such regions can reduce resolution (Gatesy *et al*. 1993) while their inclusion can support erroneous patterns of branching (Hickson *et al*. 2000).

3.2.5. Combined *trn*L-F and ITS data set construction

The combined molecular data set of 75 taxa consisted of 72 ingroup and three outgroup taxa. Only taxa common to both the individual data sets, were used in the combined analysis.

3.2.6. Phylogenetic Analyses

3.2.6.1. Maximum Parsimony (MP) analyses

Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2003) with all characters treated as unordered with equal weighting (Fitch parsimony; Fitch 1971). A second search strategy using successive approximations weighting (Farris 1969) was employed to create a new data set in which characters that are more consistent are replicated more than others (Kitching *et al*. 1998). Characters were reweighted according to the rescaled consistency index (base weight = 10) on the best tree(s). The

data matrices for each of the two gene regions were analyzed separately and as a combined data matrix.

An initial run was performed using the heuristic search option and tree-bisection reconnection (TBR) branch-swapping with 1000 random addition replicates, saving five trees per replicate to minimize the time spent searching through large numbers of trees, steepest descent off and MULTREES in effect. Branches were collapsed if their maximum length equaled zero. All the trees obtained were then used as starting trees in a second analysis with same parameters as above, saving all optimal trees with a limit of 10 000 trees. In the case of successive weighting, trees recovered were used for subsequent rounds of reweighting and analysis until the tree topology stabilized. Trees were rooted with the outgroup, comprising members of the Lobeliaceae and Cyphiaceae.

Support for each clade retrieved by the analysis was assessed using bootstrap analyses (Felsenstein 1985). The usefulness of bootstrap analyses has been intensively debated, but it remains the most commonly used method for assessing the level of internal support on phylogenetic trees (DeBry and Olmstead 2000). Bootstrap analyses entail random sampling with replacement of a set of characters until a replicate data set is constructed. This replicate data set is subsequently analyzed and a phylogenetic tree is reconstructed according to a specified search strategy. This process is repeated for a specified number of times, and the results are then summarized as a bootstrap consensus tree. The frequency at which each clade is recovered is termed the bootstrap proportion, or bootstrap support. For the bootstrap analysis, a heuristic search with 1000 replicates, simple taxon addition and TBR branch-swapping was employed. Only bootstrap values of over 50% are reported. Bootstrap values were interpreted as follows: 50–74 % weakly supported, 75-89% moderately supported, 90-100% strongly supported.

Mort *et al*. (2000) demonstrated that bootstrap and jackknife analyses generally provide similar estimates of support. Jackknife analyses were not employed in this study.

For each analysis the consistency (CI) (Kluge and Farris 1969) and retention (RI) (Farris 1989) indices were calculated to give an indication of the measure of fit between the data and the tree topologies. This in fact gives an estimation of the involvement of characters showing convergence and parallelism (i.e. homoplasy) in the cladogram construction. The CI indicates the ratio between the minimum number of transformations theoretically expected, given the number of character states in the data, and the actual number of transformations observed in the calculated cladogram. Problems with the CI are that uninformative characters will inflate its value, the value is affected by the number of taxa included, and its value can never reach zero (Kitching *et al*. 1998). Farris (1989) recognized the problems with the CI and introduced the RI to address the limitations of the CI. The RI measures the amount of synapomorphy expected from the data set that is retained as synapomorphy on a cladogram. It is calculated as:

RI= (*g*-*s*)/(*g*-*m*),
where *g* is the maximum possible number of character transformations, *s* is the actual number of transformations observed in the calculated cladogram, and *m* is the minimum number of character transformations in the data.

In both cases values approaching one indicate a low level of homoplasy in the data set.

3.2.6.2. Data combinability

To assess topological congruence between the *trn*L-F and ITS data sets, an incongruence test was performed using the incongruence length difference (ILD test; Farris *et al.* 1995). In this test, character congruence is measured by comparing tree length differences between trees derived from resampled data partitions of the

combined data sets and the trees derived from the defined data partition. The test uses the partition homogeneity test as implemented in PAUP* 4.0b10 (Swofford 2003). One hundred partition homogeneity replicates were used with 100 replicates of random addition sequence, TBR branch swapping, saving 10 trees per replicate.

If the probability of obtaining a smaller sum of tree lengths from the randomly generated data sets is lower ($p \leq 0.05$) than that of the original data sets, the two data sets are interpreted as incongruent.

To further evaluate incongruence, agreement subtrees (common pruned trees) were constructed using the 'agreement subtrees'option of PAUP, to identify 'unstable' sequences in the data set – those that appear in different places in different trees - need to be excluded from the analysis so that the remaining sequences pass the partition homogeneity test.

3.2.6.3. Bayesian Analysis

Bayesian analyses were conducted separately for each of the two gene regions and as a combined matrix using MrBayes 3.1 (Huelsenbeck and Ronquist 2001). The software Modeltest (version 3.06; Posada and Crandall 1998) was used to determine the best model of DNA substitution from a comparison of 56 models using the Akaike information criterion (Akaike 1974) for each of these data sets. Modeltest selected different models for each data set. The transversion model (TVM) +G was selected for *trn*L-F and the TrN model (Tamura and Nei 1993) +I+G for ITS. For the combined analysis, parameters applying to more than one partition were unlinked to allow values to differ among partitions. Five million generations were run with four independent chains (Markov chain Monte Carlo) and were sampled every hundred generations, resulting in an overall sampling of 50 000 trees.

Stationarity was established visually by plotting the negative log-likelihood (-LnL) values against generation time in Microsoft Excel to determine the burn-in period. For the first analysis stationarity was reached after 9000, for the second after 4300, and for

the third after 10 300 generations of trees. All trees were transferred to PAUP* and trees visited prior to stationarity were discarded. The remaining trees were used to generate a 50% majority-rule consensus tree with posterior probability values (PP-values) shown as percentages above the branches. PP-values of ≥95% are considered evidence of significant support for a group (Miller *et al*. 2004).

3.2.7. Estimation of the divergence times of the major South African clades

The dating method described in Chapter 2 (section 2.2.4) was used here.

For the age estimates the combined *trn*L-F and ITS data set was used because it represents the complete molecular evidence available. The tree was calibrated using the age calculated for the South African clade in Chapter 2. This was set at 28 mya on the node that includes the most recent ancestor of the ingroup for which monophyly was also enforced.

Table 3.1. List of taxa investigated for the molecular phylogenetics with their voucher information and GenBank accession numbers (where applicable).

Taxa	Voucher	*trn*L-F	ITS
Ingroup			
Craterocapsa congesta Hilliard and Burtt	Hirst and Webster, Lesotho		x (AY322049, Ay331462)
Craterocapsa montana (A.DC.) Hilliard and Burtt	Goldblatt s.n, Eastern Cape, Keiskamahoek	x	x
Craterocapsa tarsodes Hilliard and Burtt	Cupido 306, KwaZulu-Natal, Himeville	x	x
'Malmesbury plant'	Cupido 83, Western Cape, Malmesbury	x	x
Merciera azurea Schltr.	Cupido 111, Western Cape, Bredasdorp	x	x
Merciera brevifolia A.DC.	Cupido 235, Western Cape, Caledon	x	x
Merciera eckloniana H.Buek	Cupido 76, Western Cape, Villiersdorp	x	x
Merciera leptoloba A.DC.	Cupido 108, Western Cape, Bredasdorp	x	x
Microcodon glomeratus A.DC.	Cupido 105, Western Cape, Kraaifontein	x	x
Microcodon sp. 'pygmaeum'	Cupido 82, Western Cape, Malmesbury	x	
Microcodon sp. 'sparsiflorus'	Cupido 197, Western Cape, Hopefield	x	x
Microcodon sp.	Cupido 257, Western Cape, Clanwilliam	x	
Prismatocarpus brevilobus A.DC.	Duckitt s.n., Western Cape, Darling	x	x
Prismatocarpus campanuloides (L.f.) Sond.	Cupido 219, Western Cape, Genadendal	x	x
Prismatocarpus crispus L'Hér.	Manning 2651E, Western Cape, Clanwilliam	x	x

Prismatocarpus diffusus (L.f.) A.DC.	Cupido 220, Western Cape, Genadendal	x	x
Prismatocarpus fruticosus L'Hér.	Cupido 118, Western Cape, Somerset West	x	x
Prismatocarpus nitidus L'Hér.	Cupido 228, Western Cape, Cape Town	x	x
Prismatocarpus pedunculatus (P.J.Bergius) A.DC	Cupido273, Western Cape, Citrusdal	x	x
Prismatocarpus schlechteri Adamson	Cupido237, Western Cape, Caledon	x	x
Prismatocarpus sessilis Eckl. ex A.DC.	Cupido 112, Western Cape, Bredasdorp	x	x
Prismatocarpus sp. 'Vil'	Cupido 241, Western Cape, Villiersdorp	x	
Rhigiophyllum squarrosum Hochst.	Cupido 106, Western Cape, Napier	x	x
Roella amplexicaulis Wolley-Dod	Cupido 122, Western Cape, Cape Town	x	x
Roella arenaria Schltr.	Cupido s.n., Western Cape, Napier	x	x
Roella ciliata L.	Cupido 213, Western Cape, Cape Town	x	
Roella ciliata L.	T.Ayers s.n		x (AY322074, AY331487)
Roella cuspidata Adamson	Cupido 234, Western Cape, Caledon	x	x
Roella incurva A.DC.	Cupido 200, Western Cape, Hermanus	x	x
Roella muscosa L.f.	Cupido 232, Western Cape, Cape Town	x	x
Roella prostrata E.Mey. ex A.DC.	Cupido208, Western Cape, Malmesbury	x	x
Roella psammophila Schltr.	Cupido 216, Western Cape, Genadendal	x	x
Roella secunda H.Buek	Cupido 285, Eastern Cape, Joubertina	x	x
Roella squarrosa P.J.Bergius	Cupido 229, Western Cape, Cape Town	x	x
Roella triflora (R.D.Good) Adamson	Cupido 226, Western Cape, Cape Town	x	

Roella sp. 'genadendal'	Cupido 223, Western Cape, Genadendal	x	x
Siphocodon debilis Schltr.	Cupido 139, Western Cape, Napier	x	x
Siphocodon spartioides Turcz.	Cupido 133, Western Cape, Villiersdorp	x	x
Treichelia longibracteata (H.Buek) Vatke	Cupido 199, Western Cape, Hermanus	x	x
Theilera guthriei (L.Bolus) Phillips	Cupido 279, Western Cape, Prins Albert	x	x
Theilera robusta (A.DC.) Cupido	Cupido 317, Eastern Cape, Willowmore	x	x
Wahlenbergia acaulis E.Mey.	Cupido 267, Northen Cape, Kamiesberg	x	x
Wahlenbergia adpressa (Thunb.) Sond.	Cupido 210, Western Cape, Hopefield	x	x
Wahlenbergia androsacea A.DC.	Cupido183, Western Cape, Melkbos	x	x
Wahlenbergia annularis A.DC.	Cupido 251, Western Cape, Elandsbaai	x	x
Wahlenbergia axillaris Sond.	Cupido 107, Western Cape, Bredasdorp	x	x
Wahlenbergia buseriana Schltr. and Brehmer	Cupido 263, Northern Cape, Platbakkies	x	
Wahlenbergia capensis (L.) A.DC.	Cupido 184, Western Cape, Malmesbury	x	x
Wahlenbergia capillacea (L.f.) A.DC.	Cupido 313, Western Cape, Uniondale	x	x
Wahlenbergia cernua (Thunb.) A.DC.	Cupido188, Western Cape, Cape Town	x	x
Wahlenbergia cinerea (L.f.) Sond.	Cupido 222, Western Cape, Genadendal	x	x
Wahlenbergia cuspidata Brehmer	Cupido 302, KwaZulu-Natal, Himeville	x	x
Wahlenbergia depressa J.M. Wood and M.S. Evans	Roux 3350, Free State, Baker's Kop	x	x
Wahlenbergia desmantha Lammes	Cupido 310, Western Cape, Albertinia	x	x
Wahlenbergia ecklonii H. Buek	Cupido 206, Western Cape, Paarl	x	x

Wahlenbergia exilis A.DC.	Cupido 81, Western Cape, Malmesbury	x	x
Wahlenbergia fruticosa Brehmer	Cupido 311, Western Cape, Riversdale	x	x
Wahlenbergia huttonii (Sond.) Thulin	Cupido 304, KwaZulu-Natal, Himeville	x	x
Wahlenbergia juncea (H.Buek) Lammers	Cupido 296, Eastern Cape, Sterkstroom	x	
Wahlenbergia krebsii Cham.	Cupido 294, Eastern Cape, Hogsback	x	x
Wahlenbergia longifolia A.DC.	Cupido 212, Western Cape, Darling	x	x
Wahlenbergia neoridiga Lammers	Cupido 278, Western Cape, Prins Albert	x	x
Wahlenbergia nodosa H. Buek	Cupido 144, Western Cape, Worcester	x	
Wahlenbergia oxyphylla A.DC.	Cupido 259, Western Cape, Vanrhynsdorp	x	x
Wahlenbergia paniculata (Thunb.) A.DC.	Cupido 181, Western Cape, Yzerfontein	x	x
Wahlenbergia parvifolia (P.J.Bergius) Adamson	Cupido 119, Western Cape, Cape Town	x	x
Wahlenbergia pilosa H.Buek	Cupido 272, Northern Cape, Calvinia		x
Wahlenbergia polyantha Lammers	Cupido 287, Western Cape, Albertinia	x	x
Wahlenbergia procumbens (Thunb.) A.DC.	Cupido 244, Western Cape, Napier	x	x
Wahlenbergia psammophila Schltr.	Cupido 260, Western Cape, Vanrhynsdorp	x	x
Wahlenbergia rubioides A.DC.	Cupido 215, Western Cape, Genadendal	x	
Wahlenbergia stellarioides Cham. and Schltdl.	Cupido 295, Eastern Cape, Sterkstroom	x	
Wahlenbergia subulata (L'Hér.) Lammers	Cupido 207, Western Cape, Somerset West	x	x
Wahlenbergia tenella (L.f.) Lammers	Cupido 194, Western Cape, Cape Town	x	x
Wahlenbergia tenerrima H.Buek	Cupido 277, Western Cape, Prins Albert	x	x

Wahlenbergia thunbergiana H.Buek	Cupido 250, Western Cape, Elandsbaai	x	x
Wahlenbergia thunbergii (Schult.) B.Nordenstam	Forest s.n., Eastern Cape, Port Elizabeth		x
Wahlenbergia undulata (L.f.) A.DC.	Cupido s.n., Eastern Cape, Hogsback	x	x
Wahlenbergia unidentata (Thunb.) A.DC.	Cupido 274, Western Cape, Caledon	x	
Wahlenbergia virgata Engl.	Cupido 299, KwaZulu-Natal, Himeville	x	x
Wahlenbergia sp. 'ann andro'	Roux 3169, Northern Cape, Nieuwoudtville	x	
Wahlenbergia sp. 'ann nama'	Cupido 269, Northern Cape, Kamiesberg	x	
Wahlenbergia sp. 'BK'	Roux 3349, Free State, Baker's Kop	x	x
Wahlenbergia sp. 'chatsworth'	Cupido 209, Western Cape, Malmesbury	x	x
Wahlenbergia sp. 'genadendal'	Cupido 217, Western Cape, Genadendal	x	x
Wahlenbergia sp. 'leliefontein'	Cupido 268, Northern Cape, Leliefontein	x	
Wahlenbergia sp. 'Sani Rd'	Cupido 309, KwaZulu-Natal, Sani Road	x	
Wahlenbergia sp. 'UH'	Cupido 293, KwaZulu-Natal, Himeville	x	
Wahlenbergia sp	Cupido 252, Western Cape, Clanwilliam	x	
Wahlenbergia sp.	Cupido 253, Western Cape, Clanwilliam	x	x
Wahlenbergia sp.	Cupido 256, Western Cape, Clanwilliam	x	
Wahlenbergia sp.	Cupido 261, Western Cape, Vanrhynsdorp	x	x
Wahlenbergia sp.	Cupido 264, Northern Cape, Platbakkies	x	x
Wahlenbergia sp.	Cupido 265, Northern Cape, Platbakkies	x	

Outgroup

Cyphia bulbosa (L.) P.J.Bergius	Cupido s.n., Western Cape, Cape Town	x	
Cyphia comptonii Bond	Manning s.n., Western Cape, Katbakkies	x	x
Cyphia volubilis (Burm.f.) Willd.	Cupido 249, Western Cape, Paarl	x	
Lobelia comosa L.	Cupido s.n., Western Cape, Cape Town	x	x
Lobelia coronopifolia L.	Mannie s.n., Western Cape, Villiersdorp		x
Lobelia jasionoides (A.DC.) E.Wimm.	Cupido 120, Western Cape, Cape Town	x	x
Monopsis debilis (L.f.) C.Presl	Cupido s.n., Western Cape, Stellenbosch	x	

3.3. Results

3.3.1. *trn*L-F analysis

Of the 848 characters included for *trn*L-F, 386 were constant, 163 (19%) variable but parsimony uninformative and 299 (35%) parsimony informative. For details about indel positions, see matrix '*trn*L-F-chapter 3' in Appendix A.

Under the parsimony criterion 415 trees were found of 945 steps, a CI of 0.684 and a RI of 0.872.

The topology and support retrieved by the parsimony- and Bayesian analyses are similar, except that greater support for one clade (clade C) was obtained under Bayesian inference. The model parameter estimates and their 95% credible intervals are shown in Table 3.2.

In the strict consensus (Figure 3.2) the ingroup is split into two main groups, supported by bootstrap (BS) values of 100% and 83%, and posterior probability (PP) values of 99% and 95% respectively. The first includes the single species of *Rhigiophyllum* and the two species of *Siphocodon*; relationships among the three are unresolved.

The second of the two groups includes all remaining exemplars, comprising representatives of eight genera. *Wahlenbergia krebsii* is sister to the rest of the species in this group. The remainder is resolved into three clades (A, B and C), the relationships among which are unresolved. Clade A is strongly supported (BS= 100, PP= 99) and is formed by species of *Roella*, *Prismatocarpus* (except *P. crispus* in clade B) and *Merciera*. Relationships in this clade are largely unresolved. Given the general lack of well-supported resolution the monophyly of neither of these genera can be rejected by this data set. However some well-supported groupings are formed, e.g. three species of *Merciera* (BS= 85, PP= 99) and a weaker *Roella* group (BS= 74, PP= 99).

Clade B is strongly supported (BS= 100, PP= 99) and is formed by species of *Wahlenbergia* with *Prismatocarpus crispus*. This clade comprises a polytomy within which three well supported groupings of species are formed. There are two groups of annual species supported by BS values of 85% and 97%, PP 99% and 99% respectively, and a group of perennial herbs with 95% bootstrap and PP= 100% support.

The largest of the three clades, C, is weakly supported under parsimony (BS= 68), strongly supported under Bayesian inference (PP= 99) and resolved into two subclades. The first one is moderately supported (BS= 85, PP= 77) and includes species of *Wahlenbergia*, *Theilera* and *Craterocapsa.* The *Craterocapsa* species formed a clade with 99% PP and100% bootstrap support whereas the *Theilera* species formed a polytomy with six *Wahlenbergia* species. The second one has a bootstrap support of 100% and comprises species of *Wahlenbergia*, *Microcodon*, *Treichelia* and an unnamed plant from Malmesbury (referred to throughout as the Malmesbury plant). The species of *Microcodon* formed a moderately supported (BS= 89, PP= 69) clade. The position of *Wahlenbergia* in Clades B and C renders this genus paraphyletic.

Not surprisingly, given the low apparent homoplasy in the unweighted data (as evidenced by relatively high CI and RI values), successively approximated weighting of the data set did not substantially change the tree topology (tree number= 10 000, tree length= 5270, CI= 0.853, RI= 0.932).

Table 3.2. Nucleotide substitution model parameter values from the Bayesian analysis of the *trn*L-F data set for 90 taxa of the South African Campanulaceae. TL= total tree length, r(A↔C), r(A↔G), etc.= the six reversible substitution rates, pi(A), pi(C),etc.= the four stationary nucleotide frequencies, alpha= the shape parameter of the gamma distribution of rate variation across sites.

	95 % Credible Intervals		
Parameter	Lower	Upper	Median
TL	1.875000	6.439000	2.088000
r(A↔C)	0.151553	0.214696	0.181675
r(A↔G)	0.140470	0.201044	0.169431
r(A↔T)	0.040415	0.066912	0.052700
r(C↔G)	0.221381	0.306452	0.262281
r(C↔T)	0.149936	0.211472	0.179355
r(G↔T)	0.125524	0.184423	0.152624
pi(A)	0.296924	0.348143	0.322249
pi(C)	0.158455	0.199068	0.178258
pi(G)	0.157632	0.198454	0.177780
pi(T)	0.296516	0.346888	0.321503
alpha	0.341475	1.269933	0.999463

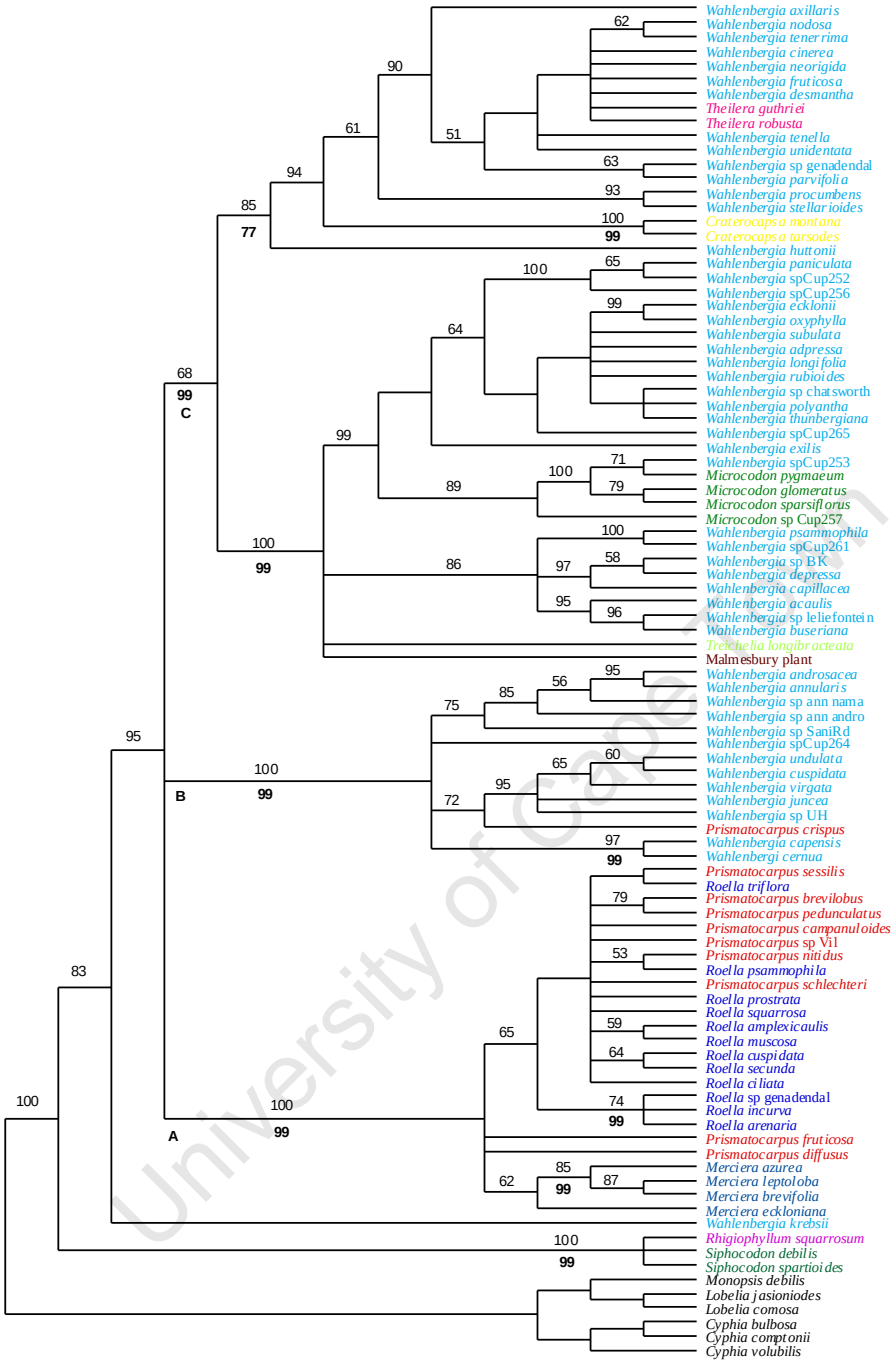


Figure 3.2. Strict consensus of 415 equally parsimonious trees (length=945, CI=0.684, RI=0.872) found after heuristic search of the *trn*L-F data set for 90 taxa of the South African Campanulaceae and six Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches. Numbers below the branches indicate posterior probability values expressed as percentages (only clades mentioned in the results are shown).

3.3.2. ITS analysis

The ITS matrix consisted of 353 characters, of which 189 were constant, 57 (16%) variable but parsimony uninformative and 107 (30%) parsimony informative. Details of the indel positions are given in the matrix 'ITS-chapter 3' in Appendix A. Under the parsimony criterion, 207 trees were found of 470 steps, a CI of 0.536 and a RI of 0.752.

The strict consensus tree (Figure 3.3) formed a dichotomy with *W. annularis* sister to a clade comprising the remaining species (BS= 87), whose relationships are largely unresolved. In the *trn*L-F analysis, *W. annularis* is part of a clade with moderate bootstrap support of 83%. This position is not strongly contradicted given the moderate bootstrap support for the individual clades. A few subclades are resolved within the large polytomy. The first, with no bootstrap support, is formed by *Theilera*, *Craterocapsa*, *Treichelia*, the Malmesbury plant and several species of *Wahlenbergia*. This clade is also resolved in the *trn*L-F analysis with weak support. The second subclade is formed by five *Wahlenbergia* species (BS= 82). *R. squarrosum* and *S. spartioides* resolved as a distinct clade with 87% bootstrap support while two species of *Merciera* form an unsupported clade.

Contrary to parsimony, the topology discovered with Bayesian inference is fully resolved, but not all relationships are well supported. The model parameter values and their 95% credible intervals obtained from this analysis are shown in Table 3.3. In the 50% majority rule consensus (Figure 3.4) *Prismatocarpus pedunculatus*, *W. androsacea*, *W. paniculata* and *W. annularis* resolved as a grade with the rest of the Campanulaceae as a terminal clade. This large clade is moderately supported (PP= 82) and is further separated into two clades (A and B). Clade A, moderately supported (PP= 90) is formed by *Rhigiophyllum*, *Siphocodon* and *W. huttonii*. In the *trn*L-F analysis *W. huttonii* does not group with these genera but is placed in clade B. Clade B, also unsupported, further resolves into two unsupported subclades (B1 and B2).

Clade B1 divides further into a strongly supported (PP= 99) group B1a formed by species of *Wahlenbergia*. This group is also retrieved in the parsimony analysis of the ITS data, but not in the *trn*L-F analysis. The other group, B1b, is unsupported and comprises species of *Roella*, *Prismatocarpus*, *Merciera* and *Wahlenbergia krebsii*. *W. krebsii* and *R. ciliata* form a clade sister to the clade consisting of the remaining *Roella* species, *Prismatocarpus* and *Merciera*. In the *trn*L-F analysis *W. krebsii* is not associated with this group of species.

Clade B2, also retrieved by the *trn*L-F analysis and the parsimony analysis of the ITS data, formed a dichotomy comprising one large clade, sister to *W.* sp. Cup 264. The large clade resolved into two groups, B2a (weakly supported, PP= 57) and B2b (strongly supported, PP= 100). The former comprises species of *Wahlenbergia*, *Theilera* and *Craterocapsa*, and the latter species of *Wahlenbergia*, *Microcodon*, *Treichelia* and the Malmesbury plant.

Three rounds of successive approximations weighting were necessary to stabilize the tree topology and tree length. The strict consensus tree (Figure 3.5) (tree number= 458, length= 1844, CI= 0.801, RI=0.880) is considerably more resolved than the unweighted analysis. It resolved similar clades to the Bayesian analysis. *W. androsacea*, *W. paniculata* and *W. annularis* are resolved as a grade, with the rest of the Campanulaceae as a terminal clade. *Prismatocarpus pedunculatus* moved to clade B1b, whereas clade A and clade B1a changed positions.

Table 3.3. Nucleotide substitution model parameter values from the Bayesian analysis of the ITS data set for 75 taxa of the South African Campanulaceae.
TL= total tree length, r(A↔C), r(A↔G), etc.= the six reversible substitution rates, pi(A), pi(C),etc.= the four stationary nucleotide frequencies, alpha= the shape parameter of the gamma distribution of rate variation across sites.

	95 % Credible Intervals		
Parameter	Lower	Upper	Median
TL	7.482000	12.638000	10.027000
r(A↔C)	0.054770	0.114756	0.080512
r(A↔G)	0.093027	0.202747	0.138357
r(A↔T)	0.061078	0.150351	0.099484
r(C↔G)	0.063368	0.121775	0.088850
r(C↔T)	0.389431	0.562282	0.479130
r(G↔T)	0.071256	0.151763	0.107019
pi(A)	0.170410	0.242611	0.205121
pi(C)	0.312430	0.391215	0.351000
pi(G)	0.260448	0.342132	0.300441
pi(T)	0.117247	0.170667	0.142056
alpha	0.189056	0.295139	0.235664

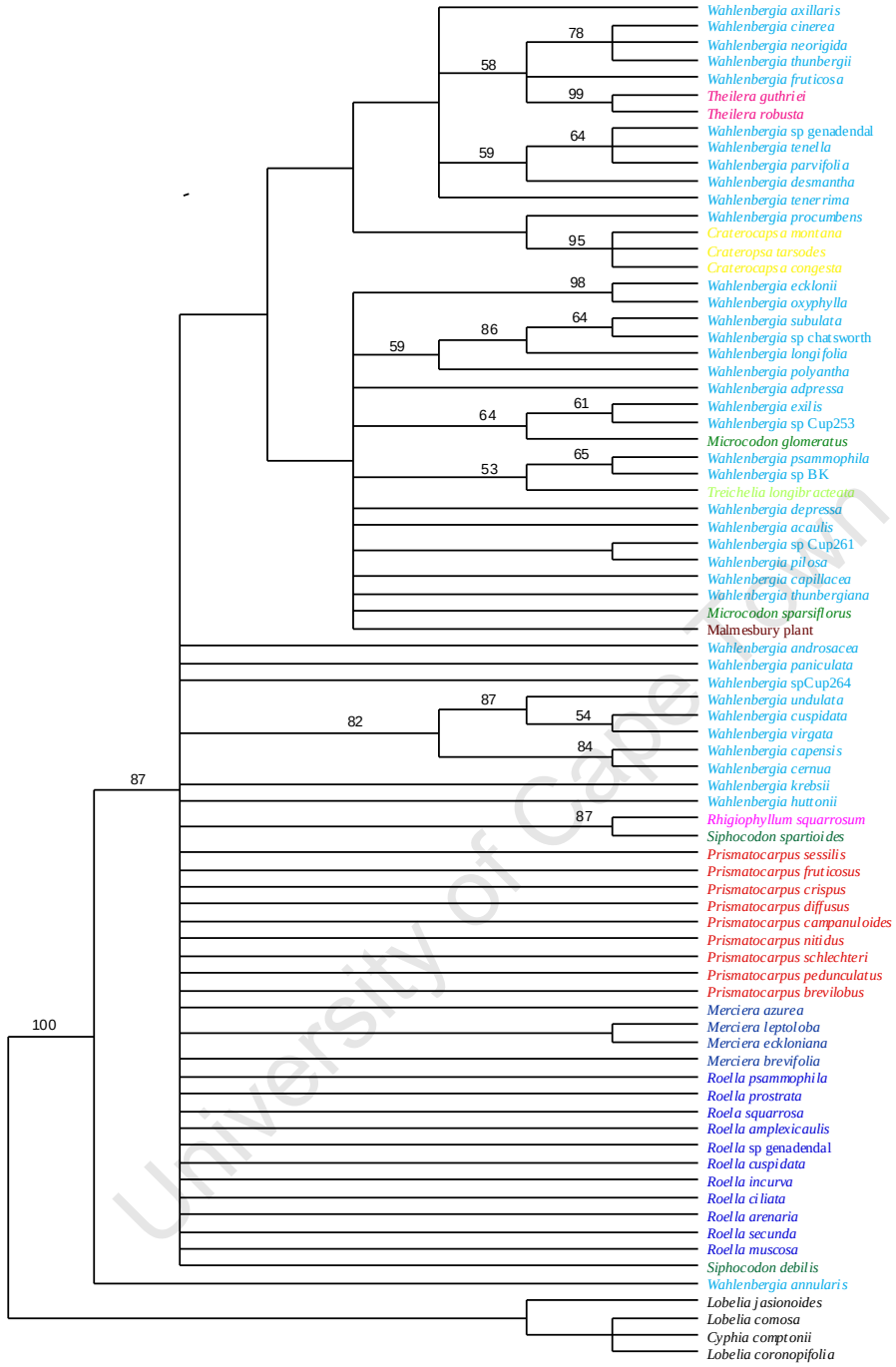


Figure 3.3. Strict consensus of 207 equally parsimonious trees (length=470, CI=0.536, RI=0.752) found after heuristic search of the ITS data set for 75 taxa of the South African Campanulaceae and four Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

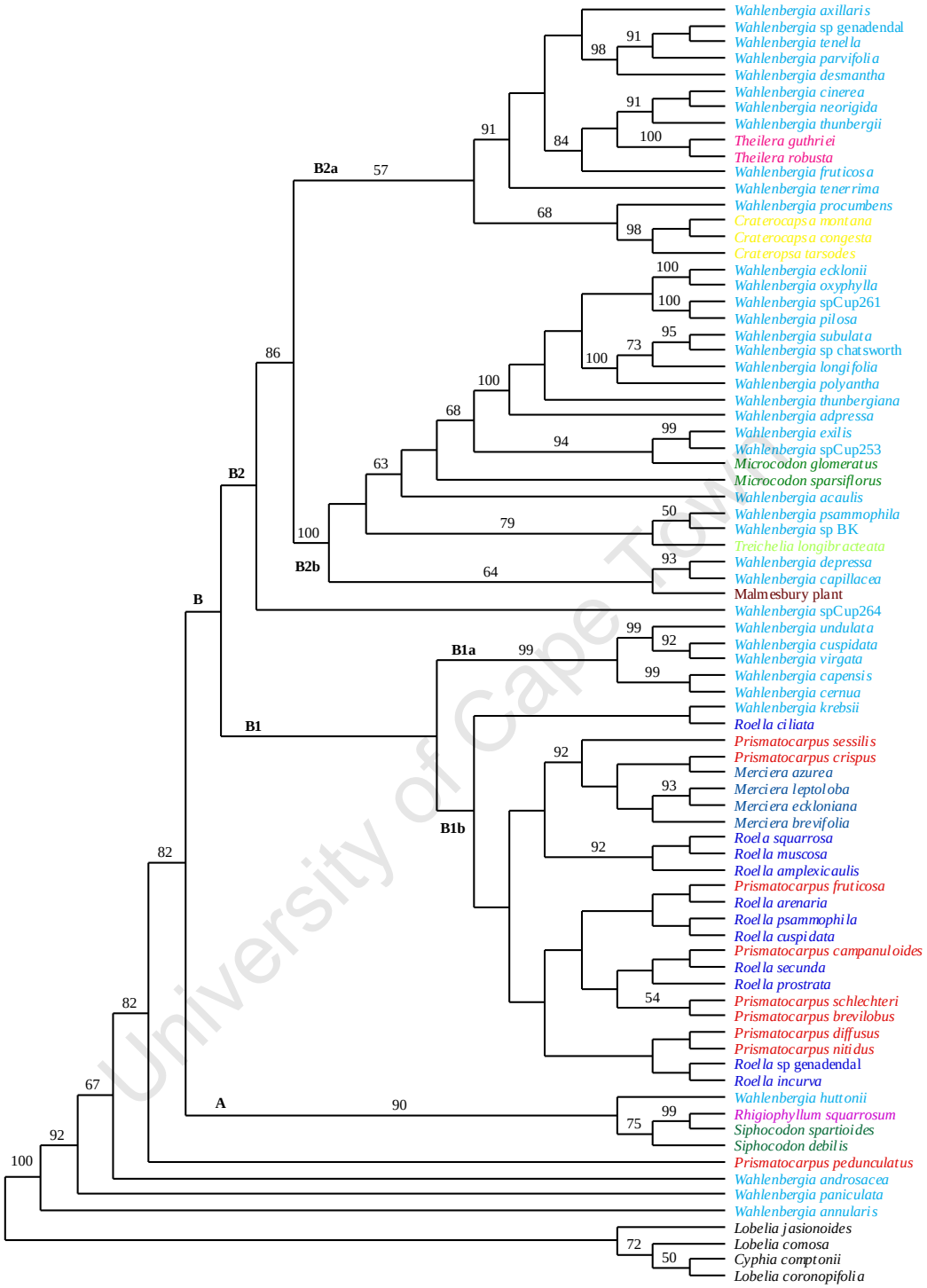


Figure 3.4. 50% majority rule consensus of trees retained in the Bayesian analysis of the ITS data set for 75 taxa of the Campanulaceae and four Lobeliaceae (outgroup). Numbers above branches indicate posterior probability values expressed as percentages.

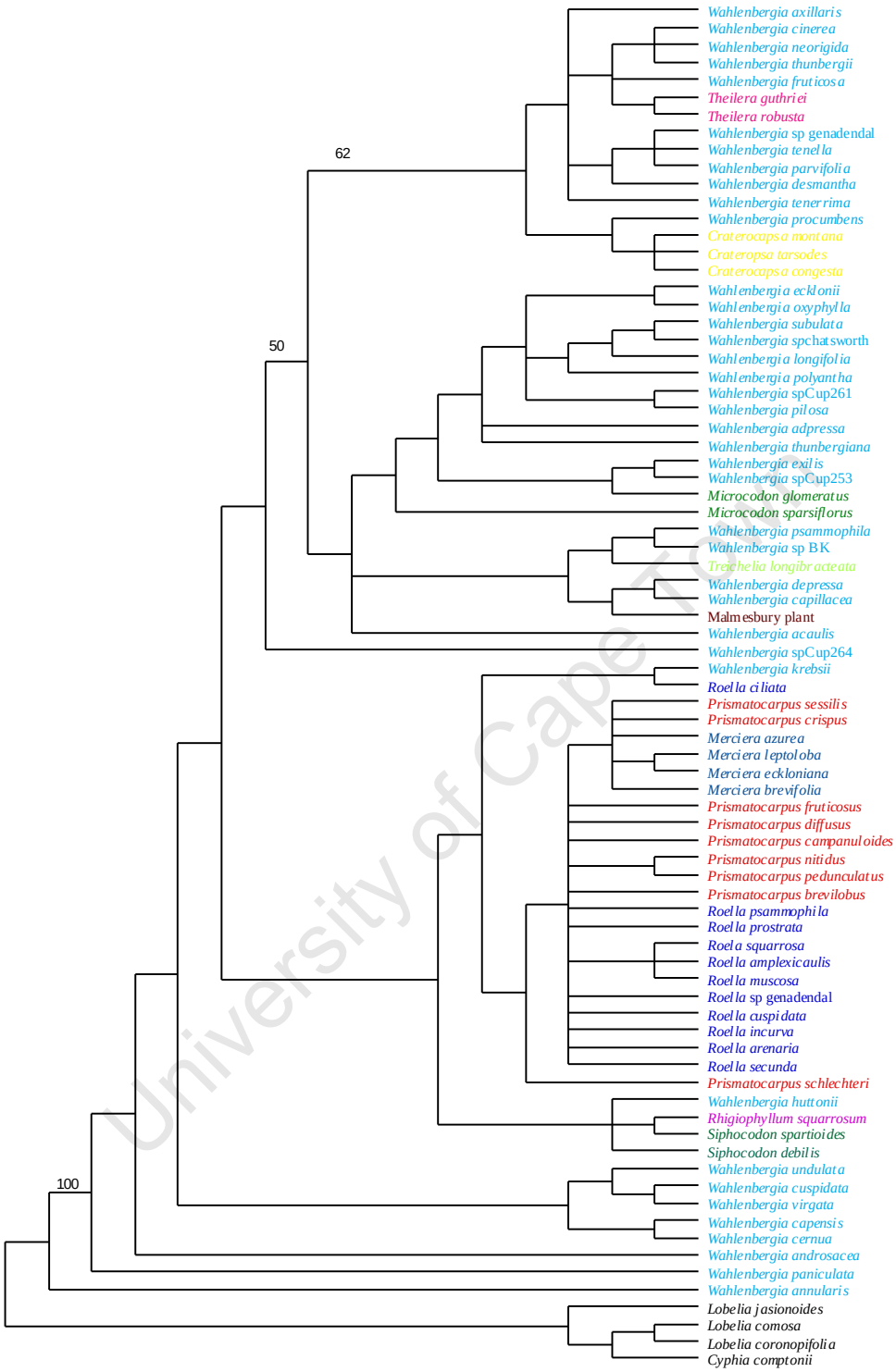


Figure 3.5. Strict consensus of 458 equally parsimonious trees (length=1844, CI=0.801, RI=0.880) found after heuristic search (weighted) of the ITS data set for 75 taxa of the South African Campanulaceae and four Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches

3.3.3. Data combinability

The partition homogeneity test found that the *trn*L-F and ITS data sets were significantly incongruent (p=0.01). However, a number of studies have shown that the results of the ILD test can be misleading (Wiens 1998, Dolphin *et al*. 2000, Reeves *et al*. 2001, Yoder *et al*. 2001 and Ramírez 2006). Despite the result of the ILD tests, the data sets were nonetheless combined because of the possibility of resolving more clades, reducing the number of parsimonious trees and obtaining better supported clades.

The poor resolution and support of the tree based on the ITS data set, makes it of limited use in comparing areas of conflict with the better resolved relationships found in the *trn*L-F analysis.

3.3.4. Combined *trn*L-F and ITS analysis

The combined data matrix consisted of 1201 characters, of which 619 were constant, 225 variable but parsimony uninformative and 357 (30%) parsimony informative. Two hundred and twenty two equally parsimonious trees were saved, of 1267 steps, a CI of 0.639 and a RI of 0.809.

The 50% majority rule consensus tree inferred from the Bayesian analysis (Figure 3.6) resolved similar clades as the maximum parsimony analysis, but is better resolved and supported. The trichotomy forming the large clade that is sister to *W. krebsii* in the parsimony analysis, is resolved (reduced to a dichotomy) under Bayesian inference. The nucleotide substitution model parameter values and their 95% credible intervals obtained from this analysis are summarized in Table 3.4.

A phylogram indicating the extent of divergence between the major clades is shown in Figure 3.7. The topology of the 50% majority rule consensus tree shows a high degree of congruence with the strict consensus trees of the separate *trn*L-F data set. As in the

*trn*L-F analysis *Wahlenbergia krebsii* appears isolated and is placed sister to the large clade that excludes *Rhigiophyllum* and *Siphocodon*. *W. annularis*, which resolved as sister to the rest of Campanulaceae in the ITS analysis, forms a clade (PP= 100) with seven *Wahlenbergia* species in which *P. crispus* is sister to the rest. The clade comprising *Wahlenbergia, Theilera, Craterocapsa, Treichelia, Microcodon* and the Malmesbury plant is discovered in both separate analyses with varying support. In the combined analysis the clade comprising *Rhigiophyllum* and *Siphocodon* is fully resolved whereas in the *trn*L-F analysis a trichotomy is formed. The *Roella-Prismatocarpus-Merciera* clade is common to the *trn*L-F analysis.

The largest common pruned tree for this combined data set (Figure 3.8) contains 48 of the original 75 taxa. This pruning resulted in the exclusion of *Siphocodon* and *Rhigiophyllum*.

Since the molecular data are not complete for all samples, the combined analysis introduces a taxon sampling concern. Separate analyses for each data set for which there is complete data were done to determine the effect of taxon sampling on the topology. In the strict consensus tree for *trn*L-F (Figure 3.9) (length= 772, CI= 0.738, RI= 0.874), clade C collapsed and together with *W. huttonii* participates in a five clade polytomy to form the large clade sister to *W. krebsii*. The collapse of clade C is influenced by the exclusion of 16 *Wahlenbergia* samples out the total of 22 excluded samples. The exclusion of a total of four ITS samples had no influence on the tree topology (Figure 3.10) (length= 444. CI= 0.541, RI= 0.743).

Table 3.4. Nucleotide substitution model parameter values from the Bayesian analysis of the combined *trn*L-F and ITS data sets for 72 taxa of the South African Campanulaceae. TL= total tree length, r(A↔C), r(A↔G), etc.= the six reversible substitution rates, pi(A), pi(C),etc.= the four stationary nucleotide frequencies, alpha= the shape parameter of the gamma distribution of rate variation across sites, {1}= partition 1, {2}= partition 2.

	95 % Credible Intervals		
Parameter	Lower	Upper	Median
TL {all}	2.020000	2.645000	2.289000
r(A↔C) {1}	0.147314	0.218798	0.180918
r(A↔G) {1}	0.133927	0.202563	0.165843
r(A↔T) {1}	0.041845	0.072940	0.055775
r(C↔G) {1}	0.213444	0.308708	0.258665
r(C↔T) {1}	0.144146	0.214634	0.176935
r(G↔T) {1}	0.126528	0.195723	0.158220
r(A↔C) {2}	0.097513	0.156083	0.124421
r(A↔G) {2}	0.115925	0.189380	0.149599
r(A↔T) {2}	0.063054	0.132507	0.094143
r(C↔G) {2}	0.103384	0.158122	0.128720
r(C↔T) {2}	0.325316	0.437775	0.380003
r(G↔T) {2}	0.088379	0.155896	0.118978
pi(A) {1}	0.294232	0.346265	0.319821
pi(C) {1}	0.158987	0.200454	0.178873
pi(G) {1}	0.159991	0.200888	0.179802
pi(T) {1}	0.295139	0.346996	0.320814
pi(A) {2}	0.166487	0.228764	0.196158
pi(C) {2}	0.344664	0.416197	0.379924
pi(G) {2}	0.258594	0.329699	0.293326
pi(T) {2}	0.108241	0.153283	0.129463
alpha {1}	0.518585	0.830770	0.656351
alpha {2}	0.639444	1.456534	0.971668
pinvar {2}	0.197161	0.411486	0.321389

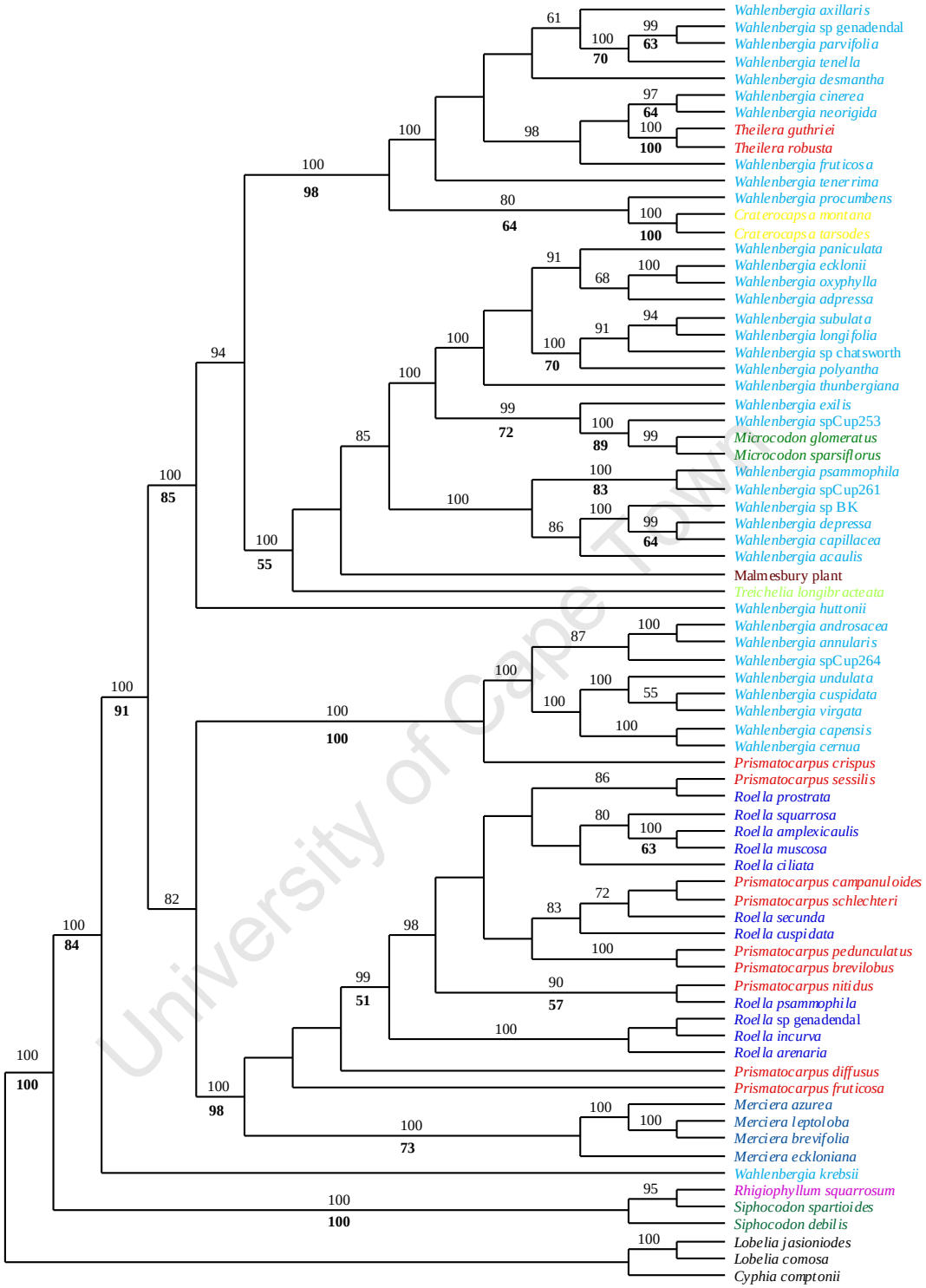


Figure 3.6. 50% majority rule consensus of trees retained in the Bayesian analysis of the combined *trn*L-F and ITS data sets for 72 taxa of the Campanulaceae and three Lobeliaceae (outgroup). Numbers above branches indicate posterior probability values expressed as percentages. Bootstrap values ≥50% are indicated below the branches.

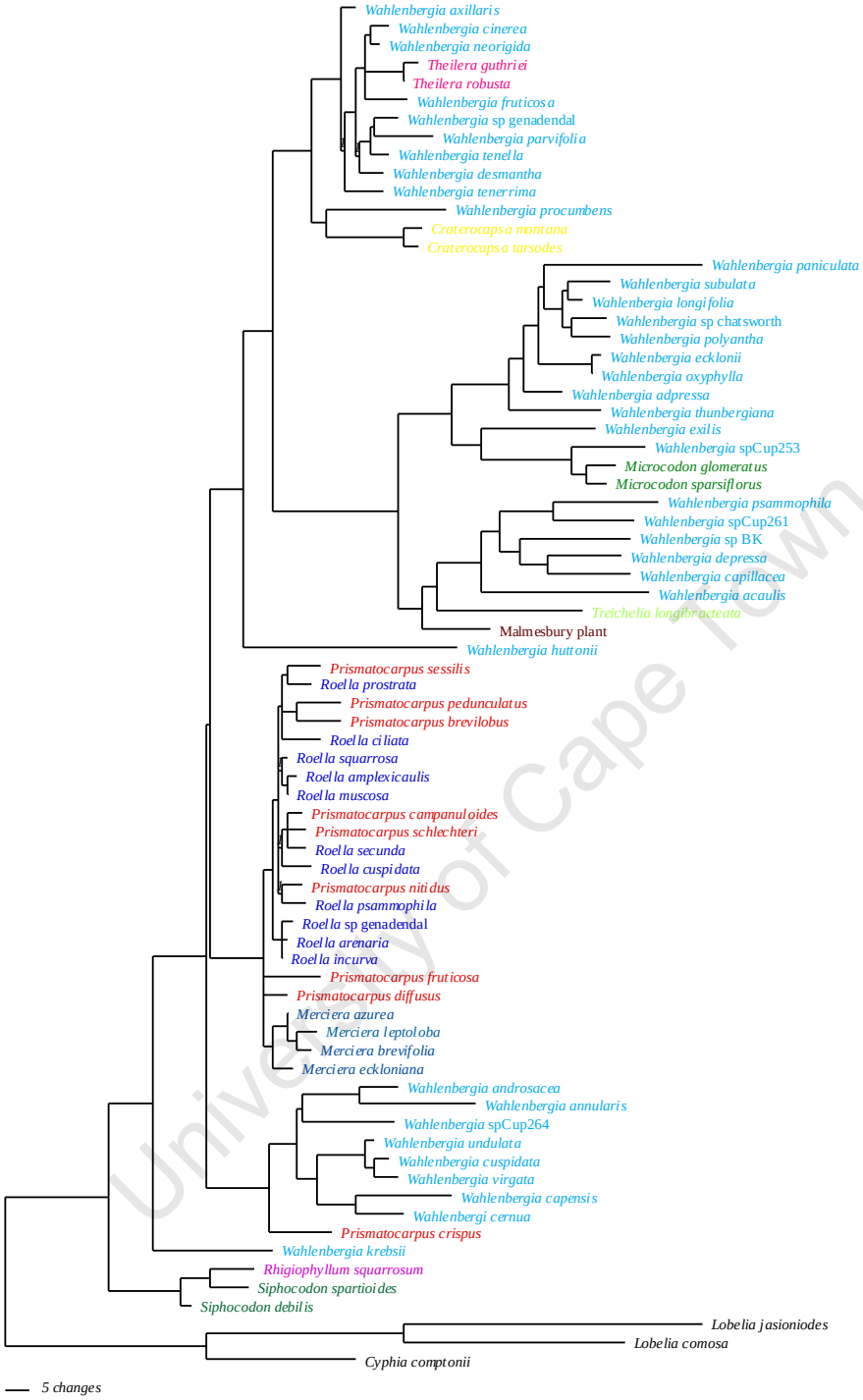


Figure 3.7. Phylogram of one of the 222 equally parsimonious trees found after heuristic search of the combined *trn*L-F and ITS data sets of 72 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup) A scale bar representing 5 changes is shown on the bottom left corner.

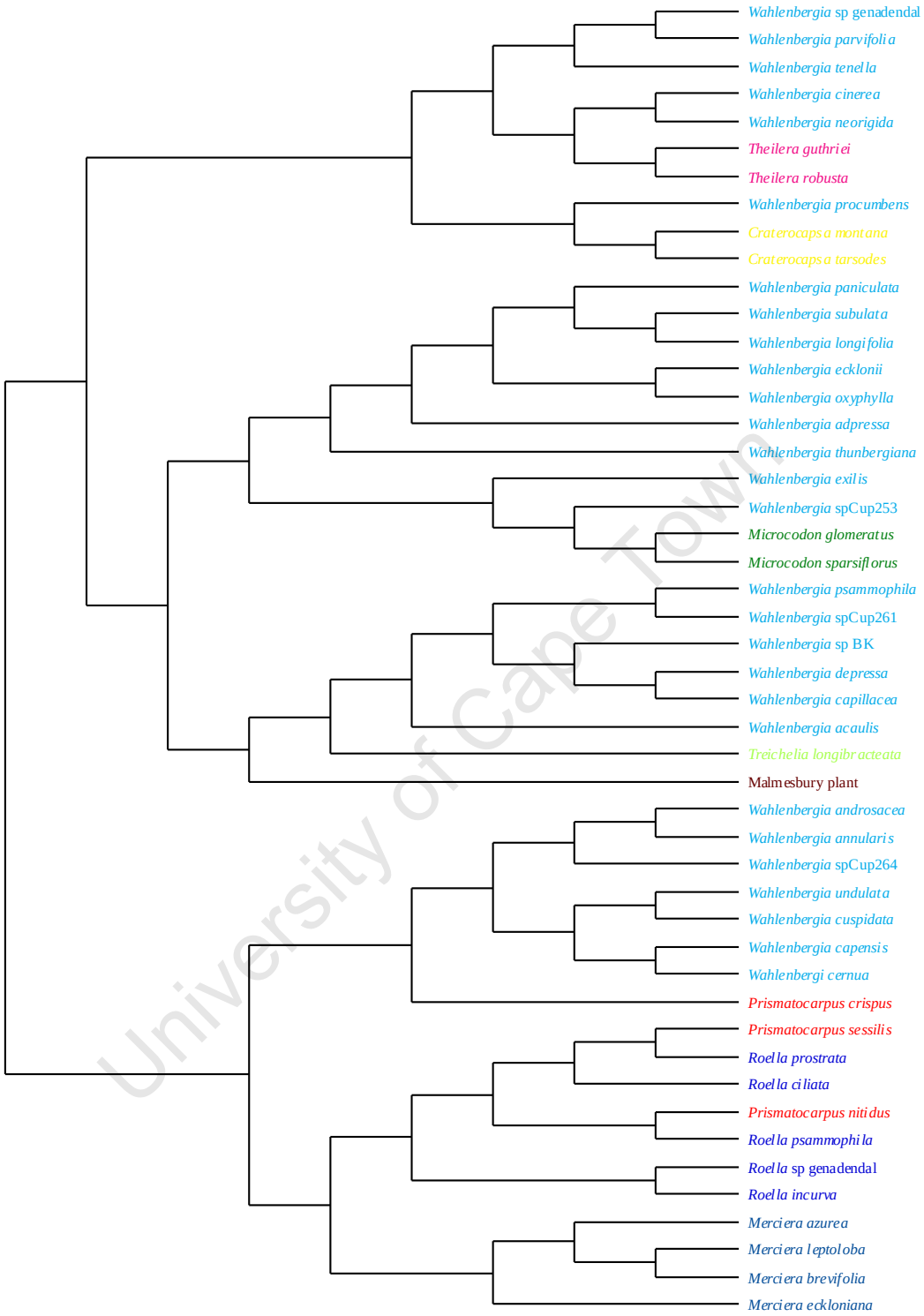


Figure 3.8. Largest common pruned tree found after agreement subtrees search of the combined *trn*L-F and ITS data sets for 72 taxa of the South African Campanulaceae and three Lobeliaceae (outgroup).

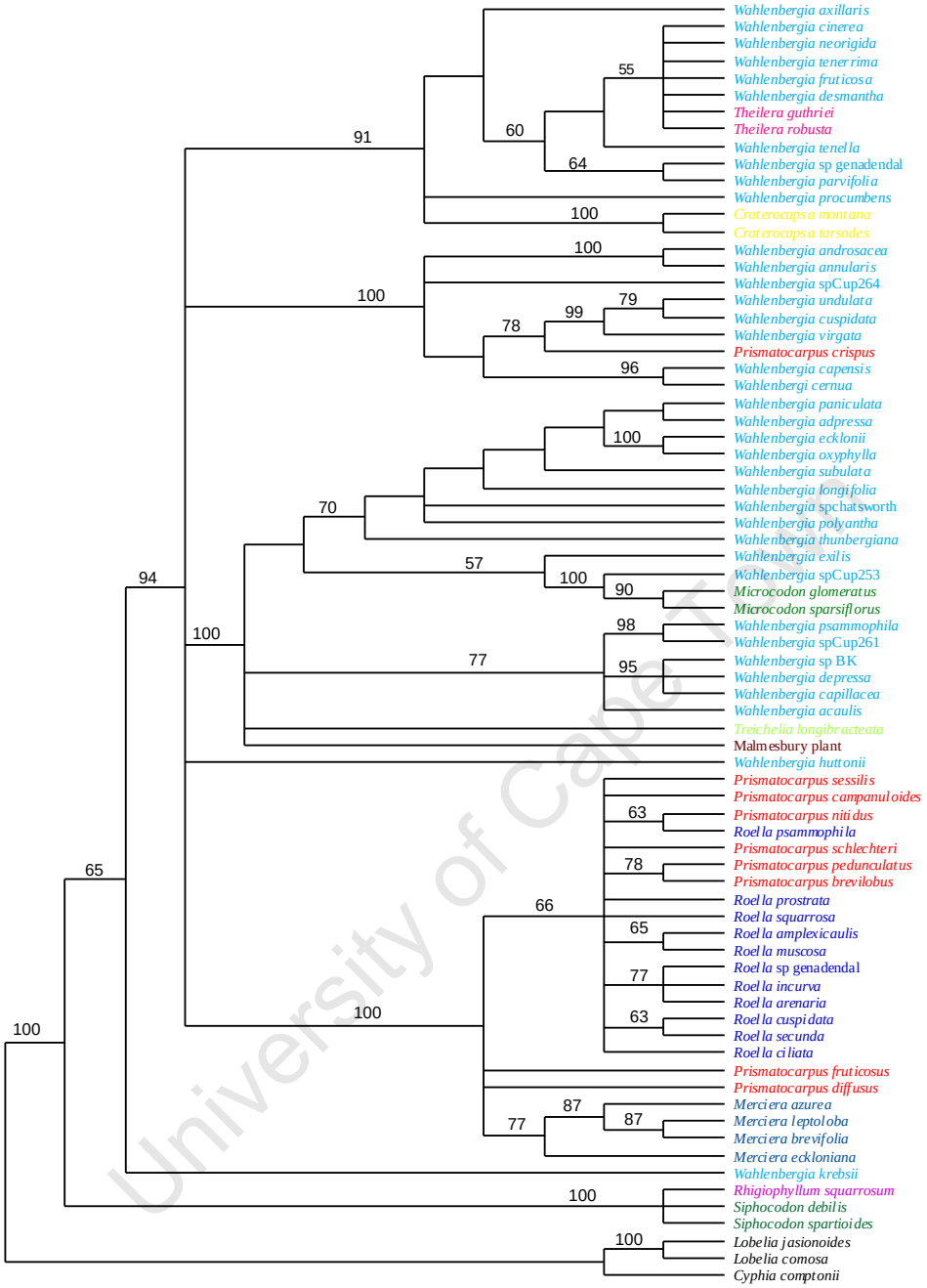


Figure 3.9. Strict consensus of 161 equally parsimonious trees (length=772, CI=0.738 RI=0.874) found after heuristic search of the reduced *trn*L-F data set for 72 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

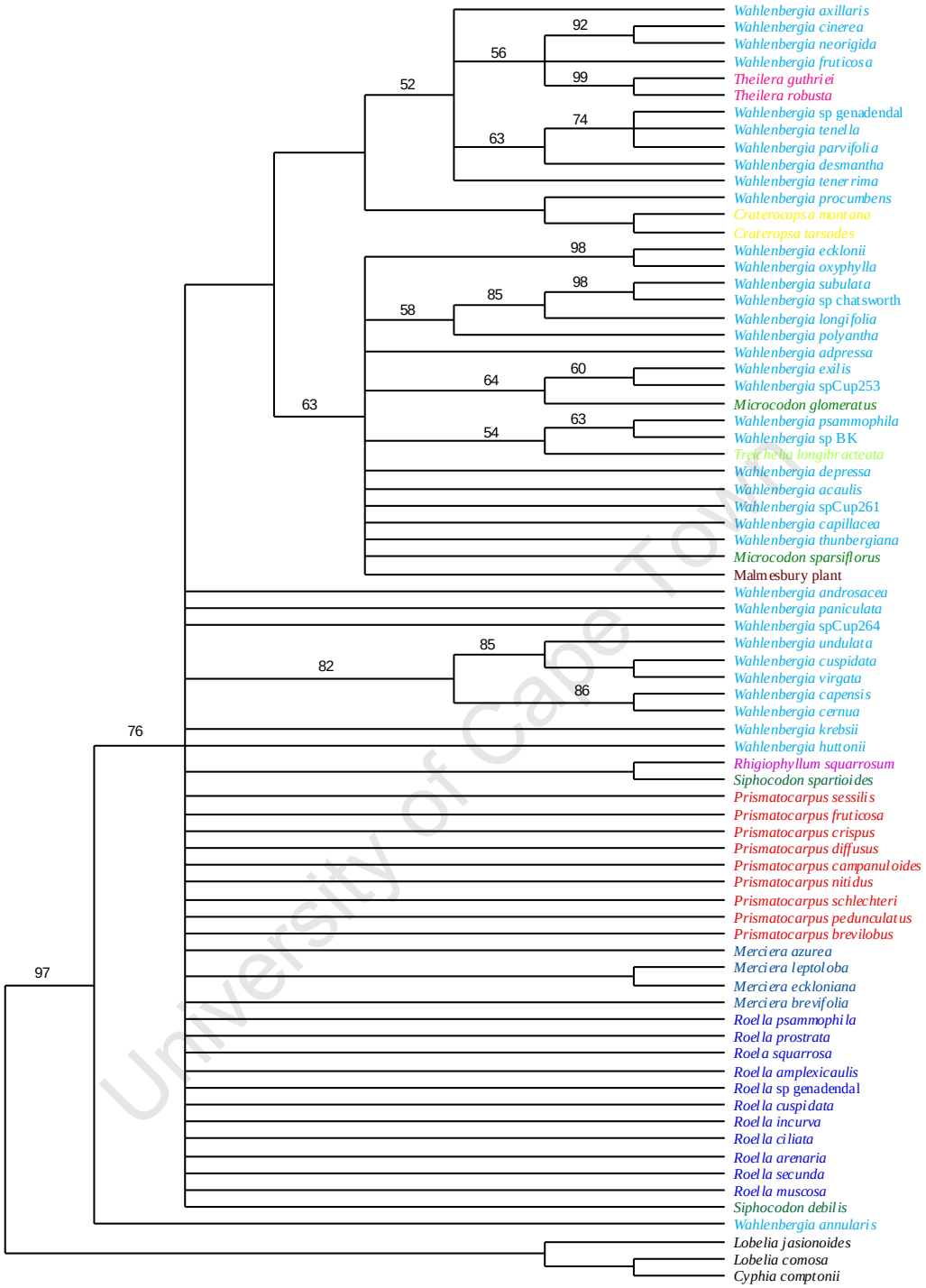


Figure 3.10. Strict consensus of 205 equally parsimonious trees (length=444, CI=0.541, RI=0.743) found after heuristic search of the ITS data set for 72 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

3.4. Age estimates

The South African Campanulaceae are estimated to have started diversifying in the mid Oligocene (28 mya) (see Chapter 2). The age estimates for each of the major clades found by the phylogenetic analyses are shown in Figure 3.11. *Rhigiophyllum-Siphocodon* split from the rest of the Campanulaceae 25 mya and started to diversify 4 mya. The remaining clades diversified between 8 and 22 mya.

Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia diversified about 19 mya at the same time as the separation of the *Roella-Prismatocarpus-Merciera* and *Wahlenbergia-P. crispus* clades from each other. The age estimates for the *Roella-Prismatocarpus-Merciera* and *Wahlenbergia-P. crispus* clades are 8 and 12 mya respectively.

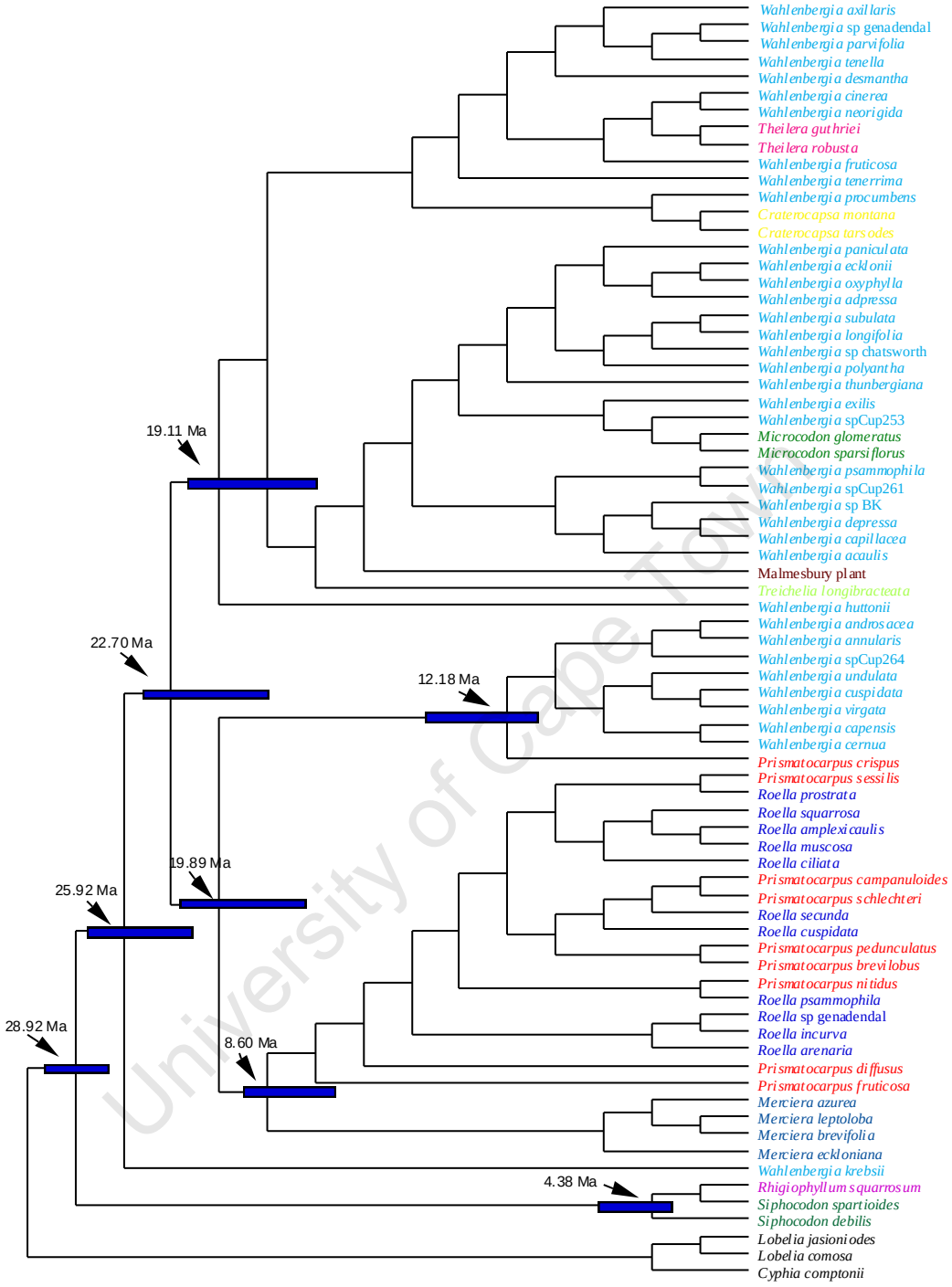


Figure 3.11. A summary of the divergence times for all major South African Campanulaceae clades, obtained using Bayesian inference as implemented by the program BEAST (Drummond *et al*. 2002, Drummond and Rambaut 2006a). The blue bars represent the 95% highest posterior density (HPD) interval for the divergence times.

3.5. Discussion

3.5.1. Effects of character weighting on topology

In phylogenetic analysis every attempt should be made to find the best phylogenetic estimate for a given data set, with a fully resolved, well-supported tree topology the ultimate goal. Two character weighting schemes, equal weighting (Fitch 1971) and successively approximated weighting (Farris 1969) were used in this study in an attempt to obtain good phylogenetic signal. Successive weighting reduces the effect of homoplasious characters on the tree topology, but is often criticized for introducing assumptions into the data set because certain characters are excluded and others replicated.

The topologies of the equally and successively weighted trees for *trn*L-F are identical, but in the case of the ITS gene region the successively weighted tree is better resolved than the unweighted tree (Figure 3.5). However the successively weighted trees have higher CI and RI values. This is expected as successive weighting is designed to minimize the involvement of homoplasious characters in estimating phylogenies. Support for the major clades is not significantly different between the weighting schemes. None of the two weighting schemes conclusively provides a better phylogenetic signal than the other. Since neither of the unweighted analyses has much apparent homoplasy, one would not expect weighting to have a significant effect.

3.5.2. Comparative utility of the two gene regions

Of the two regions used, *trn*L-F produced more informative and robust results under both maximum parsimony and Bayesian approaches. However, there are two instances where Bayesian approaches recovered higher support for major clades. The ITS data set, lack resolution under maximum parsimony, but the clades recovered under Bayesian approaches lack support and therefore do not strongly contradict the maximum parsimony results. The lack of resolution may be ascribed to either character conflict or a scarcity of characters. In this study, the removal of unalignable regions

across the complete ITS data set might have contributed to the scarcity of characters that potentially could have resolved relationships.

The *trn*L-F data set resolved 21 strongly supported nodes, whilst the one supported by the ITS data set was among these 21. The ITS data set provided a slightly lower percentage of parsimony informative characters (30% of 353 for ITS, 35% of 848 for *trn*L-F) than the *trn*L-F data set. The number of South African accessions sequenced for the ITS gene region was increased from 12 in Chapter 2 to 75 in this chapter. This represents 30% of known species in South Africa. Although this sampling is by no means comprehensive, it represents all known genera, all life forms, and species from across the geographical range of the family. Those species that are absent are morphologically similar to one of the included representatives of their respective genera. Relationships within the South African clade in Chapter 2, are ambiguous but when the sample size is increased the resolution decreases (under maximum parsimony) which made relationships between taxa even more ambiguous. Even though the addition of more taxa affected the topology, it is evident that compared to the combined analysis, adding more characters is unlikely to change the topology.

In the *trn*L-F data set the consistency and retention indices obtained are slightly higher than in the ITS data set, suggesting a lower lever of homoplasy. The percentage of variable characters is almost identical for the two data sets.

Neither region was useful in resolving relationships among *Roella*, *Prismatocarpus* and *Merciera*, possibly due to insufficient variation among representatives of these genera. Within this clade the alignment yielded 13% variable characters for *trn*L-F and 20% for ITS. The short branch lengths as shown by the phylogram suggest a slow-down in evolutionary rate for this group or that it radiated recently. However, *trn*L-F resolves *Merciera* as monophyletic. The *trn*L-F region appears to be useful for resolving major lineages in the Campanulaceae and is also easy to amplify and align.

3.5.3. Generic limits and age estimates

The molecular data do not support all the current generic circumscriptions in the family, but rather five species assemblages become apparent, 1. *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia*, 2. *Wahlenbergia-P. crispus*, 3. *Roella-Prismatocarpus-Merciera*, 4. *Wahlenbergia krebsii*, 5. *Rhigiophyllum-Siphocodon*.

1. *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia*

Wahlenbergia, the core genus in South Africa, is comparable to the northern hemisphere's *Campanula* in diversity and its extensive distribution. It is, however, not monophyletic, with *Theilera, Craterocapsa, Microcodon* and *Treichelia* all nested within it. These genera are part of the *Wahlenbergia* line of diversification, and each of them has probably adapted to unique ecological conditions such as fire, rainfall and soil type. The common ancestor of this clade is estimated to have lived 19 million years ago with each of these nested genera evolving either during the late Miocene or Pliocene (*Treichelia*= 8.9 mya, *Microcodon*= 5 mya, *Craterocapsa*= 1.8 mya, *Theilera*= 1.2 mya). This period coincides with the climatic shift from summer-wet to summer-dry conditions, and the eventual establishment of the present day Mediterranean-type climate (Coetzee 1983). Towards the end of the Miocene, an east-west rainfall gradient developed due to uneven uplift of the margins of the southern African pediplain (Linder 2003). It is assumed that the summer-dry climate increased the frequency of fire that ultimately became an important ecological factor, particularly in the Cape Floristic Region. These climatic and topographical changes provided diverse habitats, each with its unique set of selective pressures on the species that occupy them. Most of the *Wahlenbergia* species adapted to the summer-dry conditions and fire by developing a shrubby habit that allows them to die back and resprout. *Treichelia* and *Microcodon* have adapted to the same conditions as spring flowering annuals that survive the harsh summer as seed, whereas *Craterocapsa* and *Theilera* occupy areas where these conditions are absent.

Previous authors separated these genera from *Wahlenbergia* because of the importance placed on differences in the mode of capsule dehiscence or floral morphology. However, *Theilera* and *Microcodon* share the same mode of capsule dehiscence with *Wahlenbergia*. *Theilera* was most likely separated from *Wahlenbergia* because of its tubular corolla. The genus is also restricted to the drier montane areas from the Swartberg near Oudtshoorn, to Willowmore where the rainfall is mainly in summer and fire absent. In *Microcodon*, the locules alternate with the calyx lobes instead of being opposite to them, as in the case of *Wahlenbergia* species with a five locular ovary.

The close relationship between *Craterocapsa* and *Wahlenbergia* is interesting since two of the four species of *Craterocapsa* were originally described as either *Wahlenbergia* or *Roella*. Thulin (1975) suggested a close relationship between *Craterocapsa* and *Roella* based on the resemblance in capsule dehiscence, which takes place by an apical operculum. This suggestion is surprising because *Roella* comprises shrublets (except *R. muscosa* which is herbaceous) and *Craterocapsa* herbs. In addition *Roella* occurs mainly in the south-western Cape (except *R. glomerata* which extends into the Eastern Cape and KwaZulu Natal) while *Craterocapsa* occurs only in the Eastern Cape and KwaZulu Natal. The species of *Craterocapsa* separated from *Roella* was not sampled for this study. In the combined analysis, the *Craterocapsa* species are sister to *W. procumbens* with which they share a prostrate habit.

Although the molecular data suggest that these genera are most probably congeneric with *Wahlenbergia* it does however support them as coherent separate groups within a larger *Wahlenbergia*.

The recognition of *Lightfootia* as a distinct genus from *Wahlenbergia* is not supported by these data, reaffirming Thulin's (1975) union of the two. It was separated mainly on the basis of corolla structure, style length and habit. All these characters overlap between the two genera and are not useful for generic distinction. Despite its shrubby habit, several species (*W. adpressa, W. axillaris, W. cinerea, W. desmantha, W. huttonii, W. longifolia, W. neorigida, W. nodosa, W. parvifolia, W. polyantha, W.*

rubioides, *W. subulata*, *W. tenella*, *W. tenerrima*, *W. thunbergiana*, *W. unidentata*) previously treated as *Lightfootia*, are associated with herbaceous *Wahlenbergia* species as well as the shrubby *Theilera*. These data also support the view that *Theilera* is simply a *Wahlenbergia* with tubular flowers.

The close relationship between *Treichelia* and the Malmesbury plant provides insight into the classification of this plant. Efforts to identify this plant collected at Malmesbury, ±60 km North west of Cape Town led to an interesting taxonomic inquiry. It was first collected on Lion's Head in Cape Town and named *W. depressa* by Wolley-Dod (1901). Unfortunately this name was already in use for a *Wahlenbergia* species described by Wood and Evans (1897) from Van Reenen's Pass in KwaZulu Natal. Adamson (1950) realized the illegitimacy of the name and re-identified the specimen as *T. longibracteata*. However, several morphological characters separating the two species, such as leaf shape, locule number and epigynous disc shape, were ignored. The molecular data suggest that the two species form a coherent group within the larger *Wahlenbergia* group. It would therefore be appropriate to recognize the Malmesbury plant as a distinct taxonomic entity in future taxonomic treatments.

2. *Wahlenbergia-P. crispus*

Prismatocarpus crispus, one of two herbaceous (annual) species in *Prismatocarpus* is nested within a strongly supported clade comprising several herbaceous *Wahlenbergia* species. The other annual species, *P. hildebrandtii* Vatke, was not sequenced in this study because collecting efforts failed. Thulin (1974) found that this species, as treated by Adamson (1952) was heterogeneous. He then transferred all the Dinter collections from Namibia to a new genus *Namacodon.* The remaining specimens were the type collection from the Hatamberg (Meyer 1896) and a collection from Vanrhynsdorp (Esterhyusen 1422). The type was probably destroyed in Berlin during the war and the other one is deposited in the Bolus Herbarium, Cape Town. Examination of this specimen strongly suggests that *P. hildebrandtii* is conspecific with *P. crispus*. The placement of *P. crispus* is surprising, but it is similar in all analyses even after having been re-sequenced from different individuals to eliminate potential sampling errors.

Apart from the herbaceous habit, *P. crispus* also shares a funnel-shaped corolla with these *Wahlenbergia* species. However their modes of fruit dehiscence differ. In the case of *Wahlenbergia* dehiscence is by apical valves, while that of *P. crispus* is by longitudinal slits that do not correspond with the calyx lobes. The relationship between *P. crispus* and *Wahlenbergia* requires further study.

The common ancestral species of this clade is estimated to have lived 12 million years ago when the flora of southern Africa was tropical (Linder and Hardy 2004). When the climate became drier the tropical flora was largely decimated, leaving relics such as *Prionium, Metrosideros* and *Brabejum* behind. The nesting of species (*W. androsacea, W. virgata, W. undulata*) shared with tropical Africa in this clade corroborates the affinity between the two floras and perhaps suggests a northward migration of these species.

3. *Roella-Prismatocarpus-Merciera*

The close relationship between *Roella, Prismatocarpus* and *Merciera*, as suggested by Adamson (1952, 1955b), is confirmed by the molecular data of this study and that of Cosner *et al*. 2004. Adamson postulated that *Roella* and *Prismatocarpus* are derived from a common ancestor and that *Merciera* was derived from *Roella* series *Roella* (as *Ciliatae*). This series comprises eight species: *R. ciliata* L., *R. incurva* Banks ex A.DC., *R. rhodantha* Adamson, *R. maculata* Adamson, *R. triflora* (R.D.Good) Adamson, *R. dregeana* A.DC., *R. psammophila* Schltr., *R. dunantii* A.DC. All extant taxa of this clade can be trace back to a common ancestral species that lived 8 million years ago with *Merciera* appearing about 2 million years ago. This Cape floral clade, according to the definition used by Linder (2003,) can be associated with the establishment of the fynbos vegetation and radiated in response to drought and fire (Linder and Hardy 2004). *Merciera* for example, resprouts and grows prolifically after fire, but after a long absence of fire the plants become moribund and start disappearing from the veld (Cupido 2006). Vegetatively, it is not always possible to separate *Merciera* from species of *Roella* series *Roella* (Cupido 2006). Adamson (1952) also

stated that without knowledge of the mode of capsule dehiscence it is difficult to assign some species of *Roella* and *Prismatocarpus* to one genus or the other.

The extent of morphological variation within *Roella* and *Prismatocarpus* prompted Adamson (1952) to subdivide these genera. *Roella* is divided into five series and *Prismatocarpus* into two sub-genera. The one subgenus, Euprismatocarpus is further subdivided into three series. Due to the largely unresolved relationships among species of these genera, no support for the subgeneric classification of Adamson (1952) is evident. The paraphyletic nature of these two genera casts doubt on the value of the single fruit character in indicating generic limits. Only a better resolved tree topology would help to detect relationships between these. Species of *Merciera* formed a weakly supported monophyletic group in the separate *trn*L-F topology. The generic status of these taxa is further discussed in Chapter 5.

4. *Wahlenbergia krebsii*

The isolated position of *W. krebsii* needs further investigation. Thulin (1975) placed this species with *W. pusilla* in a group based on unique seed morphological features, but never doubted its wahlenbergioid nature. It is a variable species that Thulin (1975) subdivided into two subspecies. *W. krebsii* subspecies *krebsii* is southern African, occurring in Lesotho and all the South African provinces except the Western and Northern Cape. The other subspecies, *W. krebsii* subspecies *arguta* is found throughout tropical Africa. Because sampling errors were initially suspected this species was re-sequenced for the *trn*L-F data matrix, but its position on the tree topology remained unchanged. In the case of the North American Campanulaceae seed morphology proved helpful in revealing recognizable generic patterns (Shetler and Morin 1986), but such information is incomplete for the South African taxa.

5. *Rhigiophyllum-Siphocodon*

The most obvious morphological similarity between these two genera is the epipetalous stamens. Both genera are limited to the south-eastern parts of the Western

Cape. *Rhigiophyllum* is endemic to the Napier-Bredasdorp area whereas *Siphocodon* occurs from Sir Lowry's Pass to Riviersonderend. Adamson (1955b) suggested that *Rhigiophyllum* was derived from *Roella* series *Squarrosae* (*R. amplexicaulis* Wolley-Dod, *R. decurrens* L'Hér., *R. squarrosa* P.J.Bergius) possibly because of leaf structure and arrangement. *Rhigiophyllum* has the same tubular corolla structure as *Merciera*, *Theilera* and *P. diffusus* but none of these taxa was considered as a possible ancestral stock of *Rhigiophyllum* by him. The *Rhigiophyllum-Siphocodon* clade is sister to the rest of the sampled South African Campanulaceae and trace back to a common ancestor that lived 28 million years ago. After the initial split 24 million years passed before the current speciation of this lineage (4 mya) (Figure 3.11). The relatively large interval after the initial divergence could imply that the radiation during the Oligocene was followed by large-scale extinction during the wetter, warmer Miocene or to low rates of speciation.

The molecular results co-incide with the discovery of a unique pollen morphology in *Rhigiophyllum* and *Siphocodon* by Bill Eddie, John Skarvla and myself, which further supports the affinity between the genera. A paper is in preparation to further discuss the pollen structure and its taxonomic value. *Rhigiophyllum* and *S. spartioides* form a sister relationship in the ITS tree. In addition to the previously mentioned characters, these two species have the same seed morphology and number of locules. However, other than similarity in these mostly inconspicuous characters they are morphologically distinct.

The molecular evidence presented here is clearly in disagreement with the current classification in the family. In general, the results do not support the recognition of the numerous smaller genera within the Campanulaceae and it highlights the questionable classification of *P. crispus* in *Prismatocarpus*. Although some of these smaller genera are embedded within *Wahlenbergia* each of them form a coherent group that is morphologically recognizable as separate entities that have speciated recently. The age estimates of the major clades suggest that the radiation of the Campanulaceae

correlates with dramatic climatic and topographical changes in southern Africa that was initiated during the Oligocene.

CHAPTER 4

MORPHOLOGY AND MOLECULES; ANTAGONISTIC OR CORROBORATIVE EVIDENCE ON RELATIONSHIPS OF SOUTH AFRICAN CAMPANULACEAE? AN ANALYSIS AND EXPLORATION OF CHARACTER EVOLUTION

4.1. Introduction

Systematics as a synthetic scientific discipline derives its data from a variety of sources, for example anatomy, cytology, morphology, chemistry and molecular biology, to evaluate patterns of biological variation. The observed patterns are described, causes and consequences investigated, and manipulated to produce a predictive system of classification and to demonstrate evolutionary relationships among organisms. The basic units that provide taxonomic evidence are characters. The science of visible characters is morphology (Webber 2003). Many definitions of character exist in the literature. It is defined as 'any feature whose expression can be measured, counted or otherwise assessed' (Davis and Heywood 1963) or, for the purpose of phylogenetic analysis, as 'a feature that can be evaluated as a variable with two or more mutually exclusive or ordered states (Pimentel and Riggins 1987).

Phylogenetic analysis comprises two steps (Thiele 1993): exploration (discovery, selection, delimitation and resolution of characters and taxa) and analysis of the discovered data to construct a set of cladograms that explain the distribution of characters over the taxa. These two steps, also referred to as primary and secondary assessment (de Pinna 1991), are interlinked. Primary homology involves two stages (Brower and Schawaroch 1996): the first is the choice of characters by means of comparative morphological study among the taxa in question (topographic identity), whilst the second is the partitioning of characters into states that are then coded and assigned to terminal taxa as one column in the data matrix (character state identity). This data matrix represents a set of primary homology statements (Hawkins *et al*. 1997) that has often been regarded as subjective (de Pinna 1991) because of different

character coding techniques used by different investigators (see Hawkins 2000). Ultimately the primary homology statement influences the outcome of phylogenetic analyses (Pleijel 1995).

Two aspects of character coding have received attention in the literature. The first is qualitative versus quantitative data, and delineation problems of different character states within a character (Stevens 1991). According to him quantitative characters are continuous and therefore not suitable for phylogenetic analyses, in which character states have to be discrete. Pimentel and Riggins (1987) argued that quantitative variables are suitable for phylogenetic analysis when their ranges do not overlap and they can be ordered. The single quantitative character included in this analysis, meets these requirements.

The second aspect focuses on delineation problems between characters and character states (Pimentel and Riggins 1987, Pleijel 1995). The main concern is whether to code character states as multistate variables or as binary (absent/present) variables. This was particularly problematic for the capsule dehiscence character for which nine states were assigned. The first coding option is advocated by Pimentel and Riggins (1987) who considered treating cladistic characters independently as present, or absent as a bad practice, because the multistate character is given more weight and redundancy is introduced into the data. Pleijel (1995) favoured the binary method because it is simpler and avoids problems with non-applicable states. I favoured the second option to avoid making unnecessary assumptions on transformation of character states. In the three cases where multistate coding was applied, these were treated as unordered.

Primary homology assessment in DNA sequence data is straightforward because characters and character states are usually clearly defined. The characters are the positions of the bases themselves, and the character states are the bases present at the position. However, the number of positions is likely to vary, resulting in sequences of unequal lengths. These differences in length have lead to the development of procedures to line up these bases by the insertion of gaps (Wheeler 1996). The

different treatment of gaps, whether as missing data or as character states has been demonstrated to influence the resulting phylogenetic hypothesis (Simmons and Ochoterena 2000).

4.1.2. Morphological phylogenetic studies in the Campanulaceae

The current classification of the Campanulaceae, which is based on morphology, appears to be contradicted by the molecular phylogeny (See chapter 3). However, since no morphological phylogeny for the Campanulaceae exists, it is impossible to confirm this notion. Lammers' (1996) phylogenetic analysis of *Wahlenbergia* in the Juan Fernández Islands, which was used as a framework to investigate patterns of diversification and distribution within the archipelago, is the only morphology-based study for the family. The phylogenetic utility of the various possible suites of morphological characters is thus unknown for the Campanulaceae. This study explores the potential utility of macro-morphological characters as a starting point for future studies. The definition of a character as 'any feature that we think will provide information to use in phylogenetic analysis…' (Steven 2000) was used in this study.

The evolution of South African Campanulaceae appears to have centred around *Wahlenbergia*, from which several smaller genera evolved, displaying diverse characters. The variation displayed by the mode of capsule dehiscence has been crucial in Campanulaceae taxonomy. However, its importance in defining monophyletic genera is questioned in this study, as it appears to be homoplasious (See Chapter 1). However, unlike in groups such as the Brassicaceae (Mummenhoff *et al*. 2005), the evolution of the fruit has never been interpreted within a phylogenetic framework. A phylogenetic hypothesis can be used in several ways to investigate characters. For example, to determine which characters are useful for classification, i.e. identifying synapomorphies for monophyletic groups, or to provide insight into the patterns of morphological evolution (Archibald 2003).

The use of a phylogeny to interpret the evolution of morphological characters, although now accepted as standard practice, is not without inherent problems or

assumptions. It treats the phylogeny as if it existed before the evolution of the characters and assumes that the characters had no influence on the topology of the tree (Maddison 2006). The approach of using trees, based completely or partially on morphological data to optimize characters, has been considered as circular reasoning (e.g. Hedges and Maxson 1996). This argument assumes that the independence among characters is compromised and may bias the analysis (Luckow and Bruneau 1997). It is therefore desirable to exclude any character from the analysis that may be correlated with the evolutionary question (Coddington 1988, Brooks and McClennan 1990, Armbruster 1992). This act of excluding characters may lead to weaker phylogenetic hypotheses. Scharaschkin and Doyle (2006) point out that the topology of any tree is based on not only the character under investigation, but on all other characters included in the analysis, which together provide a phylogenetic hypothesis of the study group. It is further assumed that molecular phylogenies are better than morphological ones for tracing the evolution of morphological characters, because molecular characters are not subject to the same selective pressures as morphological characters (Luckow and Bruneau 1997). Bruneau (1997) demonstrated that such distinctions between molecular and morphological data are not always valid.

In principle, different types of data used in phylogenetic reconstruction should, when drawn from the same set of taxa, produce the same phylogeny. However, in practice when comparing tree topologies from independent data sets conflicting phylogenies are often produced. Some differences are due to errors in project design, data collecting and analysis. Others are due to biological processes such as hybridization, lineage sorting or orthology/paralogy conflation. Although phylogenetic incongruence is often seen as undesirable, a more fruitful interpretation is that it offers us insight into various evolutionary processes of the study group (Wendel and Doyle 1998).

Combining different character data sets increases the number of characters and maximizes phylogenetic information. It could then bring us closer to discovering the 'true' phylogeny of an organism (See chapter 3). In light of the issues raised above, the

evolution of characters is traced on a tree derived from the combined molecular analysis.

The aim of this chapter is to use a partitioned and a combined morphological and molecular phylogenetic framework:

1. to evaluate the usefulness of macro-morphological characters in proposing a phylogenetic hypothesis for the South African Campanulaceae,
2. to provide a morphological perspective on the status of the genera of the South African Campanulaceae,
3. to identify synapomorphies for revised generic limits,
4. to examine the evolution of characters, especially the fruit, previously considered important in the taxonomy of the family.

4.2. Materials and Methods

4.2.1. Taxon sampling

The sampling strategy followed for both ingroup and outgroup is as for the molecular phylogenetics, discussed in Chapter 3.

Eighty-one ingroup taxa, representing 33% of the total number of species in the group were chosen to represent the genera described by earlier workers. Six outgroup taxa were sampled, giving a final total of 87 taxa in this data set.

Gross morphological data were recorded from herbarium specimens, fresh material and field observations. Herbarium specimens from SAM, BOL, PRE and NBG (abbreviations as in Holmgren *et al*. 1990) as well as additional fresh material were examined. Specimens were selected from five different localities to include maximum geographical variation. Fresh material was examined or preserved in formalin-acetic

acid-alcohol (FAA) for later examination. A voucher herbarium specimen of each collection was made and deposited in the Compton Herbarium (NBG).

Floral morphology of flowers at anthesis was examined with the aid of a dissecting microscope. Flowers of herbarium specimens were rehydrated in boiling water for 30 seconds before the floral parts were dissected out and examined.

4.2.2. Characters and character coding

An initial set of 45 characters was studied. Many of these were excluded because they were not variable between taxa or were difficult to score because of the poor quality of herbarium specimens. In the end, 25 characters representing reproductive and vegetative morphology form the data matrix used in the phylogenetic analysis. The characters included one quantitative and 24 qualitative characters. These were selected because they were easily observable, have been previously used to separate genera, are potential synapomorphies to diagnose monophyletic groups and could be coded into discrete states. To avoid scoring the same character twice, correlated characters such the number of style lobes, which is identical to the number of ovary locules, were excluded.

All 25 characters were optimized to investigate character evolution but in the end eight floral characters and one vegetative character were selected to report on (Table 4.2). In addition to the reasons given above, this subset of characters can be hypothesized to have evolved in response to specific ecological factors, for example the shrubby habit to fire or the corolla structure to pollination syndromes.

The characters and the taxonomic distribution of their associated states are given in Table 4.1.

4.2.3. Character descriptions

1. **Habit**: erect or low growing herbs (0); shrubs (1)

The degree of woodiness and duration of above ground parts usually defines the habit of plants. In the Campanulaceae, the combination of these two features varies, making it sometimes difficult to assign a plant to a particular habit. Shrubs were defined as woody, branching perennials with persistent above ground parts, including small shrubs (subshrubs), which may have partially herbaceous stems. Herbs, plants without persistent above ground stems, include annual and perennial duration types.

2. **Leaf presence**: well developed (0); reduced (1)

Reduced leaves means leaves present and identifiable as such, but not distinct giving the plant a leafless appearance.

3. **Leaf axillary clusters**: absent (0); present (1)
The presence of smaller leaves in the axils of well-developed leaves is characteristic of many South African taxa of the Campanulaceae. The formation of the leaf clusters appears to be the result of the reduction of lateral branches along the stem. In the enrichment zone of the inflorescence, the 'bracts' may appear to be leaf clusters.

4. **Corolla shape tubular**: absent (0); present (1)

5. **Corolla shape infundibular**: absent (0); present (1)

6. **Corolla shape campanulate**: absent (0); present (1)

7. **Corolla shape stellate:** absent (0); present (1)

8. **Hypanthium shape**: linear, pedicel-like (0); various (non-linear), not pedicel-like (1)

The fused basal portion of floral parts (sepals, petals, and stamens) surrounding a ± inferior ovary is considered a hypanthium. In the fruiting stage, when the hypanthium becomes enlarged, the shape might differ from that of the flowering stage.

9. **Filament dome**: absent (0); present (1)

The filament bases are generally ciliated and variously dilated. Sometimes the expanded filament bases come into contact with each other forming an arching nectary dome over the epigynous disc (Figure 4.1A). This dome is associated with pollination and could be taxon specific.

10. **Epigynous disc**: flattened and (often) fleshy (0); dilated (swollen) and hollow (1); dilated (swollen) and solid (2) (Figure 4.1 A, B).

The epigynous disc surrounding the style base takes on various forms and is often nectiferous. When enlarged different authors interpret the disc variously, e.g. Lawrence (1951) called it a gland whereas Hilliard and Burtt (1973) refer to the same structure as a swollen style base. Morin (1983) called it a stylar disc. This disc appears to play a vital role in how capsules dehisce.

11. **Stamen fusion**: free and distinct (0); epipetalous (1)

The epipetalous condition found in flowers in this study occurs only superficially and without histological continuity, described as adherent by Porter *et.al* (1973).

12. **Stigmatic glands**: absent (0); present (1) (Figure 4.2A)

These are highly variable in size and small ones can be difficult to detect. In some taxa they are visible with the naked eye, whereas in others only at high magnification. The number and position of glands also vary. In taxa with two style lobes there is usually one gland situated at the base, on either side of the lobes. Sometimes, additional glands are present further down the style.

13. **Stigma**: lobed (0); diffuse (1)

Campanulaceae flowers are protandrous. The female phase generally starts when the style lobes separate to expose the receptive stigma (Figure 4.2 A). Before the separation the style apex is clavate. The lobes are only noticeable after the onset of the female phase or when manually separated. Very rarely does the style consist of an inseparable apex, which presents a diffuse stigma.

14. **Ovary, number of locules**: two (0); three (1); five (2)

15. **Calyx, protuberant fold**: absent (0); present (1)

These hornlike structures are found between the calyx lobes (Figure 4.2 B). They become more prominent in the fruiting stage and appear to be associated with capsule dehiscence.

16. **Placentation**: axile (0); basal (1); pendulous (2)

17. **Capsule dehiscence**: Indehiscent (0); dehiscent (1)

18. **Capsule dehiscence,** apical valves (erect): absent (0); present (1)

19. **Capsule dehiscence,** operculum: absent (0); present (1)

20. **Capsule dehiscence,** circumsessile: absent (0); present (1)

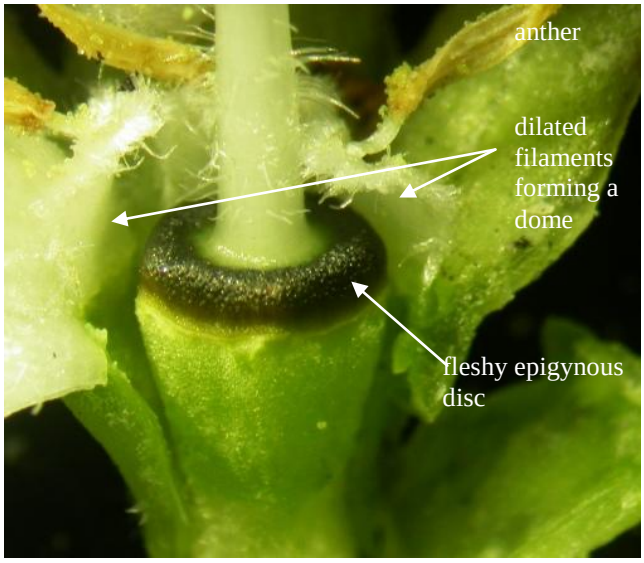
21. **Capsule dehiscence,** apical plug: absent (0); present (1)

22. **Capsule dehiscence,** protruding calyx folds: absent (0); present (1)

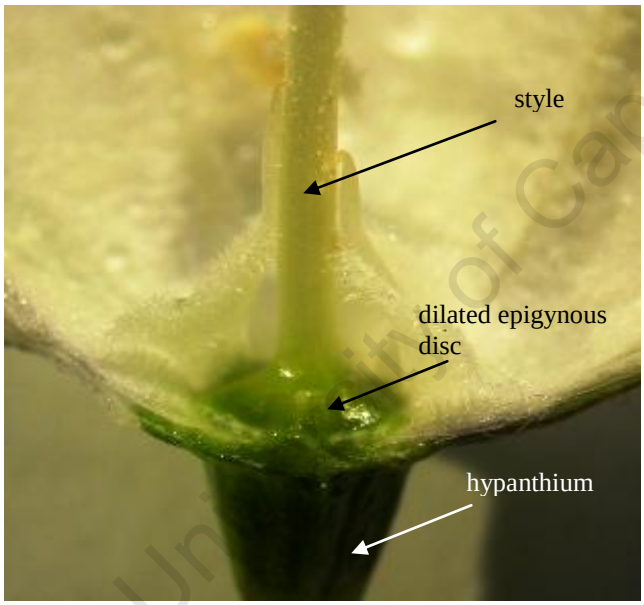
23. **Capsule dehiscence,** longitudinal slits (corresponding with calyx lobes): absent (0); present (1)

24. **Capsule dehiscence,** longitudinal slits (not corresponding with calyx lobes): absent (0); present (1)

25. **Capsule dehiscence,** apical slits (depressed valves): absent (0); present (1)

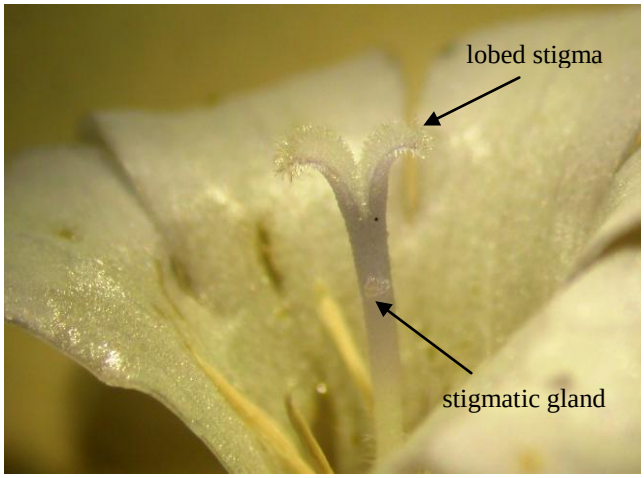


A



B

Figure 4.1. Floral characters in Campanulaceae. A, Filament dome and fleshy epigynous disc; B, Dilated epigynous disc.



A

B

Figure 4.2. Floral characters in Campanulaceae. A, Stigmatic gland and lobed style; B, Calyx folds.

Table 4.1. Morphological data matrix used for the phylogenetic analysis of the South African Campanulaceae. (missing data are indicated by '?', / indicates polymorphism).

Taxa	**Characters**																								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Ingroup																									
Craterocapsa montana	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Craterocapsa tarsodes	0	0	0	0	0	1	0	1	1	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Malmesbury plant	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0
Merciera azurea	1	0	0/1	1	0	0	0	1	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Merciera brevifolia	1	0	1	1	0	0	0	1	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Merciera eckloniana	1	0	1	1	0	0	0	1	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Merciera leptoloba	1	0	1	1	0	0	0	1	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Microcodon glomeratus	0	0	0	0	0	1	0	1	0	2	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Microcodon sp. 'pygmaeum'	0	0	0	0	0	1	0	1	0	2	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Prismatocarpus campanuloides	1	0	0	0	1	0	0	0	1	?	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus crispus	0	0	0	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Prismatocarpus diffusus	1	0	1	1	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus fruticosus	1	0	1	0	0	1	0	0	1	?	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus nitidus	1	0	0	0	0	1	0	0	1	?	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus pedunculatus	1	0	1	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus schlechteri	1	0	0	0	0	1	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus sessilis	0	0	0	0	0	1	0	0	1	?	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Prismatocarpus sp. 'Vil'	1	0	0	0	0	1	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Rhigiophyllum squarrosum	1	0	0	1	0	0	0	1	0	1	1	0	0	1	0	2	0	0	1	0	0	0	0	0	0
Roella amplexicaulis	1	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella arenaria	1	0	1	0	0	1	0	1	1	?	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella ciliata	1	0	1	0	0	1	0	1	1	?	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella cuspidata	1	0	1	0	0	1	0	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Roella incurva	1	0	1	0	0	1	0	1	1	?	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella muscosa	0	0	0	0	0	1	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Roella prostrata	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella psammophila	1	0	1	0	0	1	0	1	1	?	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella squarrosa	1	0	1	0	0	1	0	1	1	?	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella secunda	1	0	1	0	0	1	0	1	1	2	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Roella triflora	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0

Taxa	Characters																								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Siphocodon debilis	0	1	0	0	0	1	0	1	0	?	1	0	1	0	0	2	?	0	0	?	0	0	0	0	0
Siphocodon spartioides	1	1	0	0	0	1	0	1	0	1	1	0	0	1	0	2	0	0	0	1	0	0	0	0	0
Treichelia longibracteata	0	0	0	0	0	1	0	1	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Theilera robusta	1	0	1	1	0	0	0	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Theilera guthriei	1	0	1	1	0	0	0	1	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia acaulis	0	0	0	0	0	1	0	1	0	2	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0
Wahlenbergia adpressa	1	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia androsacea	0	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia annularis	0	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia axillaris	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia buseriana	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia capensis	0	0	0	0	1	0	0	1	1	1	0	1	0	2	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia capillacea	0	0	1	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia cernua	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia cinerea	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia cuspidata	1	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia depressa	1	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia desmantha	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia ecklonii	0	0	0	0	0	1	0	1	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia exilis	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia fruticosa	1	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia huttonii	1	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia juncea	0	1	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia krebsii	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia longifolia	1	0	0	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia neoridiga	1	0	1	0	0	0	1	1	1	0	0	0	0	1	?	1	0	1	0	0	0	0	0	0	0
Wahlenbergia nodosa	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia oxyphylla	0	0	1	0	0	1	0	1	1	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia paniculata	0	1	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia parvifolia	1	0	0	0	0	0	1	1	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia polyantha	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia procumbens	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia psammophila	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia rubioides	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia stellarioides	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia subulata	1	0	0	0	0	0	1	1	1	1	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia tenella	1	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia tenerrima	1	0	1	0	0	0	1	1	1	?	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Wahlenbergia thunbergiana	1	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0

Taxa	**Characters**																								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Wahlenbergia undulata	0	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia unidentata	1	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia virgata	0	1	0	0	1	0	0	1	1	?	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp ‚chatsworth‘	0	0	1	0	0	0	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
Wahlenbergia sp ‘leliefontein’	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp ‘Sani Rd’	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp Cup252	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp Cup253	0	0	0	0	0	1	0	1	1	0	0	0	0	2	1	0	0	?	0	0	0	0	0	0	0
Wahlenbergia sp Cup256	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp Cup261	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp Cup264	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Wahlenbergia sp Cup265	0	0	0	0	0	1	0	1	0	?	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Outgroup																									
Cyphia bulbosa	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Cyphia comptonii	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Cyphia volubilis	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Lobelia comosa	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Lobelia jasionoides	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Monopsis debilis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 4.2. List of characters selected for detailed evolutionary analysis in the South African Campanulaceae.

Floral	**Vegetative**
Corolla shape	Habit
Filament dome	
Placentation	
Stigmatic glands	
Stamen fusion	
Stigma lobes	
Locule number	
Capsule dehiscence	

4.2.4. Combined data set construction

The number of taxa in the individual data sets varies. Consequently, the data set with the smallest number of taxa was used to determine the number of taxa included in the combined analysis. Furthermore, taxa for which sequence data could not be obtained were excluded from the combined data matrix.

The morphological character state codes were DNA coded in McClade version 4.0 (Maddison and Maddison 2000). The combined ITS – morphological and *trn*L-F – ITS – morphological data matrices comprised 70 taxa respectively, with *Lobelia jasionoides, L. comosa* and *Cyphia comptonii* as outgroup taxa. The *trn*L-F – morphological data matrix consisted of 87 taxa with *Monopsis debilis, L. jasionoides, L. comosa, C. bulbosa, C. comptonii* and *C. volubilis* as outgroup taxa.

4.2.5. Phylogenetic analysis

4.2.5.1. **Maximum Parsimony (MP) analyses**

The computer programs McClade version 4.0 (Maddison and Maddison 2000) and PAUP version 4.0b 10 (Swofford 2003) were used to find the most parsimonious tree from the data set. McClade was used to set-up the data matrix and to create a data file for PAUP. All characters in the analysis were given equal weights (Fitch parsimony; Fitch 1971) and treated as unordered.

Search 1. Morphological data set

An initial run was performed using the heuristic search option and tree-bisection reconnection (TBR) branch-swapping with 1000 random addition replicates, saving five trees per replicate to minimize the time spent searching through large numbers of trees, steepest descent off and MULTREES in effect. Branches were collapsed if their maximum length equaled zero. All the trees obtained were then used as starting trees

in a second analysis with the same parameters as above, saving all optimal trees with a limit of 10 000 trees. In the case of successive weighting, trees recovered were used for subsequent rounds of reweighting and analysis until the tree topology stabilized. Individual trees were rooted with the outgroup, comprising members of the Lobeliaceae and Cyphiaceae.

Support for each clade retrieved by the analysis was assessed using bootstrap analyses (Felsenstein 1985). (See section 3.2.6.1, Chapter 3).

For each analysis the Consistency (CI) (Kluge and Farris 1969) and Retention (RI) (Farris 1989) indices were calculated to give an indication of the measure of fit between the data and the tree topologies. Values approaching one indicate a low level of homoplasy in the data set.

Agreement subtrees (common pruned trees) were constructed using the 'agreement subtrees' option of PAUP, to identify problematic taxa in the data set that might be responsible for any lack of resolution.

Search 2. Morphological data set successively weighted

In order to reduce the influence of homoplasious characters on the tree topology, characters were *a posteriori* weighted by the successively approximated weighting method (Farris 1969).

In this search strategy the same conditions as in search 1 were employed. Characters were reweighted by their rescaled consistency index (RCI) with a base weight equal to 10. The search was repeated until the tree length and topology stabilized (i.e. there was no change between two successive rounds).

Combined morphological and molecular data sets

For each of the combined data sets similar search strategies (equal – and successive weighting) were employed as for the partitioned morphological data set described above.

The combinability of these data sets were assessed by an incongruence test (ILD test; Farris *et al.* 1995), according to the procedure described in Chapter 3.

4.2.5.2. **Bayesian analyses**

The same strategy was used as described in Chapter 3.

After discarding the first 201 000 generation of trees as burnin the results were summarized by a 50% majority rule consensus tree.

4.2.6. Character evolution

The evolution of the characters was traced onto the strict consensus tree from the combined molecular analysis using MacClade version 4.0 (Maddison and Maddison 2000). Characters were optimized with ACCTRAN, which favours reversals rather than parallelisms.

4.3. Results

4.3.1. Morphological data set

Search 1

Under the parsimony criterion, 2385 equally parsimonious trees of 98 steps with CI of 0.286 and a RI of 0.809 were found. The topology of the strict consensus tree (not shown) is largely unresolved forming a single polytomy. Two clades within the polytomy are resolved, the first comprising the two species of *Siphocodon* and the monotypic *Rhigiophyllum*. The second clade, comprising species of *Merciera,* is well supported (bootstrap 95%).

The 50% majority rule consensus tree obtained from the Bayesian analysis (Figure 4.3) is better resolved than that obtained from the maximum parsimony analysis. Despite the improved resolution all large clades are unsupported. *Wahlenbergia* sp. Cup264 resolve as sister to the rest of the Campanulaceae. The large Campanulaceae clade comprises three subclades A, B and C. Subclade A is formed by four *Wahlenbergia* species and sister to subclades B and C. Species of *Wahlenbergia, Microcodon, Treichelia, Craterocapsa* and the Malmesbury plant form subclade B in which the relationship between *W. acaulis*, *Treichelia longibracteata* and the Malmesbury plant is weakly supported (PP= 53). The largest subclade C is formed by a trichotomy involving two smaller *Wahlenbergia* groups and a large group comprising *Theilera, Wahlenbergia, Prismatocarpus, Roella, Merciera, Rhigiophyllum* and *Siphocodon*. Within this large group species of *Roella, Prismatocarpus* and *Merciera* form a weakly supported clade (PP= 60). This clade is also retrieved by the *trn*L-F analysis. The species of *Merciera* with 71 % posterior probability is sister to *P. diffusus*. *Rhigiophyllum* and *Siphocodon* form a strongly supported clade (PP= 91) sister to *W. depressa*.

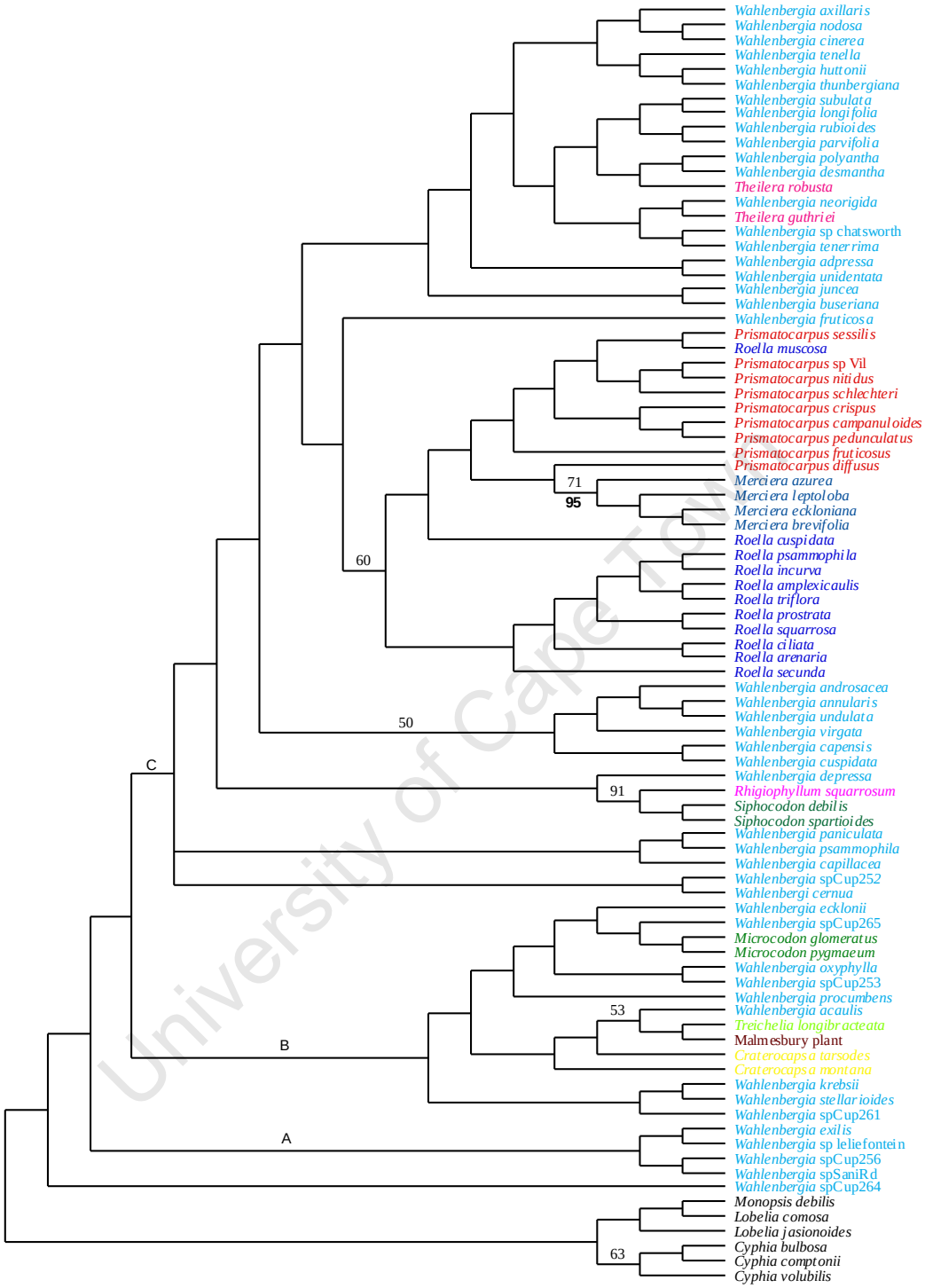


Figure 4.3. 50% majority rule consensus tree from the Bayesian analysis of the morphological data set for 81 taxa of the South African Campanulaceae and six Lobeliaceae/Cyphiaceae (outgroup). Numbers above branches indicate posterior probability values. Bootstrap values ≥50% are indicated below the branches.

Search 2. Successively Weighted Analysis of Morphological Data

Under the parsimony criterion,495 equally parsimonious trees of 325 steps, a CI of 0.487 and a RI of 0.866 were found. Five rounds of successive weighting were necessary to stabilize the topology and tree length. The strict consensus (Figure 4.4) is better resolved than the one recovered in search 1. All major clades are unsupported.

Clade A consists of species of *Wahlenbergia*, *Rhigiophyllum*, *Siphocodon*, *Theilera* and *Merciera*. Relationships within this clade are largely unresolved. Two subclades, one comprising species of *Merciera* (BS= 97) and the other *Siphocodon*, *Rhigiophyllum* and *Theilera guthriei* (BS= 58) are formed. The *trn*L-F and combined molecular analyses contradict the placement of the *Merciera* subclade. In each of these analyses, *Merciera* is nested in a clade with *Roella* and *Prismatocarpus*. With a few exceptions, the taxa forming clade A are all shrublets and the *Wahlenbergia* species were previously classified in *Lightfootia*.

Clade B, comprising species of *Wahlenbergia*, *Microcodon*, *Treichelia*, *Craterocapsa*, *Roella*, *Prismatocarpus* and the Malmesbury plant resolved as a trichotomy. The largest group in the trichotomy is formed by species of *Wahlenbergia*, *Microcodon*, *Treichelia*, *Craterocapsa* and the Malmesbury plant and a smaller group is formed by *Roella* and *Prismatocarpus*. All the *Roella* species form a subclade, except *R. muscosa*, which is placed in the *Prismatocarpus* subclade. In the *trn*L-F and combined molecular analyses, clade B (excluding *Prismatocarpus* and *Roella*) and A (excluding *Merciera*, *Rhigiophyllum* and *Siphocodon*) are subclades of a large terminal clade.

As in the molecular analyses, clade C comprises herbaceous *Wahlenbergia* species with funnel-shaped corollas. In this analysis, the clade is unresolved and in the ITS analysis *W. androsacea* and *W. annularis* are excluded from this clade.

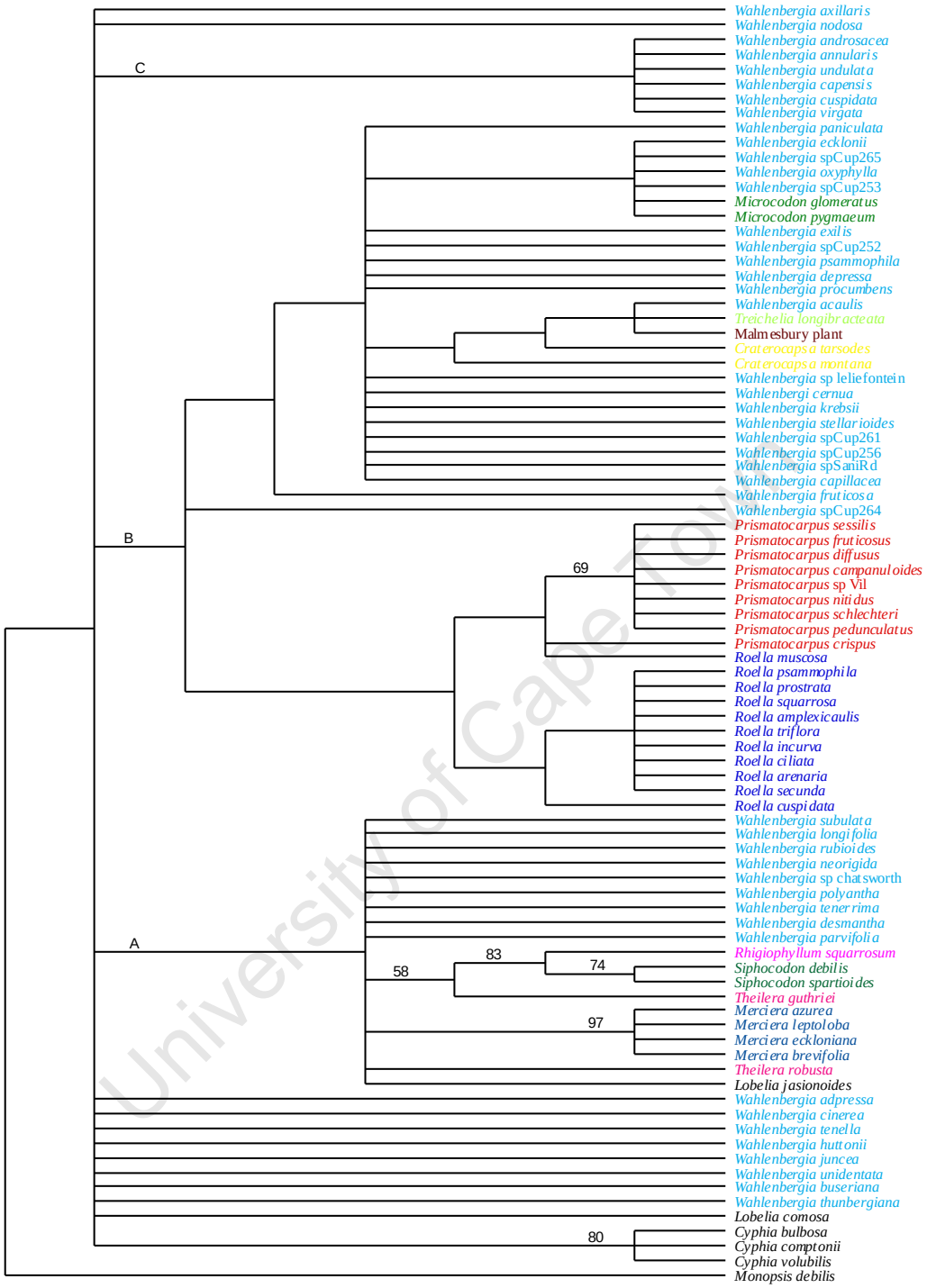


Figure 4.4. Strict consensus of 495 equally parsimonious trees (length=325, CI=0.487, RI=0.866) found after heuristic search (weighted) of the morphological data set for 81 taxa of the South African Campanulaceae and six Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

4.3.2. Combined morphological and molecular data sets

4.3.2.1. Data combinability

The results of the partition homogeneity tests for each of the combined data sets show distinct incongruence (*P*= 0.01). However, a number of studies have shown that the results of the ILD test can be misleading (Wiens 1998, Dolphin *et al*. 2000, Reeves *et al*. 2001, Yoder *et al*. 2001, Ramírez 2006 c.f. chapter 3). Therefore, despite the results of the ILD test, combining the morphological and molecular data sets provided more resolved and better supported trees. Combining the data sets is therefore justified.

(i) morphology and *trn*L-F

Under equal weights, 352 equally parsimonious trees with a length of 1047 steps, a CI of 0.617 and a RI of 0.838 were found. All major clades retrieved by this combined analysis (Figure 4.5) are also retrieved and has similar bootstrap support to, the *trn*L-F sequence analysis.

The strict consensus of 474 trees (Figure 4.6) found after five rounds of successive weighting (L= 5200, CI= 0.850 and RI= 0.942) is better resolved than the equally weighted tree, but retrieved similar major clades.

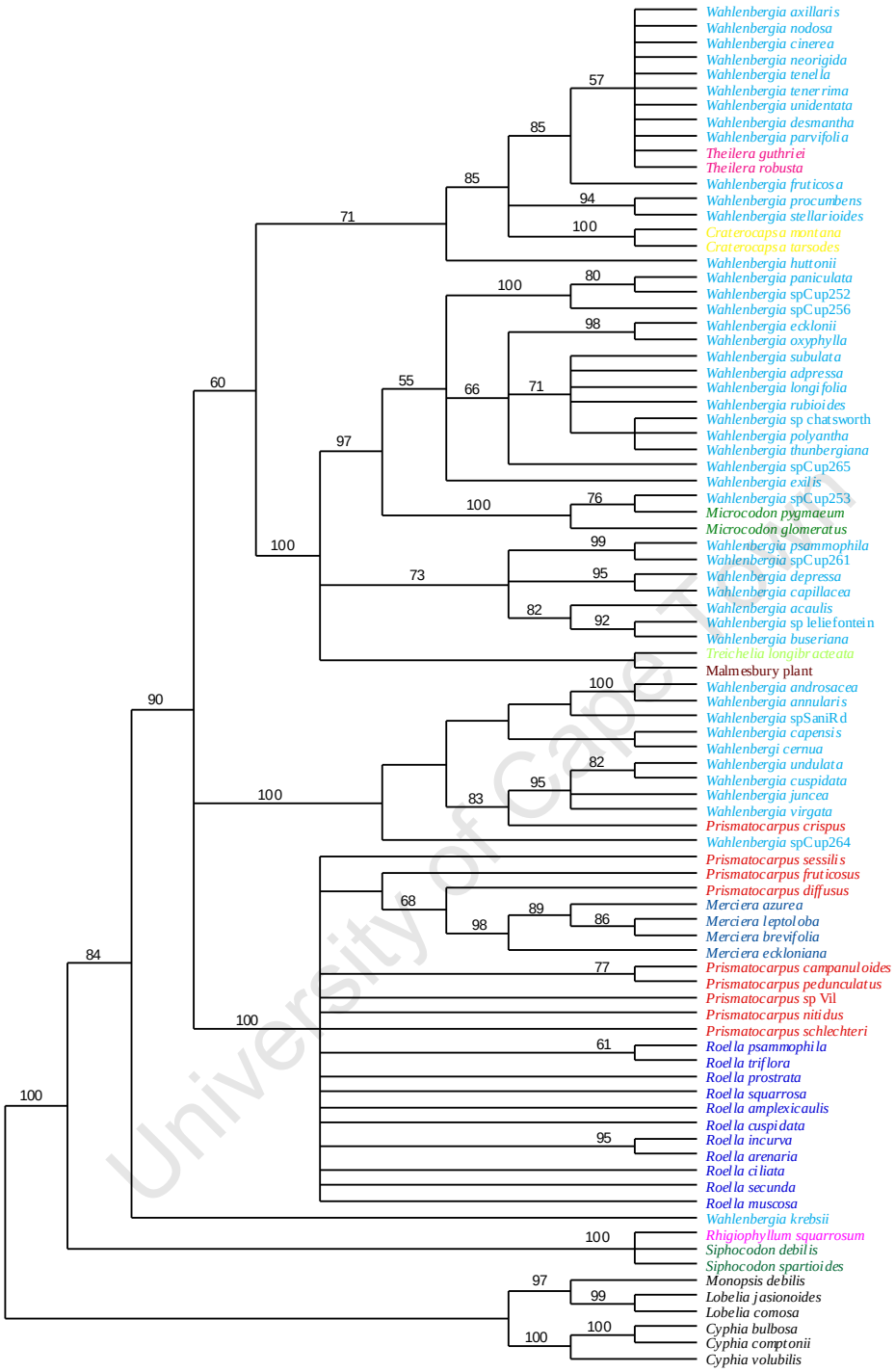


Figure 4.5. Strict consensus of 352 equally parsimonious trees (length=1047, CI=0.617, RI=0.838) found after heuristic search (unweighted) of the combined morphological and *trn*L-F data sets for 81 taxa of the South African Campanulaceae and six Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

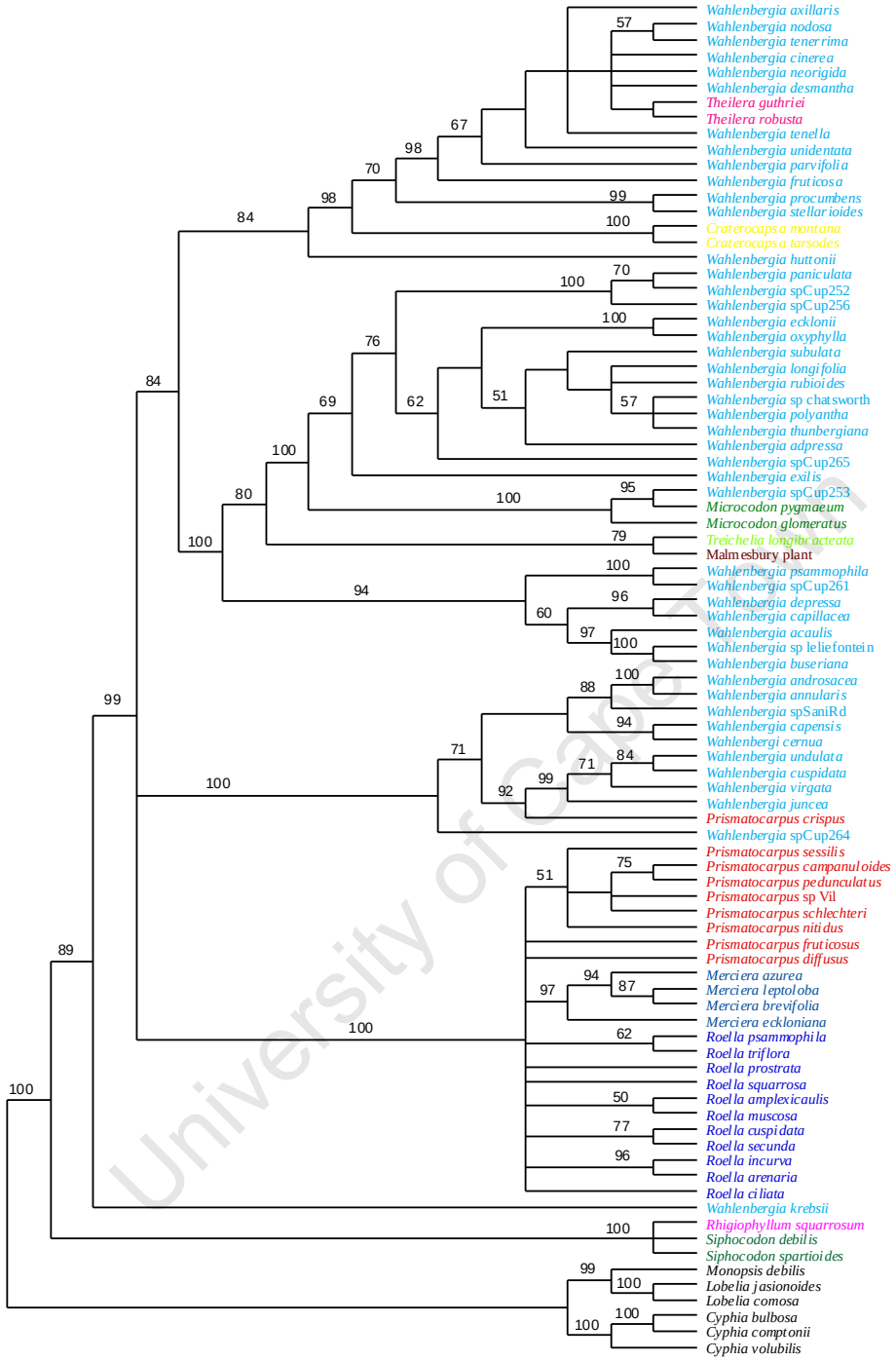


Figure 4.6. Strict consensus of 474 equally parsimonious trees (length=5200, CI=0.850, RI=0.942) found after heuristic search (weighted) of the combined morphological and *trn*L-F data sets for 81 taxa of the South African Campanulaceae and six Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

(ii) morphology and ITS

Under parsimony analysis of 70 taxa, 27 equally parsimonious trees of 574 steps, a CI of 0.460 and a RI of 0.704 were found. In the strict consensus (Figure 4.7), the polytomy produced by the separate ITS analysis persists. Within the polytomy, several clades not retrieved by the separate analysis are formed. Among these is a large clade comprising species of *Merciera*, *Prismatocarpus* and *Roella*, which is weakly supported and poorly resolved. Within this clade *Merciera* forms a strongly supported subclade sister to *P. diffusus*. *Rhigiophyllum* and *Siphocodon* form a strongly supported clade (BS=90). The *W. undulata* clade is found in both analyses, but is poorly supported in the combined analysis. Similar, is the clade comprising species of *Wahlenbergia*, *Microcodon*, *Treichelia* and the Malmesbury plant, found in both analyses. *Theilera* and *Craterocapsa*, which together with some species of *Wahlenbergia*, formed a clade sister to previously mentioned clade in the separate analysis, now formed separate clades.

The strict consensus of 382 trees (Figure 4.8) recovered after six rounds of successive weighting (L=1889, CI=0.778 and RI=0.853) is better resolved than the equally weighted tree but the support for major clades remained poor.

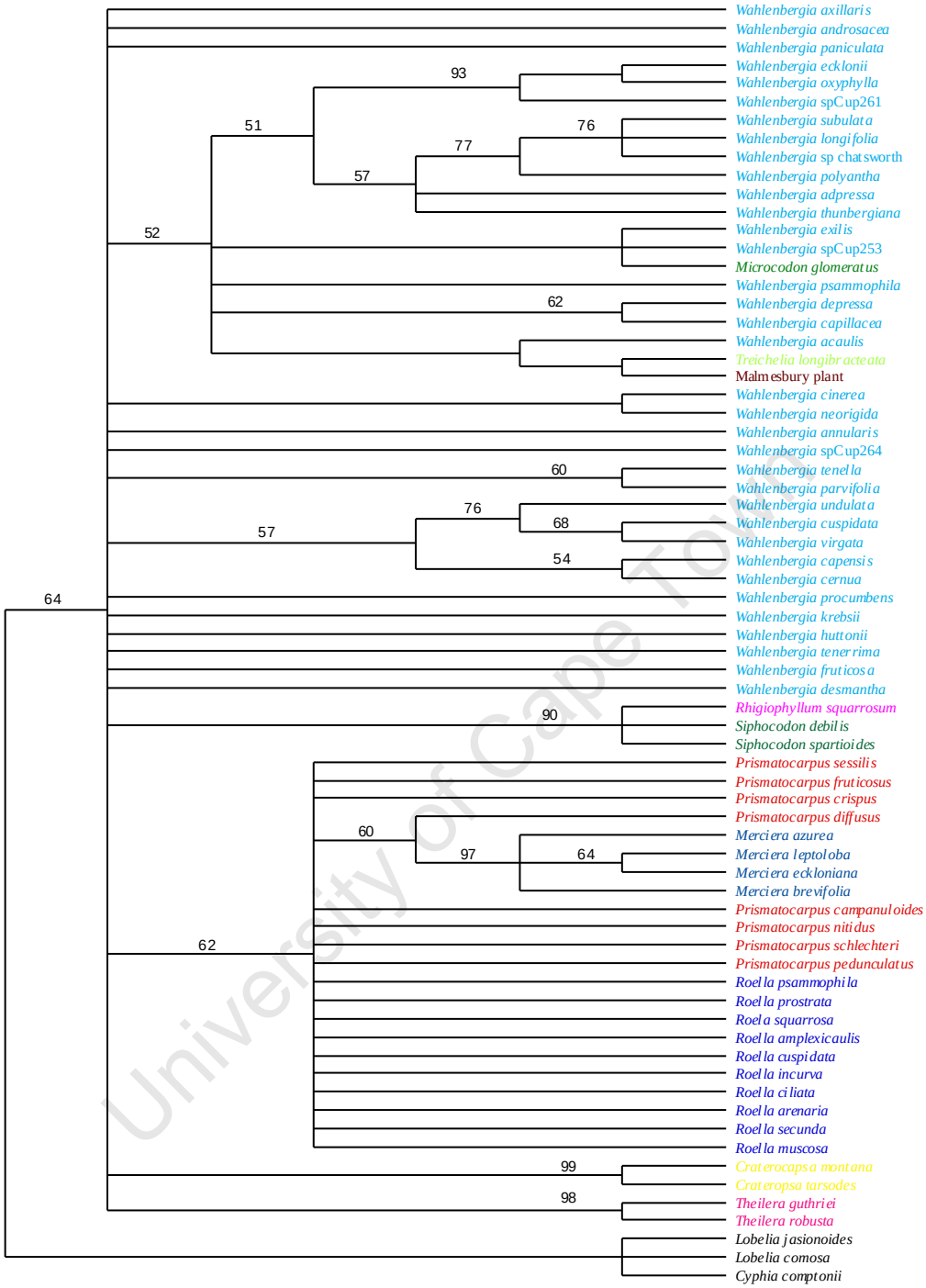


Figure 4.7. Strict consensus of 27 equally parsimonious trees (length=574, CI=0.460, RI=0.704) found after heuristic search (unweighted) of the combined morphological and ITS data sets for 67 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

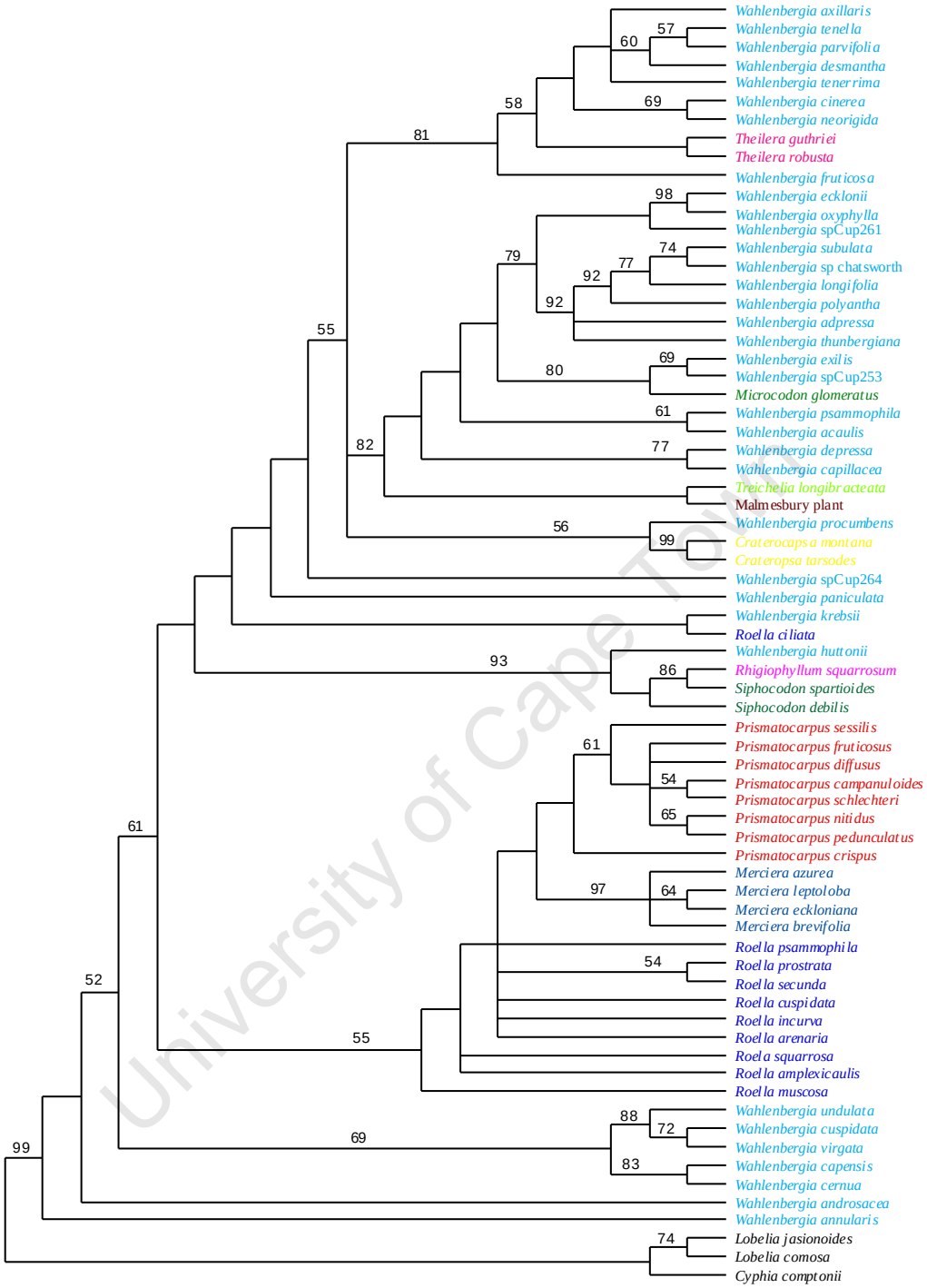


Figure 4.8. Strict consensus of 382 equally parsimonious trees found (length=1889, CI=0.778, RI=0.853) after heuristic search (weighted) of the combined morphological and ITS data sets for 67 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

(iii) morphology, *trn*L-F and ITS (Total evidence)

In the combined analysis of the two DNA sequence data sets and the morphological data set, 467 equally parsimonious trees of 1365 steps (CI=0.599 and RI=0.783) were found. The topology of the strict consensus tree (Figure 4.9) is similar to that of the combined morphological - *trn*L-F analysis.

Successive weighting of the combined data set produced a stable topology after three rounds. The strict consensus of 188 equally parsimonious trees (Figure 4.10) of 6388 steps (CI=0.854 and RI=0.915) is almost fully resolved and well supported by bootstrap values.

The topology of the total evidence Bayesian tree is better resolved than the unweighted total evidence tree of the parsimony analysis. It is largely similar to the weighted parsimony tree (Figure 4.10), except that relationships in the *Roella* clade are better resolved.

The largest common pruned tree (Figure 4.11) contains 45 of the original 70 taxa. This high cost of the pruning resulted in the exclusion of *Treichelia* from these data. A similar huge reduction in the number of taxa was found in all other analyses not presented here.

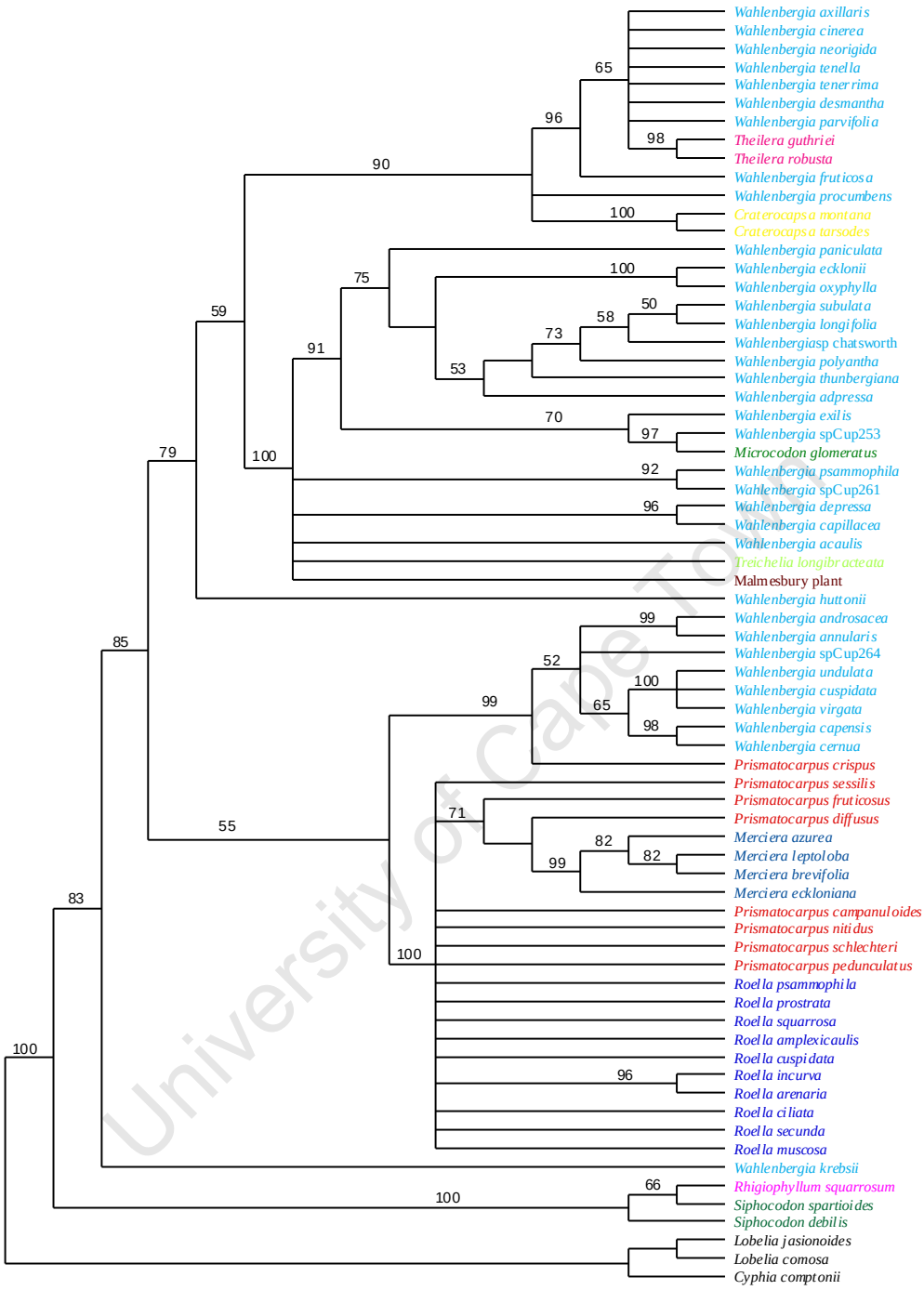


Figure 4.9. Strict consensus of 467 equally parsimonious trees found (length=1365, CI=0.599, RI=0.783) after heuristic search (unweighted) of the combined morphological, *trn*L-F and ITS data sets for 67 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches.

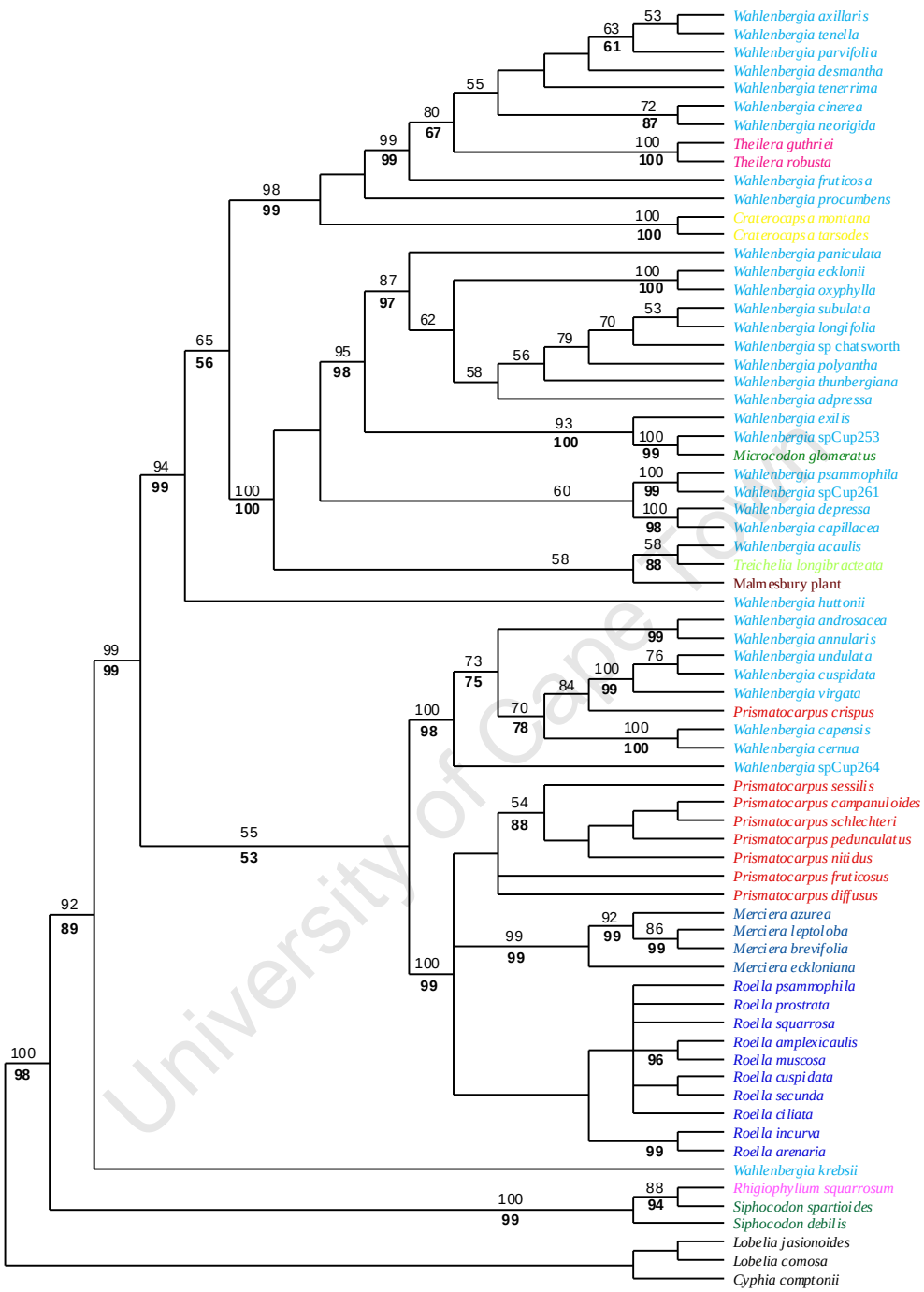


Figure 4.10. Strict consensus of 188 equally parsimonious trees (length=6388, CI=0.854, RI=0.915) found after heuristic search (weighted) of the combined morphological, *trn*L-F and ITS data sets for 67 taxa of the South African Campanulaceae and three Lobeliaceae/Cyphiaceae (outgroup). Bootstrap values ≥50% are indicated above the branches. Numbers below the branches indicate posterior probability values expressed as percentages.

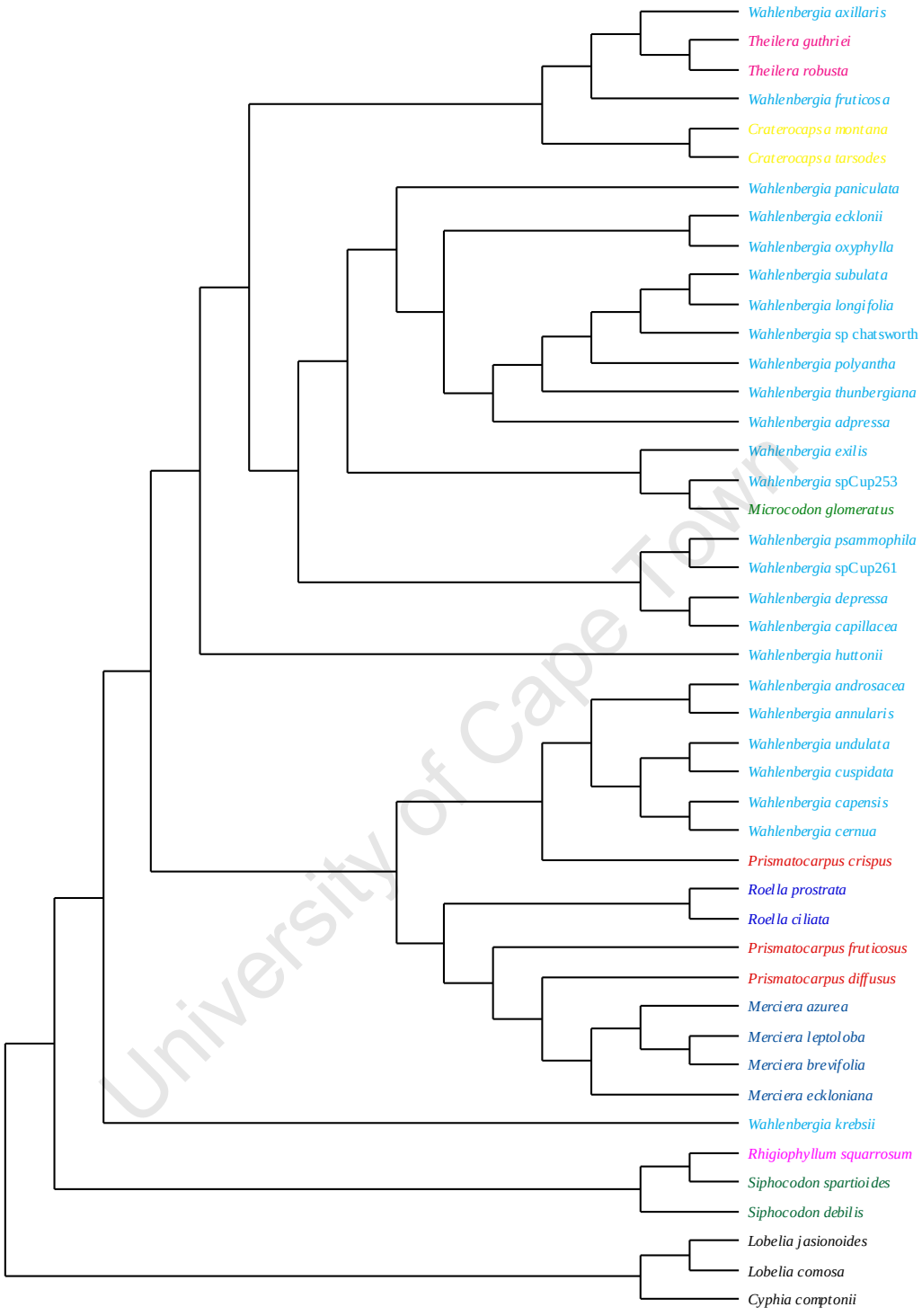


Figure 4.11. Largest common pruned tree found after agreement subtrees search of the combined morphological, *trn*L-F and ITS data sets for 67 taxa of the South African Campanulaceae and three Lobeliaceae (outgroup).

4.3.3. Character evolution

Habit

Herbaceous habit is the ancestral state within the family with several independent derivations of shrubbiness (Figure 4.12). Ambiguity is found in the *Wahlenbergia-Theilera-Craterocapsa* clade. In the *Roella-Merciera-Prismatocarpus* clade the shrubby habit was lost twice whereas in the *Siphocodon-Rhigiophyllum* clade the herbaceous habit shifted to shrubbiness.

Corolla shape

The ancestral corolla shape is ambiguous; therefore any shape could have evolved several times. The campanulate corolla type evolved in several species of *Wahlenbergia* and *Prismatocarpus*, in *Roella*, *Craterocapsa*, *Siphocodon*, *Microcodon* and the Malmesbury plant. The tubular corolla type evolved in *Merciera*, *Rhigiophyllum*, *Theilera* and *Prismatocarpus diffusus*, whereas the funnel-shaped type evolved three times, in the *Wahlenbergia-P. crispus* clade, *P. campanuloides* and *P. pendunculatus*. Ambiguity is found in the *Wahlenbergia-Theilera-Craterocapsa* clade for the stellate corolla type, as well as in the larger *Wahlenbergia-Microcodon-Treichelia* clade. The stellate corolla type also evolved in *W. huttonii* (Figure 4.13, A-D).

Filament dome

The ancestral state is ambiguous (Figure 4.14). If the absence of a filament dome is the ancestral condition, it has been retained in several genera - for example *Merciera*, *Treichelia* and *Microcodon*. The presence of a filament dome is ancestral for the large clade comprising eight genera.

Stamen fusion

Free stamens occur in most members of the family and this is the ancestral state within the family. Fused stamens evolved once in the *Siphocodon-Rhigiophyllum* clade (Figure 4.15) and thus constitute an uncontradicted synapomorphy for this group.

Stigmatic glands

The absence of stigmatic glands is the ancestral condition for the family. They appear to have evolved independently several times and, most noteworthy, are present in all species of *Roella* (Figure 4.16).

Stigma lobes

A lobed stigma is the ancestral condition for the family, with only one derivation of a diffused stigma in *Siphocodon debilis* (Figure 4.17).

Locule number

The ancestral state is ambiguous; therefore any locule number state could have evolved several times (Figure 4.18). Five-locular ovaries evolved only in a few *Wahlenbergia* species and in *Microcodon*. Two-locular ovaries evolved in all species of the *Roella-Prismatocarpus-Merciera* clade, in a few species of the *Wahlenbergia-P. crispus* clade, *S. debilis* and *Treichelia longibracteata*.

Placentation

Axile placentation is the ancestral state, with a shift to pendulous in the *Siphocodon-Rhigiophyllum* clade (Figure 4.19). Basal placentation evolved three times in the family.

Capsule dehiscence

Capsules dehiscent by apical valves are the ancestral condition within the South African members of the family (Figure 4.20B) and is retained in *Wahlenbergia*, *Theilera*, and *Microcodon*. It shifts to circumsessile dehiscence in *Siphocodon spartioides* (Figure 4.20D) followed by dehiscence via an operculum in *Rhigiophyllum* (Figure 4.20C). The operculum mode of dehiscence evolved independently in *Craterocapsa*, *Treichelia* and the Malmesbury plant. A reversal to apical dehiscence occurred in *Wahlenbergia krebsii* followed by a shift to an apical plug in *Roella* (Figure 4.20E). However, in *Roella* this condition is lost twice: in *R. muscosa* and *R. cuspidata*. In these two species the capsule is either indehiscent (*R. muscosa*) or dehisces via irregular slits (*R. cuspidata*, Figure 4.20H). Within the *Roella-Merciera-Prismatocarpus* clade, dehiscence by an apical plug shifted to indehiscent capsules in *Merciera* (Figure 4.20A) followed by longitudinal slits in *Prismatocarpus* (Figure 4.20G). Unique modes of capsule dehiscence evolved in *W. acaulis* (protruding folds, Figure 4.20F) and in *W.*sp (Chatsworth) (depressed apical slits, Figure 4.20I).

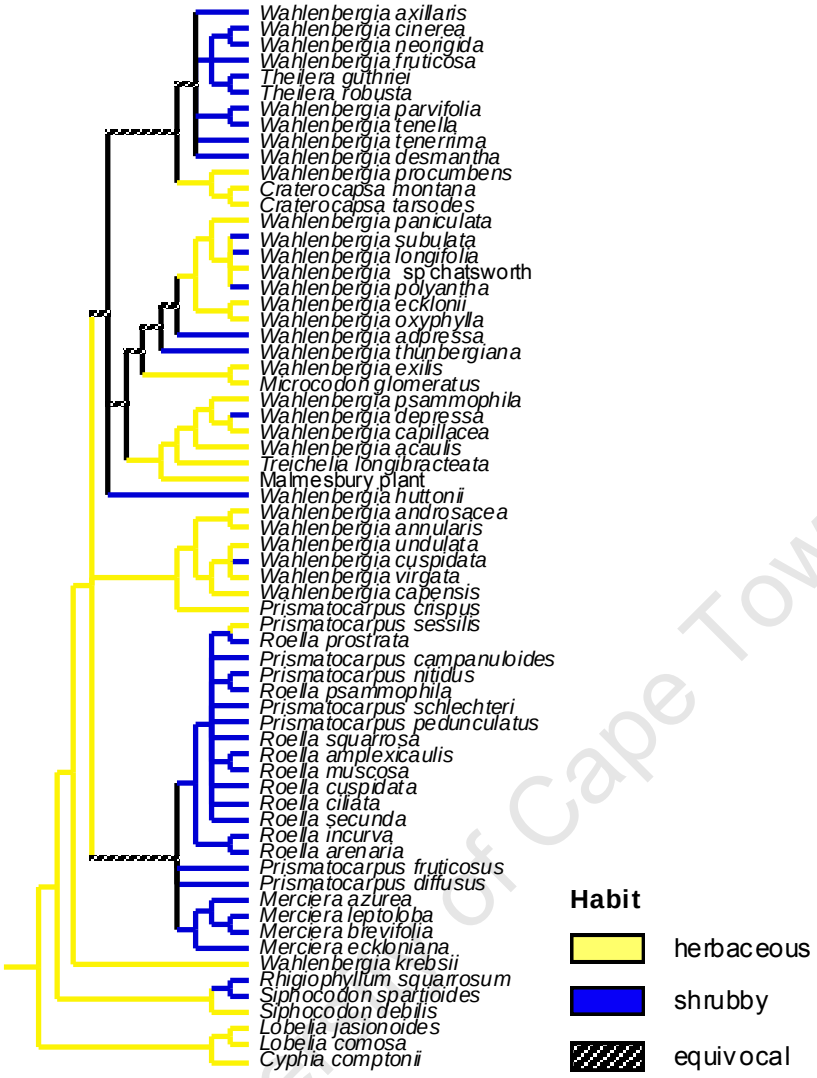
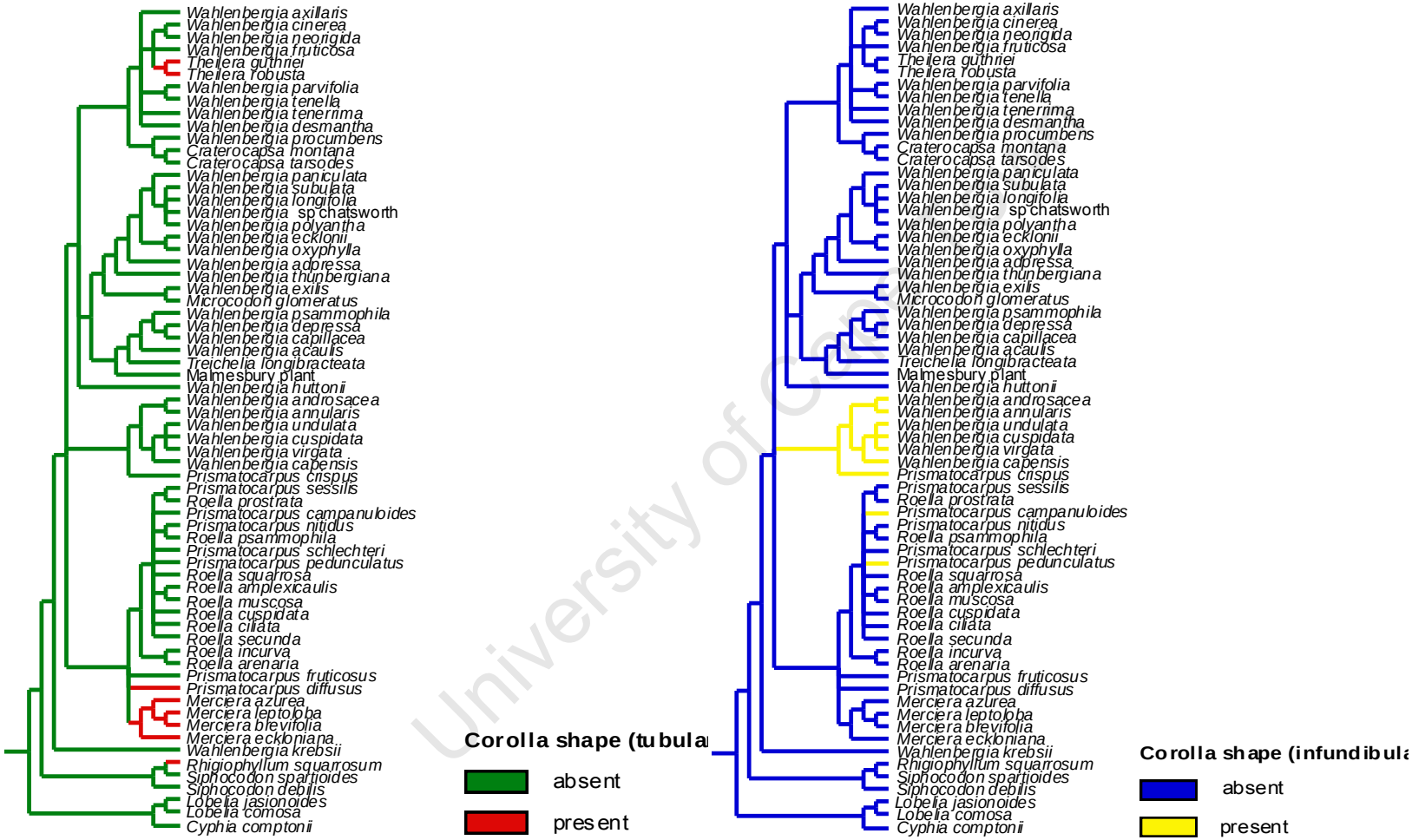


Figure 4.12. Optimization of habit character



A **B**

Figure 4.13. A and B. Optimization of corolla shape character. A, tubular; B, infundibular.

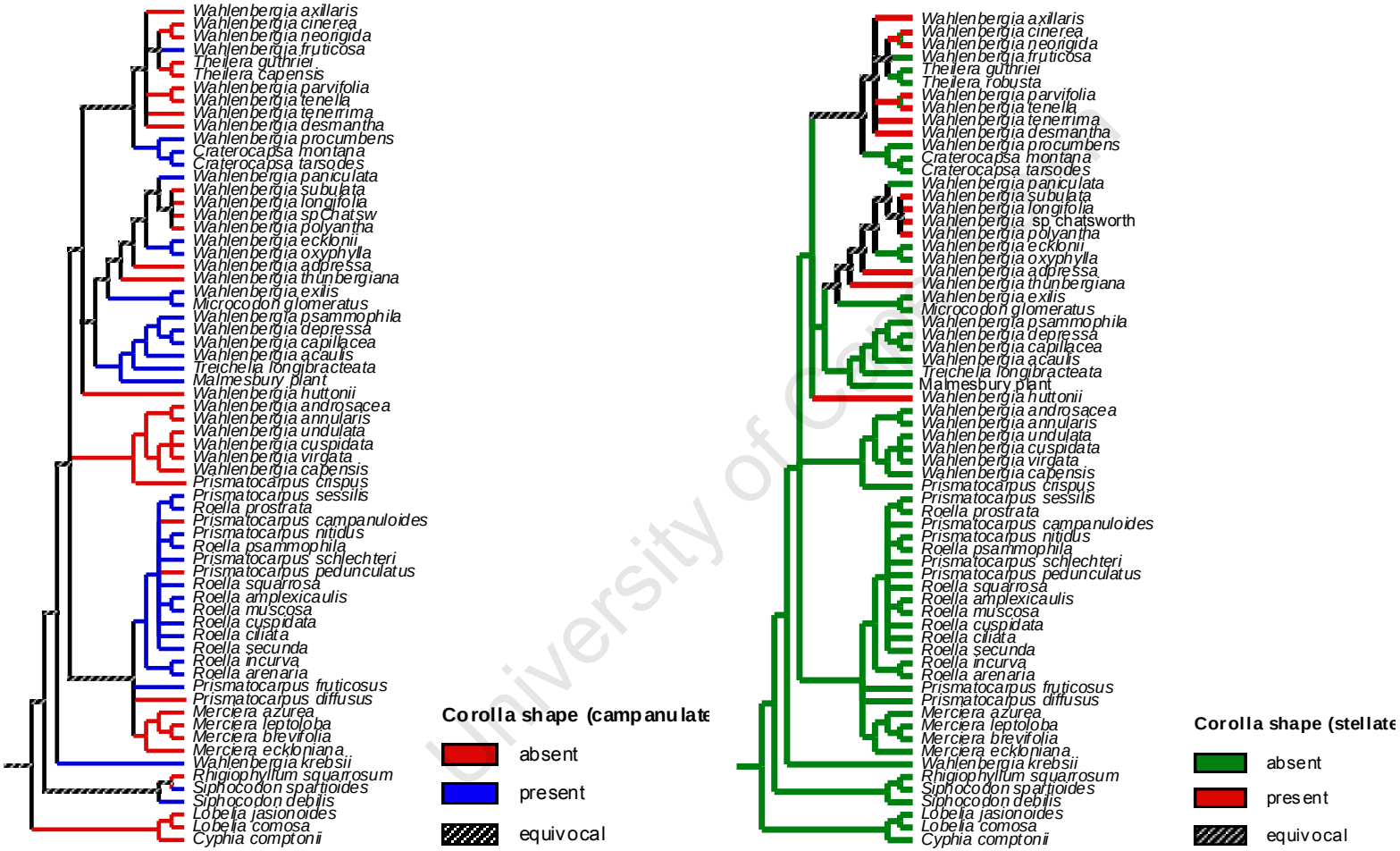


Figure 4.13. (continued) C and D. Optimization of corolla shape character. C, campanulate; D, stellate.

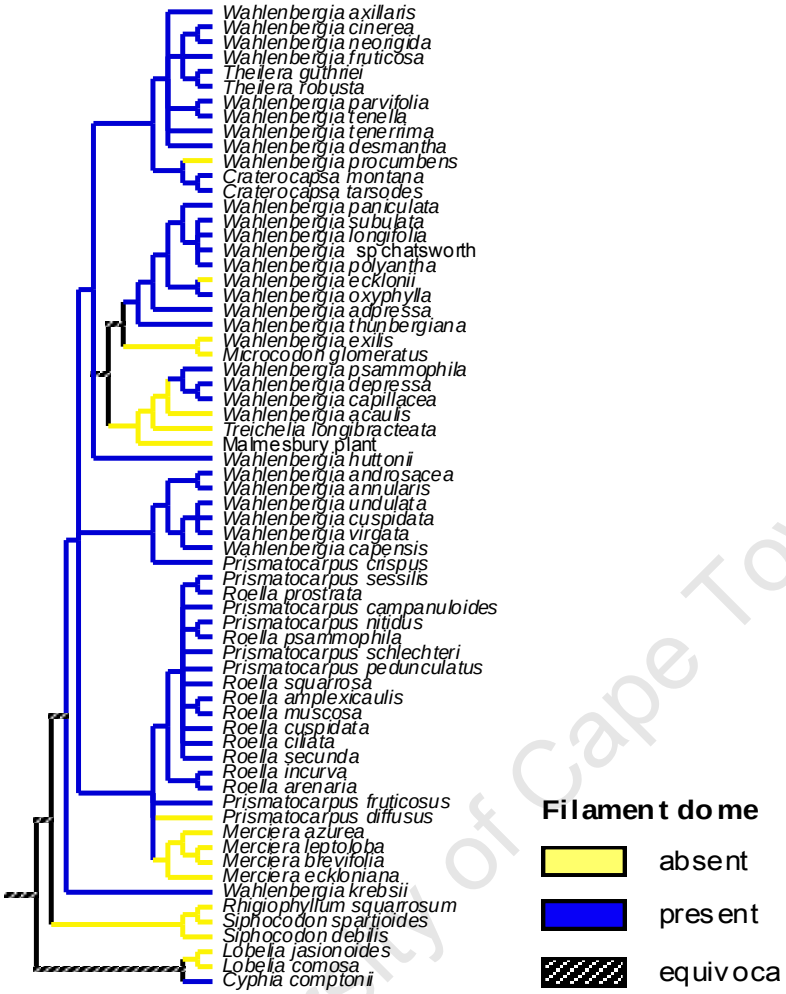


Figure 4.14. Optimization of filament dome character.

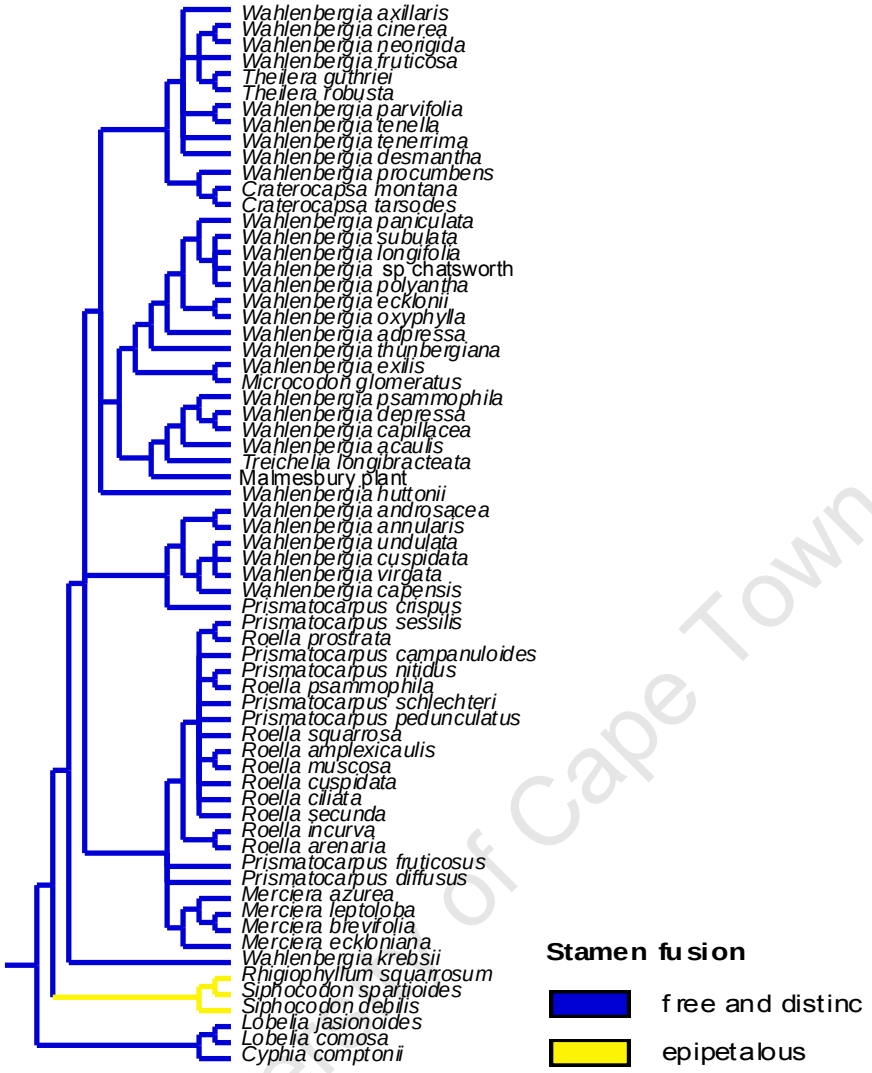


Figure 4.15. Optimization of stamen fusion character.

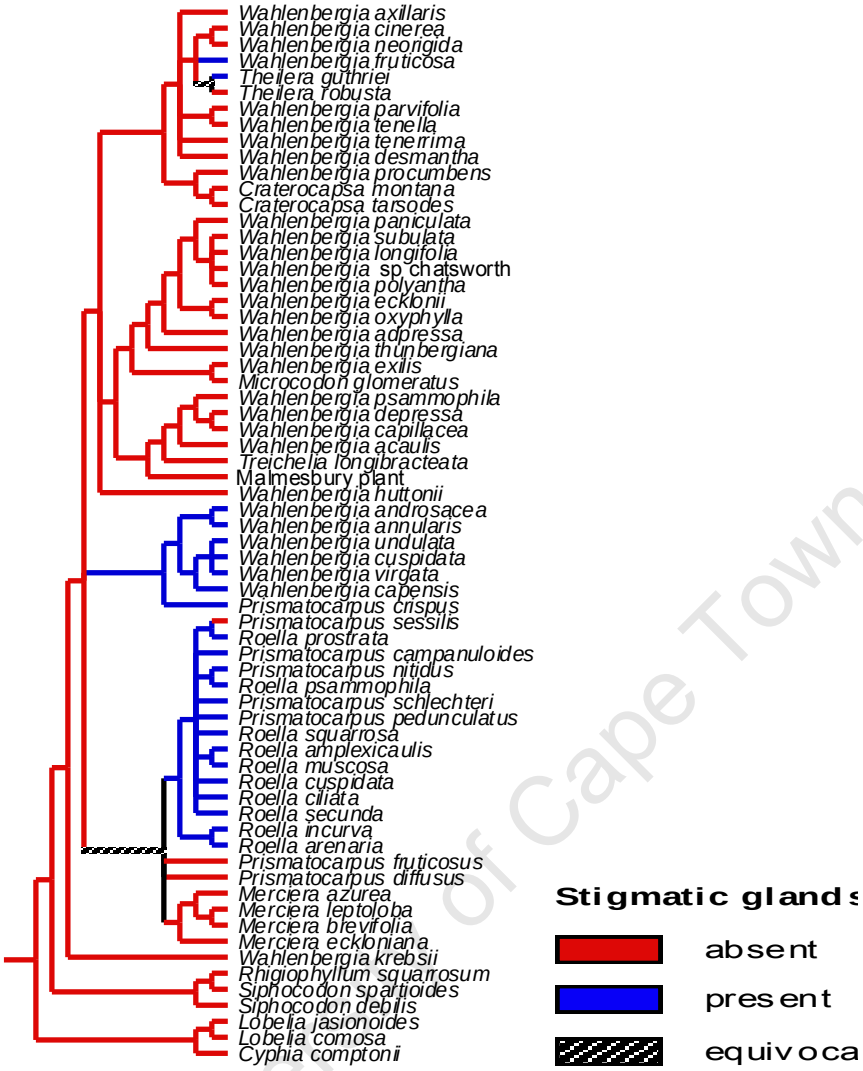


Figure 4.16. Optimization of stigmatic gland character

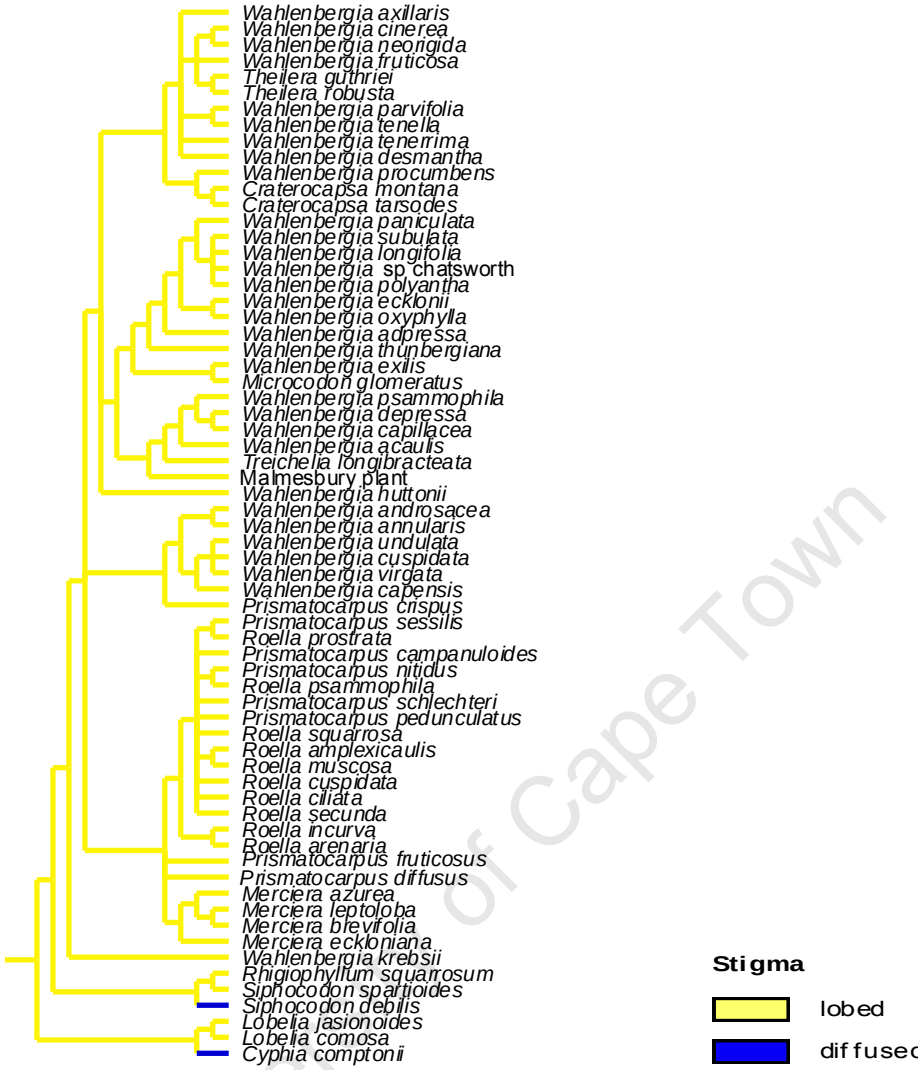


Figure 4.17. Optimization of stigma lobes character.

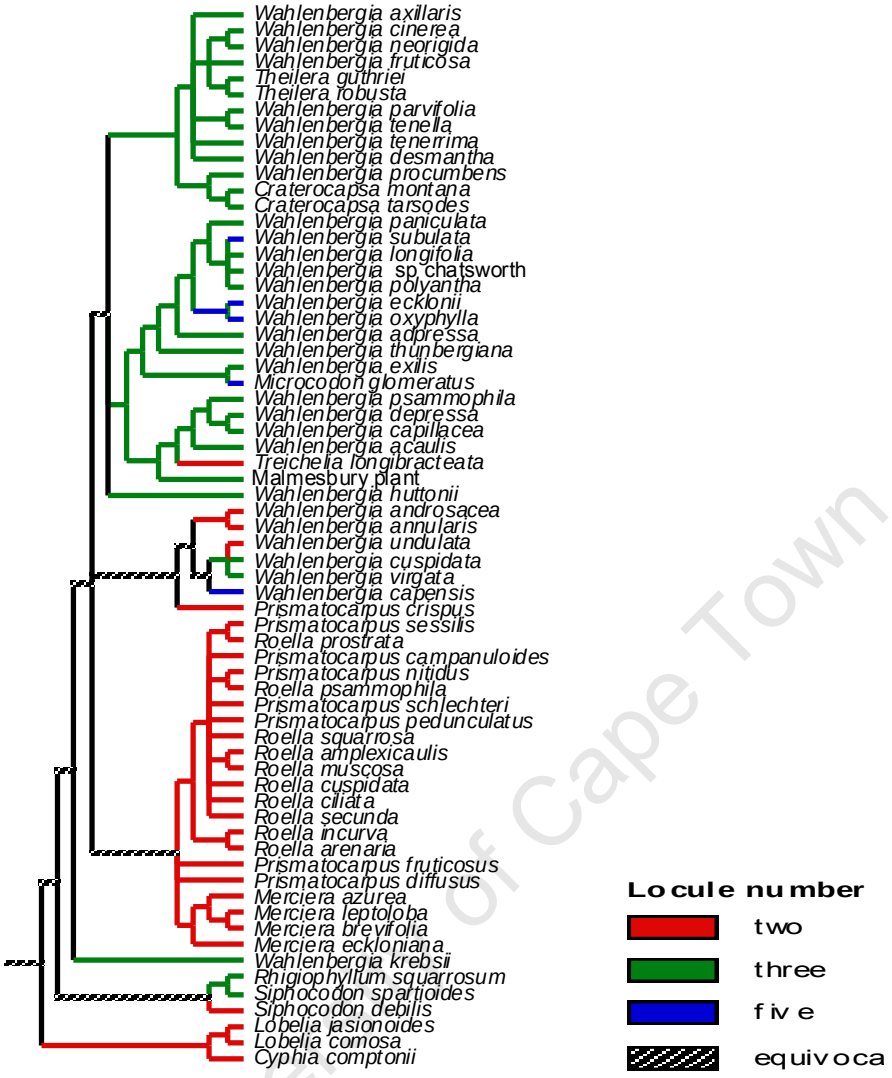


Figure 4.18. Optimization of locule number character.

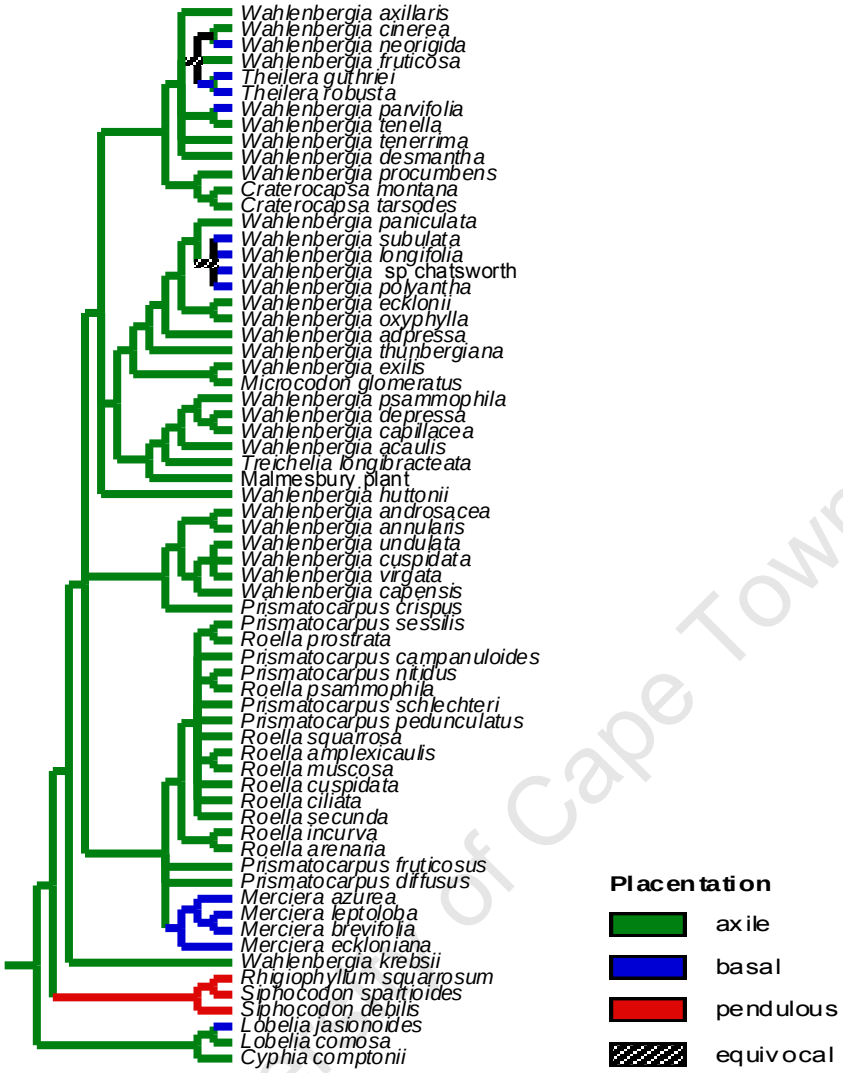


Figure 4.19. Optimization of placentation character.

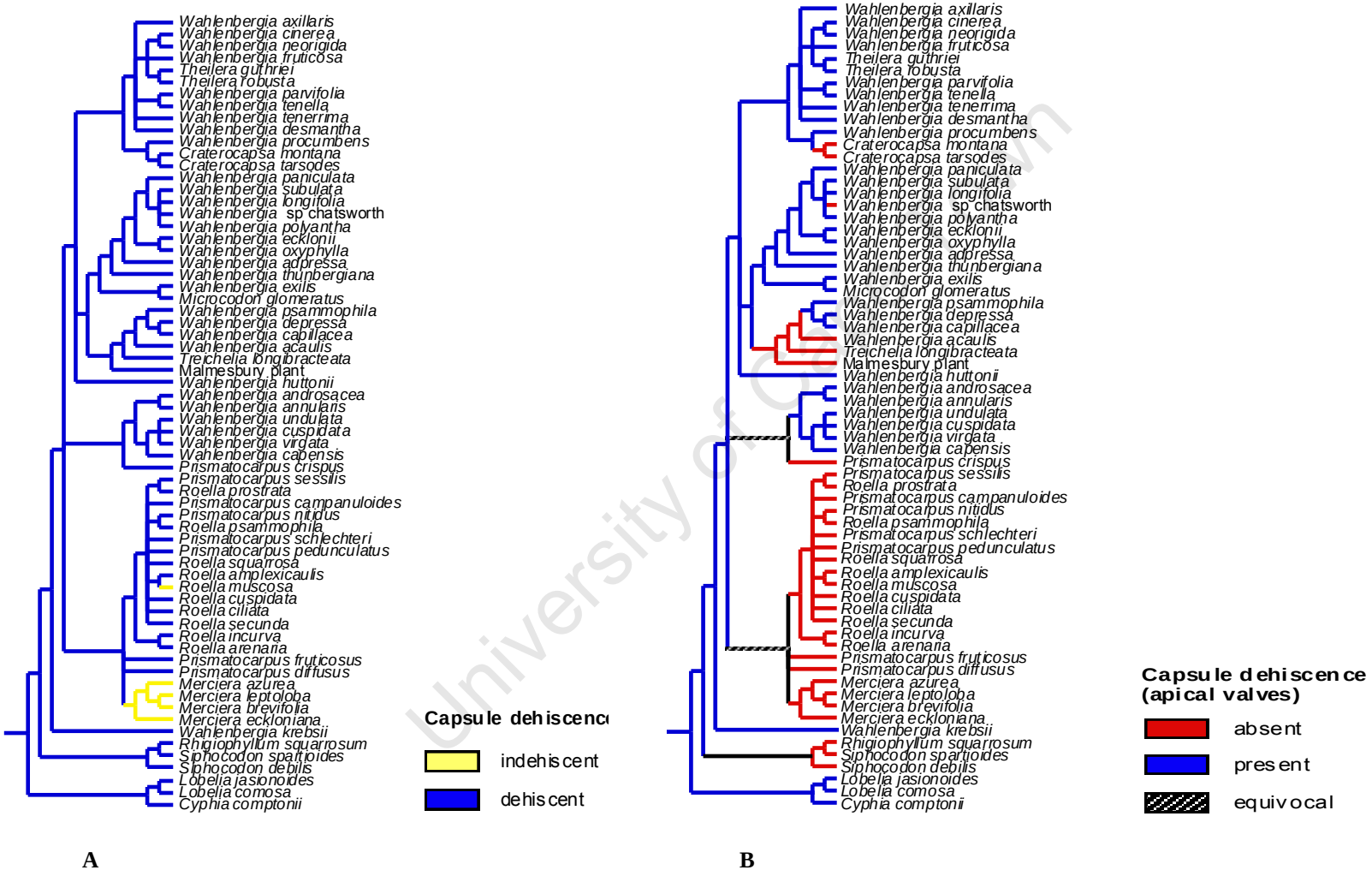


Figure 4.20. A and B. Optimization of capsule dehiscence character. A, indehiscent, B; apical valves.

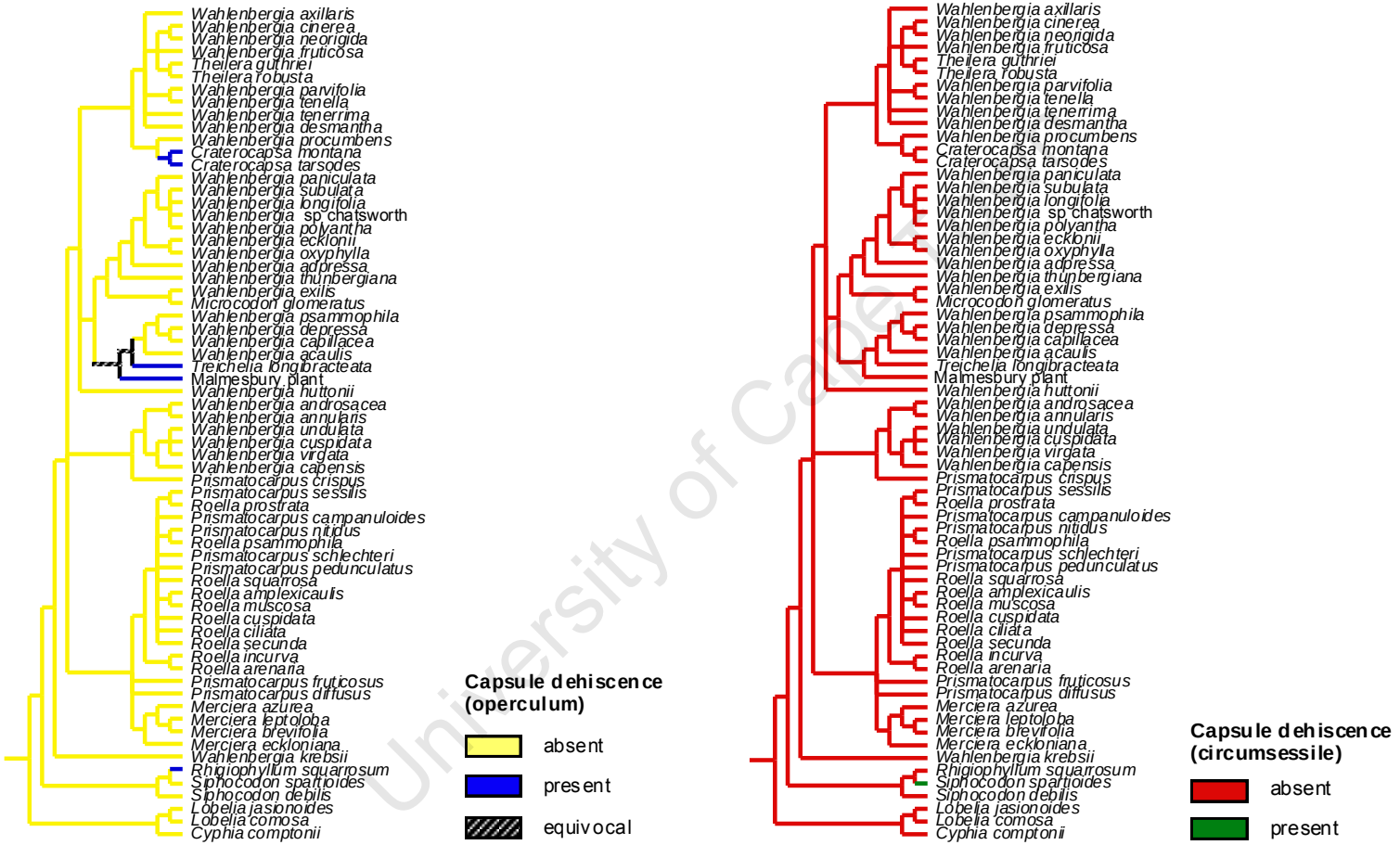


Figure 4.20. (continued) C and D. Optimization of capsule dehiscence character. C, operculum; D, circumsessile.

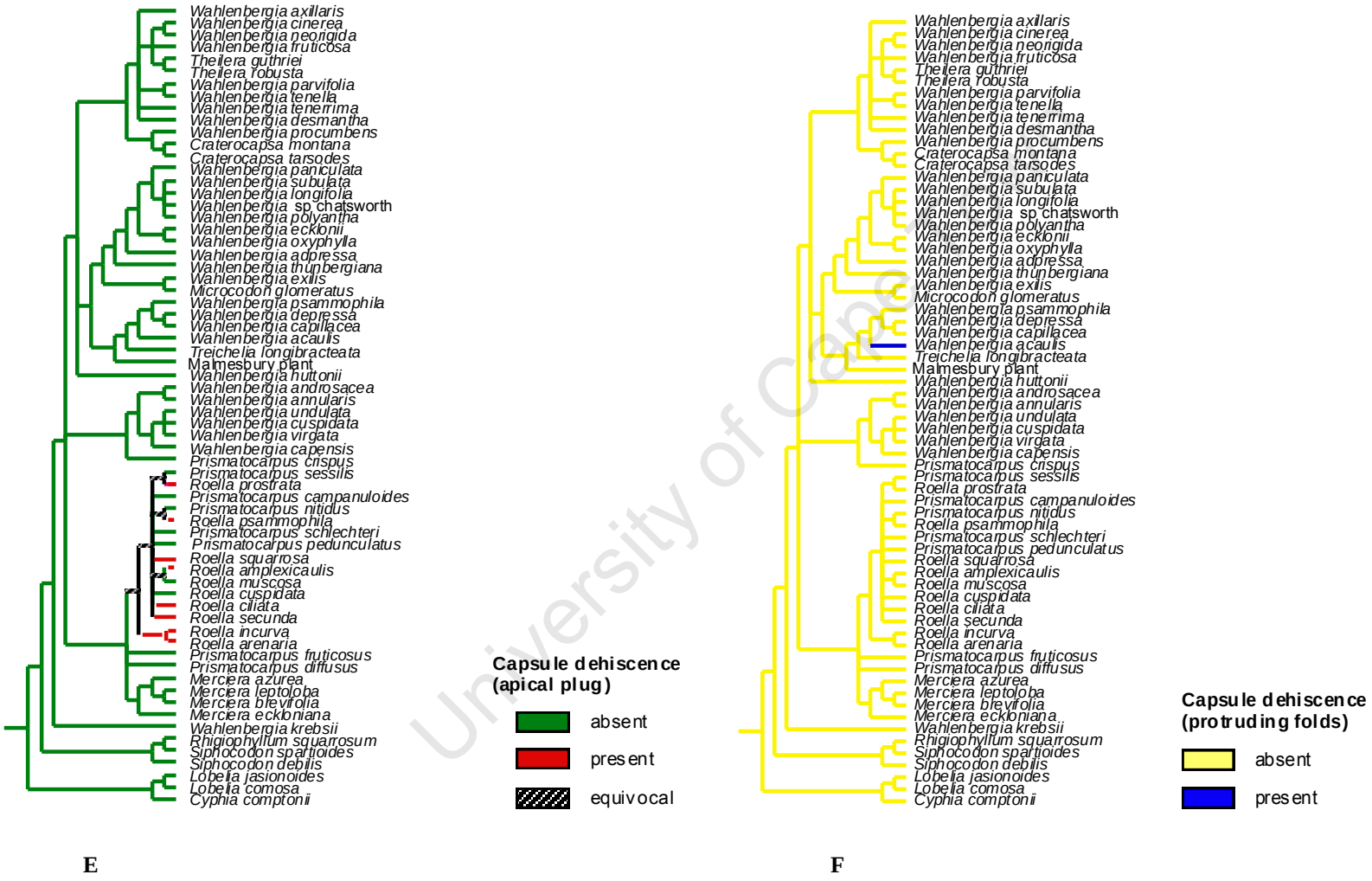


Figure 4.20. (continued) E and F. Optimization of capsule dehiscence character. E, apical plug; F, protruding folds.

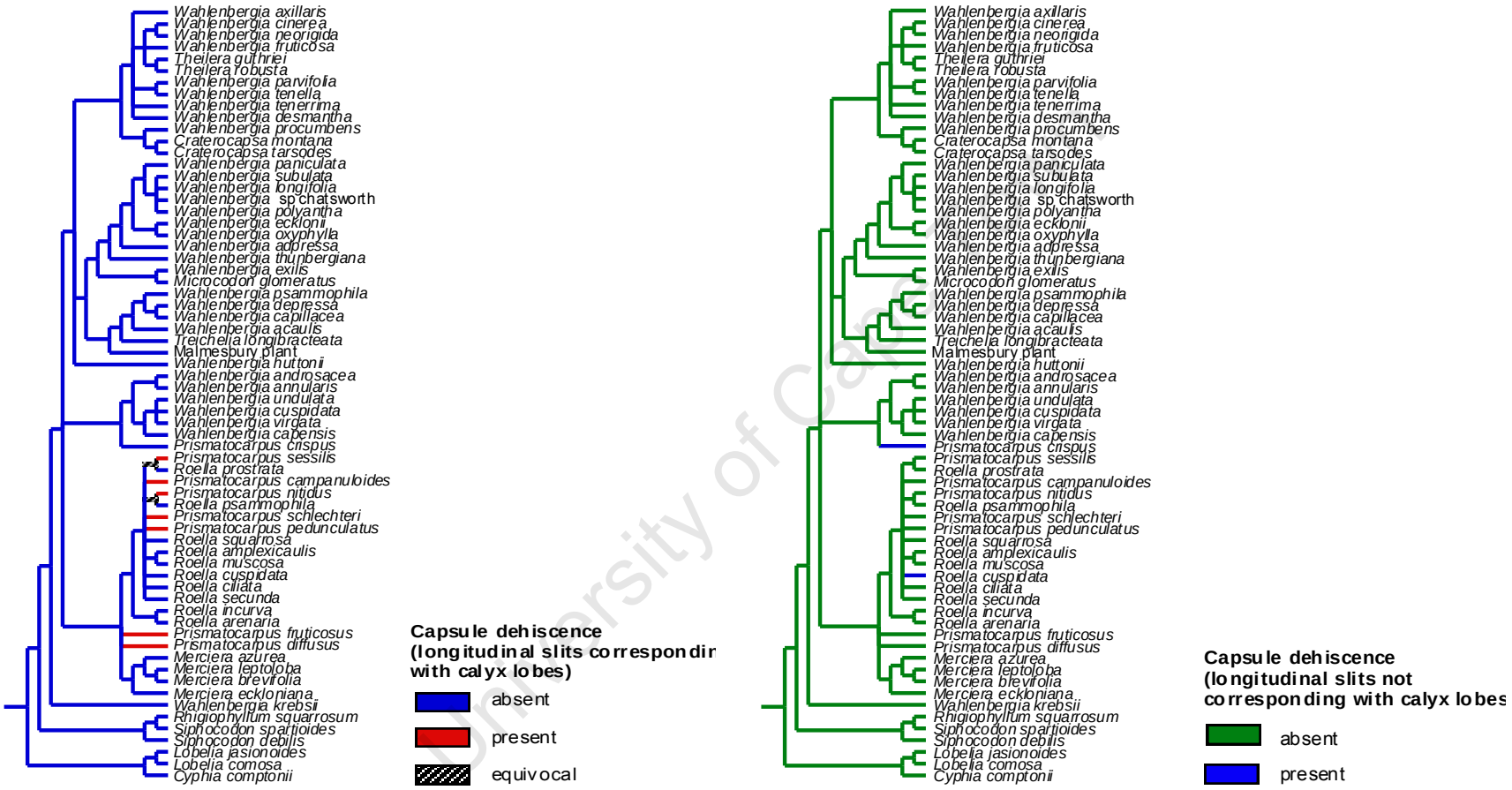


Figure 4.20. (continued) G and H. Optimization of capsule dehiscence character. G, longitudinal slits – corresponding to calyx lobes; H, longitudinal slits – irregular.

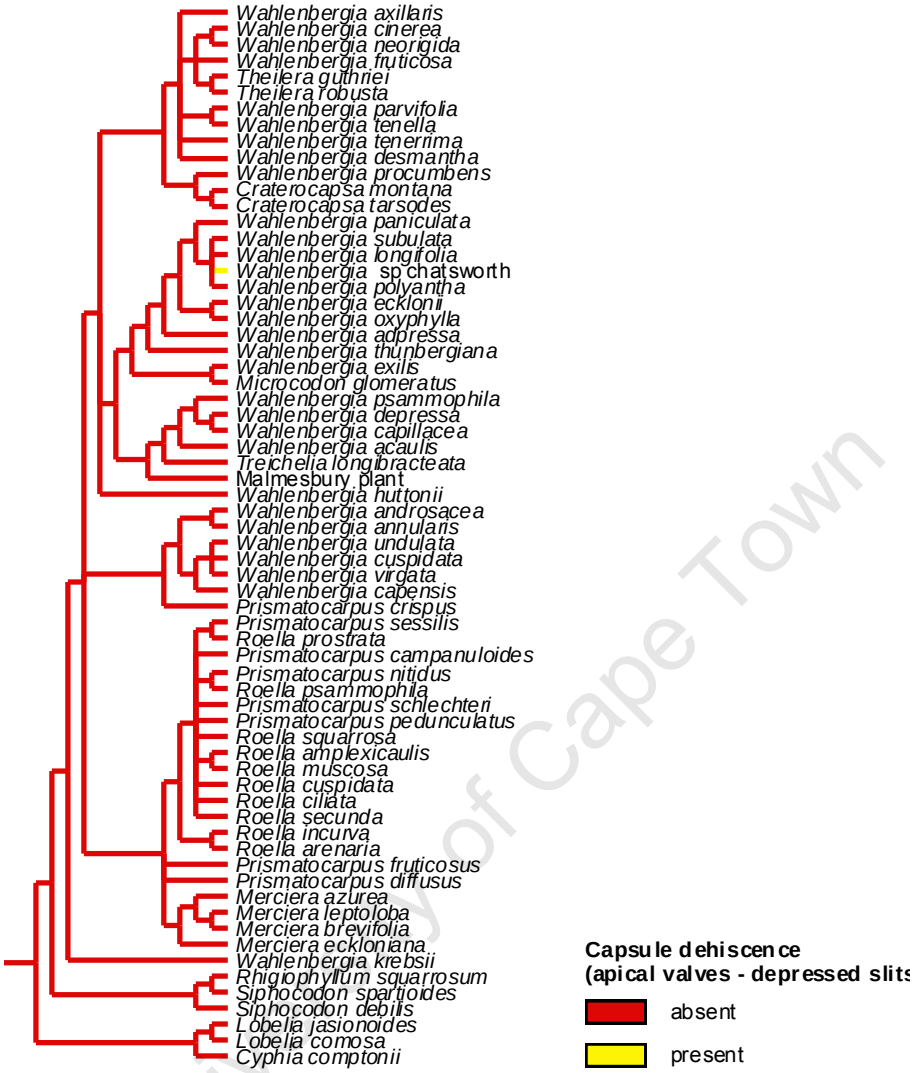


Figure 4.20.I. Optimization of capsule dehiscence character. Apical slits (depressed valves).

4.4. Discussion

4.4.1. The Morphological data set

The morphological data provided poor resolution and its usefulness in inferring phylogenetic relationships is limited. The successively weighted analysis provided better resolution, but largely without internal support. One possible reason for the lack of resolution is the high level of homoplasy present in the data set. This is evident by the low consistency indices observed for the equally weighted (CI=0.286) and successively weighted (CI=0.487) data sets.

Schulkina *et al*. (2003) reported that similarities due to convergent and parallel evolution are common in reproductive and vegetative structures of Campanulaceae. When considering the fruit character used to separate genera, it can be interpreted that the diverse capsule structures in the Campanulaceae represent a series of transformations among homologues. The next task would be to try and discover the sequence of transformation. Capsule structure could possibly be correlated with other morphological characters or environmental factors necessary to ensure successful reproduction and survival. Detailed anatomical and ontogenetic studies will provide evidence to discover such homologies. However, developmental information is often not used in phylogenetic studies (Endress 2003). The benefit of developmental studies is that they provide direct evidence for various aspects of diversity within a related group of plants. Traditional characters, such as the capsule structure in the Campanulaceae, may in fact be complex structures composed of different elements. These different elements maybe scored as separate characters thereby potentially reducing homoplasy in the data set. Endress (1970) demonstrated how detailed analysis of the inflorescence structure of the genus *Distlium* led to new interpretations of the flowers, and consequently relationships within the genus. The inflorescence structure in the Campanulaceae is complex (Thulin 1974) and consequently was not scored in this study due to the extent of such an investigation.

Other reasons that could account for the low resolution are: the small number of characters relative to the number of taxa or the character coding method employed in the primary homology assessment. For example, in the case of nominal variable coding (Pimental and Riggins 1987) also known as presence/absence coding, shared absences are assumed to be homologous. This is evident in clade B (Figure 4.4) of the weighted tree, where taxa are grouped together by a number of absences/losses.

Although the clades produced by Bayesian analysis (Figure 4.3) are not strongly supported, the individual clades do have recognizable morphological features. Some of them are consistent with the current generic boundaries in the family. An annual life form, unique intercalyx folds and seed with ribs, characterize the *W. acaulis* clade. *Rhigiophyllum* and *Siphocodon* share epipetalous stamens, triangular pollen grains and pendulous ovules. The two *Siphocodon* species have highly reduced leaves and campanulate corollas in common. The characters that *Merciera* and *P. diffusus* share, are a shrubby life form and tubular corollas. The indehiscent capsule and four basal ovules separate *Merciera* from *P. diffusus.* The clade formed by the *Roella* species is characterized by a shrubby life form, a two locular ovary and campanulate corollas. An annual life form, a five locular ovary which alternates with the calyx lobes, and a capsule with apical valves are shared characters in the *Microcodon* clade. The species forming the *W. androsacea* clade also have capsules with apical valves, but this character is accompanied by a perennial life form.

4.4.2. Combined morphology and molecular data sets

In response to the high levels of homoplasy in the Campanulaceae, Shulkina *et al.* (2003) suggested the use of all available characters in phylogenetic studies. A concern for combining the morphological and molecular data sets, is the difference in the number of characters between the two data sets. The individual molecular data sets contain more parsimony informative characters (ITS= 107, *trn*L-F= 299) than the morphological data set (22 parsimony informative characters) in each of the combined analyses. This discrepancy might result in the molecular characters swamping the

morphological characters, thereby enforcing the topology of the trees obtained from the combined analysis to reflect the topology of the partitioned molecular analysis (Pennington 1996).

When comparing the topology of the combined analyses with that of the partitioned molecular analyses, the influence of the morphological characters is evident. In the case of the *trn*L-F data set the morphological characters resolved the relationship between *P. diffusus* and *P. fruticosus*, and *Merciera* and also between the Malmesbury plant and *Treichelia*. The relationship between the *Theilera* species is better resolved in the successively weighted analysis (Figure 4.6). Among the most notable influences of the morphological characters in the ITS data set, is the formation of the *Merciera-Prismatocarpus-Roella* clade. There are also areas in the trees where the morphological characters distorted previously resolved relationships. It would therefore appear that swamping has not taken place in any of the combined analyses.

4.4.3. The systematic value and evolution of morphological characters

The five species assemblages resolved by the phylogenetic analyses (see Chapter 3 and 4) correlate poorly with current generic boundaries. The characters mapped in this study show weak potential as synapomorphies for recognition of any of these species assemblages (possibly genera). Only the clade comprising *Rhigiophyllum* and *Siphocodon* is supported by synapomorphies - epipetalous stamens and pendulous ovules. In the remaining four clades clear (uncontradicted) supporting synapomorphies are lacking. Whilst two-locular ovaries support the *Roella-Prismatocarpus-Merciera* clade, they have been lost or gained several times. This clade also possesses a shrubby habit, except for *P. sessilis* and *R. muscosa*. The shrubby habit has also evolved in several species of the *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia* clade, once in the *Wahlenbergia-Prismatocarpus crispus* clade and in the *Rhigiophyllum-Siphocodon* clade. It is common in the Campanulaceae that the same life form is present in different taxa or an individual taxon may include more than one life form (Shulkina *et al*. 2003). For instance *Campanula* includes species that are

perennials, biennials and annuals. This character has therefore limited use in the classification of the family. The tubular corolla shape, basal placentation and indehiscent capsules support the subclade *Merciera*. However, the tubular corolla shape is homoplasious, being present in *Theilera*, *Rhigiophyllum* and *P. diffusus* (Figure.4.13A). The funnel-shaped corolla, groups species of the *W. androsacea-P. crispus* clade together.

In contradiction of previous classification systems, the current analyses show the capsule dehiscence cannot be used as the primary character to separate genera, at least if the criterion of monophyly is to be strictly applied. It has to be stated that previous classification systems might not have been based only on the criterion of monophyly to erect genera. The optimization of the mode of capsule dehiscence on the phylogenetic tree, suggests that this character is variable within each of the major clades retrieved. Within the *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia* clade, apical valves are lost several times and in the *Wahlenbergia-Prismatocarpus crispus* clade they are lost once. The occurrence of the same capsule dehiscence mechanism in each of the clades, *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia*, *Wahlenbergia-Prismatocarpus crispus* and *Wahlenbergia krebsii* suggests parallel/convergent evolution. *Craterocapsa*, erected by Hilliard and Burtt (1973) on the basis of its opercular capsule, is nested in a clade in which the apically valvate capsule is dominant. In the *Roella-Prismatocarpus-Merciera clade*, the subclade *Prismatocarpus* has capsules that dehisce by longitudinal slits corresponding with the calyx lobes while *Merciera* has indehiscent capsules. Although the capsules in most species of *Roella* dehisce by apical plugs, this character state fails to separate them as a monophyletic group. Several unique modes of capsule dehiscence have evolved independently, such as the protruding folds of *W. acaulis* and the circumsessile type of *S. spartioides*. These unique modes most probably play an important role in facilitating seed dispersal. This character fails to provide diagnostic synapomorphous states for most of the major well-supported clades, thereby rendering its systematic value in delimiting genera, unsuitable. However, in certain cases it does appear diagnostic for smaller clades. The indehiscent capsule defines *Merciera,* the longitudinally dehiscent

capsules the *Prismatocarpus* clade, and members of the *Wahlenbergia* clade including *Microcodon* have apical valvate dehiscence. (Figures 4. 20 A-H, 4.21I).

The character optimizations suggest that the common ancestor of the South African Campanulaceae comprised herbaceous plants with free stamens, axile placentation and without stigmatic glands, from which other characters states were derived, most probably in response to environmental conditions. According to Levin (2005) environmental change is the main driving force behind speciation and origin of evolutionary novelty. The shrubby habit evolved several times and is particularly evident in plants occurring in the nutrient poor, fire prone fynbos region. Survival of these plants is ensured by resprouting after fire, followed by a period of prolific vegetative growth and flowering. Shrubs contribute about 53% of the flora of this region and this dominance is attributed to the nutrient poor soil that favours shrub growth (Goldblatt and Manning 2002). The development of woodiness is also consistent with flowering times. Shrubby species flower during the hot, dry Cape summer months whereas, annual species flower from late winter to mid spring when the soil moisture is still high, allowing rapid growth in the short favourable season.

One can further speculate that re productive characters that evolved from these ancestral states were important in pollinator interactions. The commonly held view is that the flowers of most angiosperms are specialized for pollination by particular animal types (Johnson and Steiner 2000), for example by beetles, moths, rodents, butterflies, long proboscid flies and sunbirds. This suggests that many of the long narrow tubular flowers (*Merciera*, *P. diffusus*, *Theilera*, *Rhigiophyllum*) have adapted to be pollinated by long-tongued fly species. However, the pollination strategies in the Campanulaceae are largely unknown.

It appears that morphology does not really contradict the molecular evidence and therefore also underscores the controversy regarding the current generic circumscription. But this does not bring us closer to defining a genus in the Campanulaceae. Perhaps the circumscription of a genus as a natural unit with its own

combination of characters as applied by De Candolle (1830) is relevant here. This means that a genus could comprise a heterogenous assemblage of species with, for example, different modes of capsule dehiscence or corolla structure but ultimately form a coherent group recognized by a combination of characters. The recognition of *Rhigiophyllum* and *Siphocodon* as a single genus seems relatively straightforward, but within the rest are more options. One option is to recognize a single variable genus with several subgroups. Another is to subdivide the clade into numerous smaller genera with subgroups where appropriate to preserve monophyly. A final decision on generic circumscriptions is still some time off since more data are required. However, a general picture is slowly emerging.

CHAPTER 5

ROELLA L., *PRISMATOCARPUS* L'HÉR. AND *MERCIERA* A.DC.: ONE GENUS OR SEVERAL, EVIDENCE FROM MORPHOLOGY AND MOLECULES

5.1. Introduction

Initial analyses of the *trn*L-F data showed a largely unresolved clade comprising species of *Roella*, *Prismatocarpus* and *Merciera.* This prompts questions about phylogenetic relationships among these genera. Attempts to increase the taxon sampling and to explore different molecular markers for resolving relationships in this clade had limited success. Financial and time constraints also curtailed efforts. In this Chapter, the available phylogenetic information from other components of this study is utilized to potentially clarify the relationships among these genera. Phylogenetic results of partitioned and combined analyses for chloroplast, nuclear and morphological data are presented.

5.1.2. History of the Genera

Adamson (1952, 1955b) was convinced that these three genera are closely related and postulated a single common ancestry for them. Several species of *Prismatocarpus* and *Merciera* have previously been placed in *Roella.* All three genera are endemic to South Africa and mostly concentrated in the fire prone Cape Floristic Region (CFR) where persistence requires some fire survival strategy. All species are re-sprouting shrublets except *P. crispus*, *P. hildebrandtii* and *R. muscosa*. These three are herbaceous, with plants generally living for about seven years, which coincides with the natural fire cycle of the CFR (van Wilgen 1981).

The structure of the capsule, especially its mode of dehiscence, has been used to separate the genera. *Merciera* has indehiscent capsules, longitudinal splitting is

characteristic of the capsules of *Prismatocarpus*, and an apical plug is the mode of dehiscence in *Roella*. Close examination of the capsule has revealed that this commonly used character is not a synapomorphy for the individual genera. In some species of *Roella* (e.g. *R. spicata*) capsules are found with longitudinal slits, while in the case of *R. muscosa* the capsules appear indehiscent. The case of *Prismatocarpus* is similar. The capsules of *P. crispus* do not convincingly display the development of longitudinal slits or the prismatic shape of other species. Thulin (1974) erected segregate genera *Namacodon* and *Guinillea* because their capsules deviate from the structure of that of the core genus in which they were previously classified.

Roella (Figure 5.1 A) contains 24 species, mostly shrubs, that are concentrated in the CFR. Of the 24 species, two extend into the Eastern Cape, one into KwaZulu Natal and one into the Northern Cape. Adamson (1952) divided the genus into five series based on habit, bract and flower characters. Species in the series *Roella* and *Prostratae* (*R. prostrata* E.Mey. ex A.DC., *R. bryoides* H.Buek, *R. arenaria* Schltr., *R. latiloba* A.DC., *R. recurvata* A.DC., *R. goodiana* Adamson) reportedly form interspecific hybrids. Putative hybrids between *R. incurva* and each of *R. maculata*, *R. psammophila* and *R. rhodantha* have been reported by Adamson (1952). Several new combinations were made and new species described by Adamson (1952) without stating the reasons for doing so, and these are difficult to identify.

Prismatocarpus (Figure 5.1 B) comprises 30 species of which two are annuals and the rest perennials. Only one species extends into the Eastern Cape. Adamson (1952) divided the genus into two subgenera, *Prismatocarpus* (as *Euprismatocarpus*) and *Afrotrachelium*. Habit and inflorescence structure were used to further divide *Prismatocarpus* into three series, *Prismatocarpus* (as *Fruticosi*) (*P. alpinus* (Bond) Adamson, *P. altiflorus* L'Hér., *P. brevilobus* A.DC., *P. crispus* L'Hér., *P. decurrens* Adamson, *P. fruticosus* L'Hér., *P. hildebrandtii* Vatke, *P. lycopodioides* A.DC., *P. pedunculatus* (P.J.Bergius) A.DC. *Stricti* (*P. campanuloides* (L.f.) Sond., *P. candolleanus* Cham., *P. cliffortioides* Adamson, *P. hispidus* Adamson, *P. schlechteri* Adamson, *P. spinosus* Adamson, *P. virgatus* Fourcade) and *Nitidi* (*P. cordifolius*

Adamson, *P. debilis* Bolus ex Adamson, *P. lasiophyllus* Adamson, *P. lycioides* Adamson, *P. nitidus* L'Hér., *P. sessilis* Eckl. ex A.DC., *P. tenellus* Oliv., *P. tenerrimus* H.Buek). *Afrotrachelium*, with its distinctive narrow, long, cylindrical corolla is not divided into series.

Merciera (Figure 5.1 C) is a CFR endemic genus of six species of dwarf shrubs. Vegetatively it resembles *Roella* series *Roella* and *Prostratae*, but *Prismatocarpus* subgenus *Afrotrachelium* in corolla structure. It has been the focus of recent taxonomic work (Cupido 2002, 2003, 2006).

5.1.3. Aims

The aim of this chapter is:

1. to explore the evidence from the molecular and morphological analyses to evaluate the generic status of *Roella, Prismatocarpus* and *Merciera*,

2. to propose a new classification for the genera.

A

Roella incurva

Roella cuspidata

Roella amplexicaulis

B

Prismatocarpus pedunculatus

Prismatocarpus diffusus

C

Merciera leptoloba

Merciera tenuifolia

Merciera tetraloba

Figure 5.1. Representative species of *Roella*, *Prismatocarpus* and *Merciera*.

5.2. Materials and methods

The sampling and phylogenetic methods (parsimony) for the individual and combined data sets are specified in Chapters 3 and 4.

5.3. Results

5.3.1. Molecular evidence

The partitioned and combined *trn*L-F-ITS analysis produced similar results for these three genera (Figure 5.2 A and B). Only *Merciera* formed a subclade. *Roella* and *Prismatocarpus* appear non-monophyletic, and together with *Merciera* is part of a polytomy. *P. crispus* which is classified in *Prismatocarpus* is not associated with this genus or with this clade but with species of *Wahlenbergia* instead. The poorly resolved ITS topology did not retrieve this clade.

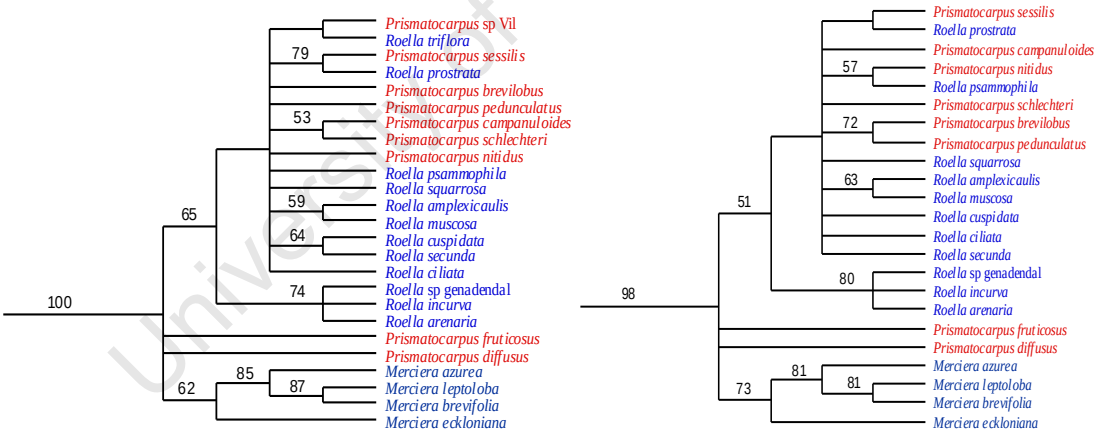
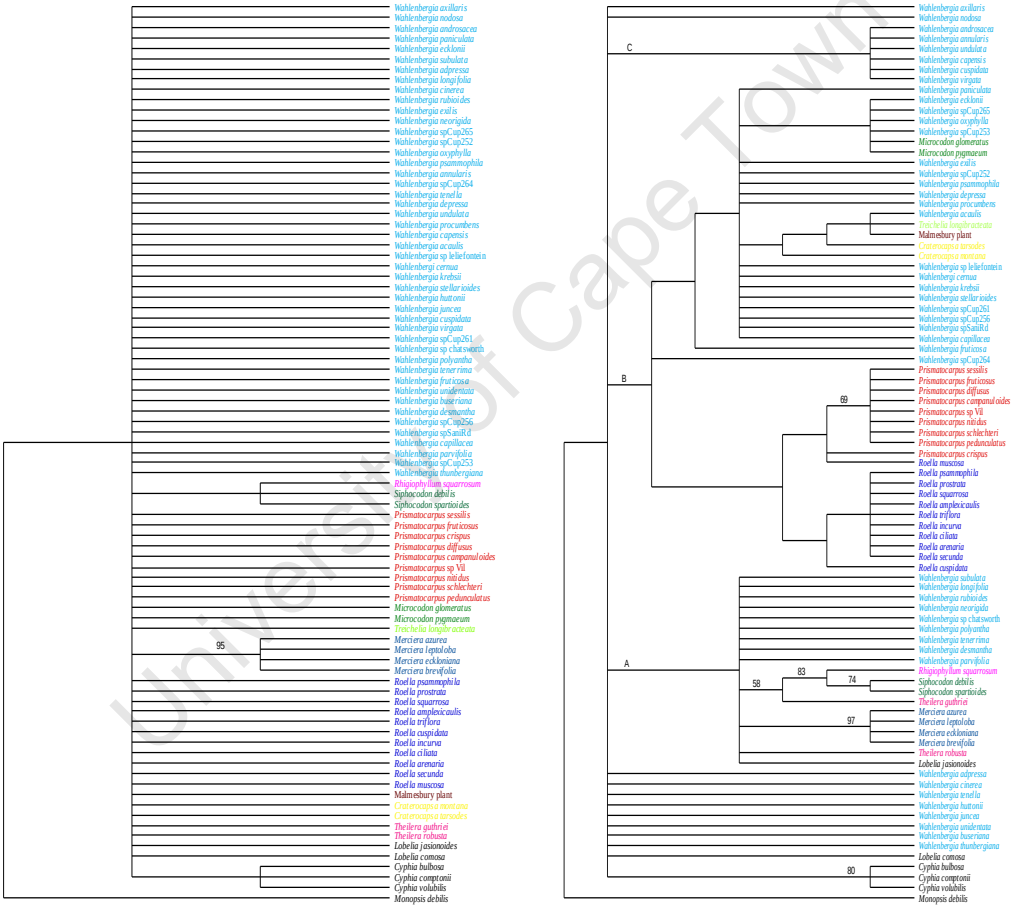


Figure 5.2. Topologies of the clades depicting *Roella*, *Prismatocarpus* and *Merciera* formed in the molecular analyses. All trees were unweighted. A; *trn*L-F data set, B; combined *trn*L-F and ITS data sets. Bootstrap values ≥50% are indicated above the branches.

5.3.2. Morphological evidence

In the weighted and unweighted tree topologies *Merciera* is monophyletic (Figure 5.3 A and B). However, in the weighted tree topology *Merciera* is not associated with *Roella* and *Prismatocarpus*, but rather participates in a polytomy with several other taxa. Species of *Roella* and *Prismatocarpus* form an unsupported clade as part of a trichotomy. This clade separates into two subclades, one with only *Roella* species and the other with species of *Prismatocarpus* and *R. muscosa*.



A **B**

Figure 5.3. Topologies of the clades depicting *Roella*, *Prismatocarpus* and *Merciera* found after heuristic research of morphological data set. A; unweighted, B; weighted. Bootstrap values ≥50% are indicated above the branches.

5.3.3. Evidence from the total evidence (combined *trn*L-F, ITS, morphology) analyses

The unweighted analysis (Figure 5.3 A) produced a poorly resolved clade, with only *Merciera* resolving as monophyletic (BS= 99) in an unsupported sister relationship with *P. crispus*. *R. incurva* and *R. arenaria* form a well supported clade (BS= 96) as part of the polytomy. In contrast, the weighted analysis resolved the clade as a trichotomy. The first subclade, with no bootstrap support, is formed by species of *Prismatocarpus*. The second is well supported (BS= 99) comprising species of *Merciera*. Species of *Roella* formed an unsupported third subclade. The phylogenetic hypothesis derived from the weighted total evidence analysis is used in subsequent discussions.

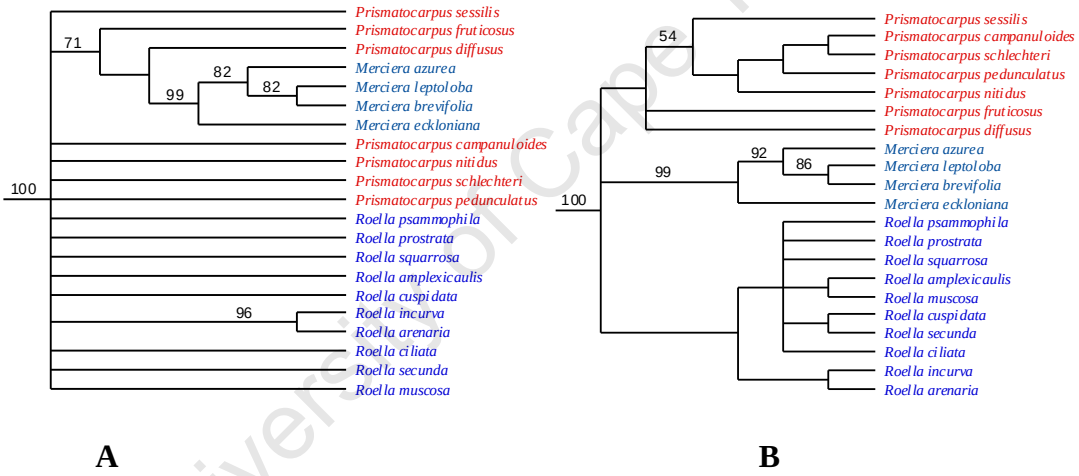


Figure 5.4. Topologies of the clades depicting *Roella*, *Prismatocarpus* and *Merciera* found after heuristic research of the total evidence analyses. A; unweighted, B; weighted. Bootstrap values ≥50% are indicated above the branches.

5.4. Discussion

Franz (2005) raised concerns regarding the increasing number of phylogenies that are not being transformed into classifications even though phylogenetic studies rely on pre-existing classifications. The next step in this study would be to try and transform the phylogenetic hypothesis into a meaningful taxonomy. The results of the phylogenetic analyses present several options for achieving this. These options require a decision between splitting and lumping, guided by the primary criterion of monophyly, followed by stability in nomenclature, strong statistical support for the taxon, maximizing phylogenetic information and ease of identification of the taxon (Backlund and Bremer 1998).

Irrespective of the option chosen it would be necessary to exclude *P. crispus* from *Prismatocarpus* to achieve a monophyletic group. *P. crispus*, probably along with *P. hildebrandtii*, appears isolated in *Prismatocarpus*, with the two species being most probably conspecific. Adamson (1952) cited a single collection (Esterhuysen 1422) from Van Rhyns Pass under *P. crispus* and *P. hildebrandtii*. The specimens cited were, however, from different herbaria. This oversight by Adamson is perhaps indicative of how morphologically similar the two species are. Specimens of Dinter cited under *P. hildebrandtii* were re-identified as *Namacodon schinzianum* (Markgr.) Thulin, an endemic species of central Namibia (Thulin 1974). The remaining specimen cited under this species, the type specimen Meyer 1869 from the Hantamsberg in Calvinia, was probably destroyed in Berlin during the war (Thulin 1974). Since Meyer's collection, no further collections of this species were made from the Hantam Mountains.

5.4.1. Option 1

The primary criterion of monophyly accompanied by strong statistical support favours the treatment of species of *Roella*, *Prismatocarpus* and *Merciera* as a single taxon. Monophyly of the group is supported by two morphological synapomorphies: the two-locular ovaries and the perennial life form. The morphological variation within this

group can be accommodated by dividing the taxon into three subtaxa. The principle of priority necessitates the application of the name *Roella* to the taxon. The re-circumscribed *Roella* would consist of 60 species, divided into three informal groups, *Roella* (24 spp.), *Prismatocarpus* (30 spp.) and *Merciera* (6 spp.). In this way the degree of morphological variation that exists within *Roella* will be highlighted. This act will necessitate new combinations for at least 36 species.

5.4.2. Option 2

Splitting the clade would result in retaining the original three genera, *Roella*, *Prismatocarpus* and *Merciera*. However the lack of statistical support does not support dividing the clade into monophyletic taxa. In fact statistical support exists for only one of these taxa, but despite this all three are morphologically distinct. The benefits of this option are that i) it achieves maximum stability as no nomenclatural changes are required and ii) each genus will be easily identifiable.

5.4.3. Option 3

Implementing this option would entail merging *Merciera* and *Roella* in a single genus and retaining *Prismatocarpus* as a separate genus. The tree topology and statistics do not readily support this treatment. However reasonable stability is achieved because new combinations will be required for only six species. *Merciera* is easily accommodated in *Roella* as a morphologically distinct subgenus. This act may prompt unnecessary future debates on the generic status of *Merciera*.

5.4.4. Option 4

In a similar action to that in option 3 *Merciera* and *Prismatocarpus* can be merged into a single genus and retaining *Roella* as a separate genus. This will also require only six new combinations, but has similar shortcomings as that of option 3.

5.4.5. Option 5

In the final permutation, merging genera entails merging *Roella* and *Prismatocarpus* into a single genus and retaining *Merciera* as a separate genus. The act will require 30 new combinations and like the previous three options the topology and lack of statistical support do not contradict or support this treatment.

5.4.6. Taxonomic approach

An alternative to the strictly phylogenetic interpretation, summarized by the "principles" set out by Backlund and Bremer (1998), is the taxonomic approach. This approach simply organizes observed patterns of character variation into similar groups thereby allowing paraphyletic groups to exist. Proponents of this approach, such as Sosef (1997) and Brummit (2002), argue that is it is not always possible to construct a taxonomy composed only of monophyletic groups. Following their approach it would be difficult to depart from Adamson's treatment of the three genera. Perhaps a few taxonomic changes will be required to refine the taxonomy at the species level. Option 2 would probably be preferred.

5.4.7. Rank-free approach (the PhyloCode)

In order to link phylogenetic philosophy with traditional Linnaean taxonomy, the phylogenetic framework is manipulated to fit traditional taxonomy. To achieve this, the principle of monophyly is applied to recognize taxa that are then named and placed in a rank-based system. A disadvantage of this approach is that ranking decisions are subjective and it leads to instability of taxon names (see option 1-3 above). The opponents of this system propose that relationships among taxa are to be presented without the use of categorical ranks (rank-free taxonomy) (de Queiroz 2006). This, according to them, will ensure that the distinction between taxonomy (representation of relationships) and nomenclature (naming of taxa) is maintained. The naming of groups (clades) discovered by phylogenetic methods is governed by the principles of phylogenetic nomenclature. These principles are formalized into a PhyloCode (Cantino and de Queiroz 2006). Clades are regarded as products of evolution that are discovered

rather than created by systematists and have an objective existence regardless of whether they are named (Cantino and de Queiroz 2006). In practice taxon names are given phylogenetic definitions which identify a clade by reference to a node, stem or apomorphy. The phylogenetic definition contains specifiers (species, specimen or apomorphy to which the name applies) and qualifying phrases (Cantino *et al*. 2007).

Within the context of the phylogenetic hypothesis presented here the following clades can be identified.

Pan-Roella clade

Definition – total clade containing all species of *Merciera, Roella* and *Prismatocarpus*.

Merciera clade

Definition (node-based)- the most inclusive clade containing *Merciera*.

Roella clade

Definition (node-based)- the most inclusive clade containing all species of *Roella*.

Prismatocarpus clade

Definition (node-based)- the most inclusive clade containing all species of *Prismatocarpus*.

5.5. Taxonomic implications

The results of the phylogenetic analysis and the principles of classification adopted here favour option 2. In choosing this option the existing genera *Roella*, *Prismatocarpus* and *Merciera* are retained. Despite the lack of support this option provides genera that are easily recognizable and requires no new combinations. This

option also compares well with the principles of phylogenetic nomenclature if the ranking of clades is ignored.

A synoptic revision of the genera *Roella*, *Prismatocarpus* and *Merciera* is presented in Chapter 6.

CHAPTER 6

A SYNOPSIS OF THE GENERA *ROELLA* L., *PRISMATOCARPUS* L'HÉR. AND *MERCIERA* A.DC.

6.1. Introduction

Adamson (1952, 1954), who last revised these genera, unfortunately never explicitly stated the species concept employed to delimit species of *Roella*, *Prismatocarpus* and *Merciera*. Nor did he provide sufficient justification for describing new species or new combinations in his taxonomic accounts. One can only assume that he applied a morphological or taxonomic species concept, which simply classifies patterns of character variation into groups. If this is so, it was generally loosely applied and has led to the recognition of many questionable species. Morphological variation within these genera, is complex and using a narrow species concept may lead to superfluous species or the overuse of infra-specific ranks to accommodate minor variation. It seems in some instances that intermediate forms were attributed to hybridization.

A comprehensive, species level taxonomic study is required to establish species boundaries within these genera, particularly in *Roella* and *Prismatocarpus*, before a confident taxonomy can be presented. In addition, the reported formation of hybrids between closely related species of the genus *Roella* requires investigation at the population level, possibly with the aid of molecular techniques. Phenotypic plasticity is common in the Campanulaceae (Eddie and Ingrouille 1999) and perhaps accounts in part for the extent of morphological variation. Often the variation induced by plasticity is mistakenly interpreted as a signal for distinct species. Notwithstanding the gaps in our knowledge, taxonomic changes are tentatively here proposed based on field experience and brief specimen examination.

A taxonomic account of the genera based on the findings of the phylogenetics studies (See Chapter 5) is presented here. In this account *Roella* comprises 19 species, *Prismatocarpus* 26 species and *Merciera* six species. In addition, to a taxonomic synopsis, a key to genera is provided. Since *Merciera* was revised recently (Cupido 2006), a key to the six species of this genus is provided.

6.2. Taxonomic treatment

Key to genera

1a Inflorescence spike-like; hypanthium hispid; ovules four; capsule indehiscent
3. *Merciera*

1b Inflorescence not spike-like; hypanthium glabrous or minutely hairy; ovules many; capsule dehiscent

2a Inflorescence 1-few flowered, closely subtended by leaves; capsule cylindrical or barrel-shaped, opening by a terminal pore or irregular longitudinal slits
1. *Roella*

2b Inflorescence usually pedunculate; capsule elongate, splitting longitudinally nearly to the base into five segments
2. *Prismatocarpus*

1. *Roella* L., Sp. pl. 170 (1753).
Type: *R. ciliata* L.

Shrublets or seldom herbaceous; branches hispidulous to hispid. *Stems* erect, suberect or decumbent, branched. *Leave*s alternate, sessile, linear, ovate, ovate-lanceolate, subulate, entire, scattered or crowded, ascending or spreading, glabrous or hairy, margins sometimes ciliate, axillary clusters of smaller, glabrous leaves often present. *Inflorescence* terminal, one to many flowered. *Flowers* sessile, axillary, actinomorphic, often surrounded or subtended by bract-like leaves; hypanthium linear, elongate, glabrous or hispid; calyx 5 lobed, often fused at the base to form a short tube, glabrous or hairy; corolla bell shaped, white, violet-blue or very rarely pale blue or pink, occasionally with darker spots on the inside, lobes 5, ovate or linear-lanceolate, , glabrous or hairy. *Stamens* 5, free, inserted at the base of the corolla tube; filaments dilated and ciliate at the base; anthers linear, basifixed. *Ovary* inferior, 2-locular, containing many axile ovules; style stout, bifid, glabrous or hairy, base discoid; stigma glabrescent. *Fruit* a capsule, glabrous or hispid, crowned with persistent calyx, many seeded, opening by longitudinal slits or apical plug.

Roella amplexicaulis Wolley-Dod

Erect, rigid. Leaves crowded, imbricate, recurved, rotund-ovate, sharply serrate on the upper part, ciliate on the lower, apiculate. Bracts orbicular. Flowers white or pale blue, 3-8 in terminal heads. Flowering from November to April.

Distribution and habitat: Endemic to the Cape Peninsula where it occurs on sandstone slopes.

Roella arenaria Schltr.

Erect or sprawling. Leaves linear, spreading, ciliate, midrib prominent beneath, axillary clusters of smaller leaves present. Bracts leaf-like, rigid, recurved. Flowers

white or pale blue, solitary at branch tips. Calyx lobes strongly recurved and hairy. Flowering from December to March.

Distribution and habitat: Malmesbury to Bredasdorp, on sandy flats.

Roella bryoides H.Buek

Erect or sprawling. Leaves linear, spreading, short, 3-6 mm long, ciliate, midrib prominent beneath, axillary clusters of smaller leaves present. Bracts leaf-like, rigid. Flowers white or pale blue, solitary at branch tips. Flowering from December to February.

Distribution and habitat: Clanwilliam to Caledon Swartberg, on sandy slopes.

Roella ciliata L.

Erect or sprawling. Leaves linear, subulate, ciliate, axillary clusters of smaller leaves present. Flowers white or blue with a dark ring or spots on lobes, the dark part often edged with white, terminal, solitary, bell-shaped. Flowering from August to March.

Distribution and habitat: Cape Peninsula to Caledon where it grows on stony slopes.

Roella compacta Schltr.
=R.cuspidata Adamson

Sprawling or decumbent. Leaves linear, pungent, coarsely ciliate, axillary clusters of smaller leaves present, margins revolute. Bracts ovate-acuminate. Flowers white, pale blue or yellowish in terminal heads. Flowering from December to February.

Distribution and habitat: Cape Peninsula to Bredasdorp, on rocky coastal limestones.

Roella dregeana A.DC.
=*R. psammophila* Schltr.

Erect or sprawling. Leaves linear, small, ciliate near the base, axillary clusters of smaller leaves present. Bracts with many stiff wire-like hairs. Flowers white or pale blue, solitary or in groups at branch tips. Calyx and hypanthium hairy. Flowering from January to March.

Distribution and habitat: Paarl to Hermanus and Riviersonderend Mountains, on sandstone slopes.

Roella dunantii A.DC.

Erect or prostrate. Leaves linear, conspicuously white ciliate, midrib prominent beneath. Flowers white or blue, occasionally with small spots on petals, solitary or in groups. Flowering from November to January.

Distribution and habitat: Mamre to Caledon Swartberg, on sandy lower slopes.

Roella glomerata A.DC.

Erect, branching from the base. Leaves linear, ciliate or toothed, axillary clusters of smaller glabrous leaves often present. Flowers white, pale blue or pinkish, in dense heads at branch tips. Flowering from January to February.

Distribution and habitat: East London to the southern KwaZulu-Natal Coast where it is found in coastal sandy flats and grasslands.

Roella goodiana Adamson

Erect, branching from the base. Leaves crowded, imbricate, spreading or recurved when young. Bracts leaf-like but not recurved, 3-toothed at the tip. Flowers white, less than 1 cm long. Flowering from February to April.

Distribution and habitat: Endemic to the Cape Peninsula: Klaver Valley, on sandy flats.

Roella incurva Banks ex A.DC.
=*R .rhodantha* Adamson

Erect or sprawling. Leaves linear, ciliate, axillary clusters of smaller leaves present. Bracts usually forming a distinct bulge below the flower. Flowers white, blue, pink or red mostly with dark spots on petal lobes, solitary or in groups at branch tips. Flowering from October to January.

Distribution and habitat: Somerset West to Bredasdorp, on sandy lower slopes.

Roella latiloba A.DC.

Erect with ascending branches. Leaves linear, ciliate near the base. Bracts leaf-like with linear pinnate teeth. Flowers white or pale blue, solitary at branch tips. Calyx lobes broadly triangular, shortly hairy. Flowering from December to February.

Distribution and habitat: Only known from Clanwilliam and Bredasdorp where it grows on sandy slopes.

Roella maculata Adamson

Erect, much branched. Leaves linear, finely ciliate, usually with distant teeth, midrib prominent beneath, axillary clusters of smaller leaves present. Bracts lanceolate-acuminate with slender distant pinnate teeth on upper part, ciliate at the base, finely hairy. Flowers blue with dark spots between corolla lobes, solitary or in groups at branch tips. Flowering from December to February.

Distribution and habitat: Kleinmond to Bredasdorp where it occurs on sandy coastal slopes.

Roella muscosa L.f.

Prostrate, mat-forming perennial herb. Leaves crowded towards tips of stems, ovate-elliptic, margins prickly toothed, ciliate near the base, narrowing to a short petiole sheathing the stem. Flowers blue, pale blue or white, solitary, terminal. Flowering from November to February.

Distribution and habitat: Cape Peninsula, on upperparts of mountain in sand between rocks.

Roella prostrata E.Mey. ex A.DC.

Erect or sprawling. Leaves linear, ciliate, midrib prominent beneath, axillary clusters of smaller leaves present. Flowers white or pale blue, solitary at branch tips. Flowering from December to March.

Distribution and habitat: Hopefield to Caledon, on sandy flats.

Roella recurvata A.DC.

Erect, branching from the base. Leaves crowded, imbricate, spreading or recurved, elliptic, apiculate, ciliate, slightly decurrent. Bracts leaf-like, recurved. Flowers white or blue, solitary at branch tips. Flowering from January to February.

Distribution and habitat: Endemic to the Cape Peninsula, on sandy flats.

Roella secunda H.Buek

Prostrate or sprawling with many short, often secund branches. Leaves more or less squarrose, flat, ciliate, axillary clusters of smaller glabrous leaves present. Flowers white, solitary or in terminal heads, heads sometimes grouped. Flowering from December to March.

Distribution and habitat: Montagu to Uitenhage, on dry sandy or stony slopes.

Roella spicata L.f.

=*R. lightfootioides* Schltr.

Erect or diffuse. Leaves crowded, linear, axillary clusters of smaller glabrous leaves often present. Bracts leaf-like, broad at the base. Flowers white, in terminal or lateral heads aggregated into a spike-like inflorescence. Flowering from January to March.

Distribution and habitat: Genadendal to Port Elizabeth where it occurs on rocky mountain slopes.

Roella squarrosa P.J.Bergius
=*R. decurrens* L'Hér.

Annual or perennial, erect or sprawling. Leaves scattered, spreading or recurved, ovate-lanceolate, sharply tooted, apiculate, ciliate near the base and decurrent. Bracts

broadly lanceolate or rotund-ovate. Flowers white or pale blue, solitary or 2-5 in terminal heads. Flowering from December to April.

Distribution and habitat: Endemic to the Cape Peninsula where it grows on sandy or sandstone slopes.

Roella triflora (R.D.Good) Adamson

Erect with ascending branches. Leaves linear, ciliate near the base, toothed on the upper part, axillary clusters of smaller leaves present. Bracts finely hairy, margins with stiff wire-like hairs. Flowers pale blue with a dark band at the base, solitary or in groups at branch tips.

Distribution and habitat: Endemic to the Cape Peninsula where is occurs on sandy lower slopes.

2. ***Prismatocarpu****s* L'Hér. in Sert. Ang. 1 (1789) nom. cons. *Campanula* sect. *Prismatocarpus* (L'Hér.) Schult., Syst. Veg. 5: 152 (1819.
Type [conserved]: *Prismatocarpus paniculatus* L'Hér.

Shrublets or seldom herbaceous; branches hispidulous to hispid. *Stems* erect, suberect or decumbent, branched. *Leave*s alternate, sessile, linear, ovate, ovate-lanceolate, subulate, entire, scattered or crowded, ascending or spreading, glabrous or hairy, margins sometimes ciliate, axillary clusters of smaller, glabrous leaves often present. *Inflorescence* terminal or axillary, one to many flowered. *Flowers* sessile, axillary, actinomorphic or seldom subactinomorphic, often surrounded or subtended by bract-like leaves; hypanthium linear, elongate, glabrous or hispid; calyx 5 lobed, often fused at the base to form a short tube, glabrous or hairy; corolla narrowly tubular, funnel-or bell shaped, white, occasionally with purple tips, violet-blue or very rarely pale blue or pink, occasionally with darker spots on the inside, lobes 5, ovate or linear-lanceolate, occasionally unequal, glabrous or hairy. *Stamens* 5, free, inserted at the base of the

corolla tube; filaments dilated and ciliate at the base; anthers linear, basifixed. *Ovary* inferior, 2-locular, containing many axile ovules; style stout, bifid, glabrous or hairy, base swollen or discoid; stigma glabrescent. *Fruit* a capsule, glabrous or hispid, crowned with persistent calyx, many seeded, opening by longitudinal slits.

Prismatocarpus alpinus (Bond) Adamson

Prostrate or mat-forming. Leaves crowded, linear, often with a recurved tip, coarsely ciliate near the base. Flowers blue, sessile or on a peduncle up to 10 cm long, funnel-shaped. Flowering in December and January.

Distribution and habitat: Occurs from the Cederberg to the Hottentots Holland Mountains on sandstone ledges at high altitudes.

Prismatocarpus altiflorus L'Hér.

Erect or sprawling, up to 1.5 m tall. Leaves linear, subulate, coarsely ciliate near the base, often crowded, axillary clusters of smaller leaves usually present. Flowers white to blue, aggregated in subumbellate, pedunculate terminal cymes, cup-shaped; hypanthium usually with coarse dense hairs. Flowering from November to December.

Distribution and habitat: Cederberg and Cold Bokkeveld mountains, on sandstone slopes.

Prismatocarpus campanuloides (L.f.) Sond.

Erect or sprawling, 0.2 – 0.8 m tall. Leaves alternate, linear or linear-lanceolate, flat or revolute, ciliate or toothed. Flowers white or tinged with pink or violet, sessile, solitary in upper axils, crowded at branch tips, funnel-shaped. Flowering from December to April.

Distribution and habitat: Worcester to East London, on sandy or limestone flats or slopes.

Prismatocarpus candolleanus Cham.
= *Prismatocarpus virgatus* Fourcade

Erect, rigid and branched, up 0.5 m tall. Leaves linear-lanceolate, margins revolute, entire or commonly toothed; bracts broad and pinnately lobed. Flowers white to pale violet, sessile in upper axils, bell-shaped. Flowering from December to January.

Distribution and habitat: Swellendam to Uniondale, on sandstone slopes.

Prismatocarpus cliffortioides Adamson

Erect, rigid and branched, up 1 m tall. Leaves linear-lanceolate, margins revolute and toothed, pungent; bracts broad and pinnately lobed. Flowers pale blue, sessile, crowded in axillary clusters, funnel-shaped with short lobes. Flowering from December to April.

Distribution and habitat: Riversdale to Mossel Bay, on stony or shale slopes.

Prismatocarpus cordifolius Adamson

Prostrate, hispid, branched from the base. Leaves ovate, hairy, toothed; bracts distinctly toothed. Flowers white, solitary or in pairs in upper axils, bell-shaped. Flowering in January.

Distribution and habitat: Kogelberg and Betty's Bay Mountains where it grows in sheltered sandstone crevices.

Prismatocarpus debilis Bolus ex Adamson

Prostrate, slender, forming loose tangles. Leaves opposite, the upper alternate, ovate, toothed. Flowers white, pale blue or pinkish, solitary or in pairs in the upper axils. Flowering from January to March.

Distribution and habitat: Ceres to Swellendam, growing in sheltered sandstone crevices.

Prismatocarpus decurrens Adamson

Decumbent. Leaves decurrent, lanceolate, toothed and ciliate near the base. Flowers white or shaded with blue, in leafless terminal cymes, cup-shaped. Flowering from December to March.

Distribution and habitat: Endemic to the Cederberg Mountains, occurring on sandstones slopes above 1000 m.

Prismatocarpus diffusus (L.f.) A.DC.

Diffuse or rounded, shortly hairy on young stems. Leaves crowded, linear, sparsely ciliate near the base. Flowers blue-violet or occasionally white, in leafless divaricate terminal cymes, tubular with somewhat unequal lobes. Flowering from November to February.

Distribution and habitat: Namaqualand to Riviersonderend, on lower sandstone slopes.

Prismatocarpus fastigiatus C.Presl ex A.DC.

Diffuse or rounded, shortly hairy on young stems. Leaves scattered, linear, sparsely ciliate near the base. Flowers blue-violet or occasionally white in leafless, divaricate terminal cymes, tubular with somewhat unequal lobes, hypanthium hairy. Flowering time unknown.

Distribution and habitat: Only know from the Uienvallei in Bredasdorp where is grows on sandstone slopes.

Prismatocarpus fruticosus (L.) L'Hér.
= *P. brevilobus* A.DC.

Diffuse and slender, up to 0.9 m tall. Leaves linear, subulate, coarsely ciliate near the base, often crowded, axillary clusters of smaller leaves usually present. Flowers white with brown or purple reverse, in leafless terminal cymes, cup-shaped. Calyx lobes shorter than corolla tube. Flowering from November to May.

Distribution and habitat: Cederberg to Bredasdorp and Langkloof. Sandy flats and rocky slopes.

Prismatocarpus hispidus Adamson

Sprawling hispid shrublet. Leaves scattered, ovate, margins slightly revolute and toothed, hispid; bracts pinnately lobed, hispid. Flowers white, in small terminal heads, narrowly funnel-shaped or tubular. Flowering in January.

Distribution and habitat: Langeberg: Cloete's Pass to Outeniqua Mountains, on sandstone slopes.

Prismatocarpus implicatus Adamson

Sprawling, delicate with wiry stems. Leaves scattered, opposite, linear. Flowers white or tinged with pink or purple at the tips, on slender divaricately spreading peduncles in upper axils, commonly tetramerous, bell-shaped. Flowering from January to March.

Distribution and habitat: Limited to the Grootwinterhoek Mountains in the Tulbagh area where is occurs on sheltered sandstone slopes.

Prismatocarpus lasiophyllus Adamson

Prostrate. Leaves ovate to lanceolate, hairy, margins slightly revolute and toothed. Flowers pale blue, terminal, bell-shaped. Calyx lobes hairy. Flowering in January.

Distribution and habitat: Only known from the Langeberg Mountains in Swellendam where it grows in sheltered sandstone crevices.

Prismatocarpus lycioides Adamson

Erect, spiny, branched. Leaves ovate, margins revolute, hispid on midrib beneath, axillary clusters of oblong leaves present. Flowers white, axillary on divaricate spiny branchlets, funnel-shaped. Flowering from January to April.

Distribution and habitat: Hammanshof between Worcester and Villiersdorp, on dry sandstone slopes.

Prismatocarpus lycopodiodes A.DC.

Sprawling or forming small tufts. Leaves imbricate, spreading-incurved or reflexed, short, linear to oblong, coarsely ciliate. Flowers white to pale pink, in subracemose

terminal cymes on slender peduncles, cup-shaped. Flowering from November to January.

Distribution and habitat: Bainskloof to Stellenbosch Mountains in sheltered places on sandstone slopes.

Prismatocarpus nitidus L'Hér.

Prostrate. Leaves alternate or subopposite, ovate to lanceolate, margins slightly revolute and toothed. Flowers white to pale blue, sessile, solitary or in groups of two to five in upper axils. Flowering from January to March.

Distribution and habitat: Endemic to the Cape Peninsula, growing in sheltered sandstone crevices.

Prismatocarpus pauciflorus Adamson

Diffuse or rounded, hairy. Leaves crowded, adpressed, linear, pilose, axillary cluster of smaller leaves occasionally present. Flowers pale violet, in groups of three to six in a secund raceme, tubular. Flowering in January and February.

Distribution and habitat: Endemic to the northern Cederberg Mountains where it grows on sandstone slopes.

Prismatocarpus pedunculatus (P.J.Bergius) A.DC.

Erect or sprawling. Leaves linear, subulate, coarsely ciliate near the base, often crowded, axillary clusters of smaller leaves usually present. Flowers white to blue, in leafless terminal cymes, widely funnel-shaped. Flowering from September to January.

Distribution and habitat: occurs from Vanrhyndorp to Malmesbury and then east to Riversdale, on stony or shale flats and slopes.

Prismatocarpus pilosus Adamson

Diffuse, rigid, hairy. Leaves rigid, linear, ciliate near the base, axillary clusters of smaller leaves occasionally present. Flowers white or pale blue, in leafless divaricate terminal cymes, tubular, hypanthium hairy. Flowering in January.

Distribution and habitat: Endemic to the Cold Bokkeveld Mountains, on sandstone slopes.

Prismatocarpus rogersii Fourcade

Erect or sprawling and slender. Leaves scattered, linear-lanceolate or linear-oblong, margins slightly revolute, entire or toothed, ciliate at the base; bracts broad, the margins with deep narrow spreading pinnate lobes. Flowers white to pale blue, sessile, aggregated at branch tips, bell-shaped. Flowering from December to April.

Distribution and habitat: Outeniqua Mountains in George, on sheltered sandstone slopes.

Prismatocarpus schlectheri Adamson

Erect, branched and slender. Leaves scattered linear-lanceolate, spreading, margins slightly revolute and toothed, ciliate near the base. Flowers white to pale blue, sessile, solitary in upper axils, crowded at branch tips, bell-shaped. Flowering from December to April.

Distribution and habitat: Paarl to Bredasdorp Mountains, on sandy slopes.

Prismatocarpus sessilis Eckl. ex A.DC.

Sprawling and wiry. Leaves linear-lanceolate, margins slightly revolute and ciliate at the base. Flowers white, pink or pale blue, sessile or pedicellate, solitary or in groups of two or three in axils, bell-shaped. Flowering from December to March.

Distribution and habitat: Cape Peninsula to Bredasdorp, on sheltered sandstones slopes.

Prismatocarpus spinosus Adamson

Erect, rigid, hairy and branched, up 1 m tall. Leaves ovate, margins revolute and toothed, pungent; bracts hairy, pungent, pinnately lobed. Flowers white, terminal, usually solitary, narrowly funnel-shaped or tubular. Flowering in January.

Distribution and habitat: Endemic to Potberg, Bredasdorp where it grows on sandstone slopes.

Prismatocarpus tenellus Oliv.

Sprawling, delicate, often forming tangled masses. Leaves scattered, opposite, linear. Flowers white, on slender divaricately spreading peduncles in upper axils, bell-shaped. Flowering from January to March.

Distribution and habitat: Limited to the Hex River Mountains in the Worcester area where is occurs on sheltered sandstone slopes.

Prismatocarpus tenerrimus H.Buek

Sprawling, minutely hairy, up 0.3 m tall. Leaves scattered, ovate-lanceolate, margins thickened, slightly revolute and toothed. Flowers white or pinkish, solitary or in groups of two or three in axils, bell-shaped. Flowering from January to March.

Distribution and habitat: Paarl (Wemmershoek) to Swellendam (Langeberg) and Prince Albert (Swartberg Mountains), on sandstone slopes.

3. ***Merciera*** A.DC. in Monog. Camp. 369 (1830).
Type: *M. tenuifolia* (L.f.) A.DC. (=*Trachelium tenuifolium* L.f.)

Subshrubs; branches hispidulous to hispid. *Stems* decumbent or suberect, branched. *Leaves* alternate, linear, subulate, entire, scattered or crowded, ascending or spreading, sessile, glabrous or hairy abaxially, margins ± ciliate, axillary clusters of smaller, glabrous leaves often present. *Inflorescence* 3-flowered, with 1 terminal, and 2 rudimentary flowers lateral, on highly reduced lateral branches with bract-like leaves, aggregated into spike-like synflorescences towards ends of main branches. *Flowers* sessile, axillary, actinomorphic; bract-like leaves 2, succulent, subtending each of rudimentary flowers, absent in terminal flower; hypanthium obconical, hispid with clavate, filiform, uncinate or circinate trichomes; calyx 4- or 5-lobed, often fused at base to form short tube, glabrous or hairy on hyaline tips and margins; corolla narrowly tubular or funnel-shaped, white, occasionally with purple tips, or violet-blue, or very rarely pale blue, lobes 4 or 5, ovate or linear–lanceolate, occasionally unequal, glabrous, or hairy on back. *Stamens* 4 or 5, free, inserted at base of corolla tube; filaments flattened, wider and pilose ±middle, narrower towards apex; anthers linear, basifixed. *Ovary* inferior, 2-locular, containing 4 erect basal ovules; style filiform, bifid, exserted, glabrous, swollen at base; stigmas glabrescent, bluish purple. *Fruit* a hispid capsule, crowned with persistent calyx, 1-seeded, indehiscent. *Seed* elliptic to ovate.

Key to species

1a Corolla tube more than 7 mm long; flowers blue, violet or purple, rarely white; flowers pentamerous:

2a Plants slender (stem equal to or less than 1 mm thick); leaves scattered; corolla lobes glabrous adaxially; distributed from Groenlandberg (Grabouw, 3419 AA) northwards to Tulbagh (3319AC)……..……...*M. eckloniana*

2b Plants stout (stem more than 1 mm thick); leaves crowded; corolla lobes hairy adaxially; distributed south of Groenlandberg (Grabouw, 3419AA):

3a Stems suberect; leaves ascending, abaxial surface hairy, axillary clusters of smaller leaves always present; corolla tube 11–26 mm long; five times as long as the lobes ……...*M. tenuifolia*

3b Stems decumbent; leaves spreading, abaxial surface glabrescent, axillary clusters of smaller leaves occasionally present on lower parts of stem; corolla tube 7–14 mm long; less than three times as long as the lobes ……...*M. azurea*

1b Corolla tube less than 7 mm long; flowers white, occasionally with purple tips; flowers tetramerous or pentamerous:

4a Flowers tetramerous, margins of calyx lobes ciliate; hypanthium trichomes uncinate to circinate; plants growing in clayey soil; distributed west of Hottentots Holland Mountains……..……*M. tetraloba*

4b Flowers pentamerous; margins of calyx lobes glabrous; hypanthium trichomes clavate or filiform; plants growing in sandy or stony soil; distributed southeast of Hottentots Holland Mountains:

5a Plants decumbent, stout; lower leaves more than 8 mm long, crowded; corolla lobes, linear-lanceolate; 2–6 mm long, almost as long as tube; hypanthium trichomes clavate……*M. leptoloba*

5b Plants suberect, slender; lower leaves less than 8 mm long, scattered; corolla lobes ovate, 2–3 mm long, up to half as long as tube; hypanthium trichomes filiform …….*M. brevifolia*

Merciera azurea Schltr.

Decumbent and stout. Leaves crowded, spreading, glabrous or hairy on abaxial surface, axillary cluster of smaller leaves occasionally present. Flowers violet-blue, rarely white; hypanthium hispid with clavate or filiform trichomes; corolla tube wide, 7–14 mm long. Flowering from November to February.

Distribution and habitat: *M. azurea* ranges from Sir Lowry's Pass to Bredasdorp and occurs on sandy or stony soil at altitudes between 100 and 650 m

Merciera brevifolia A.DC.

Semi-erect and slender. Leaves scattered to crowded, less than 8 mm long, glabrous to hairy on abaxial surface, with axillary cluster of smaller leaves. Flowers white; hypanthium hispid with filiform trichomes; corolla tube 3–6 mm long. Flowering from November to February.

Distribution: *M. brevifolia* is a montane species occurring on the Babylons Tower, and on the Bot River, Houwhoek, Shaw's and Caledon Swartberg Mountains.

Merciera eckloniana H.Buek
=*M. tenuifolia* (L.f.) A.DC. var. eckloniana (H.Buek) Sond.

Semi-erect and slender. Leaves scattered, spreading, glabrous, or hairy on abaxial surface, axillary cluster of smaller leaves occasionally present. Flowers violet-blue, rarely white; hypanthium hispid with filiform trichomes; corolla tube narrow, 7.5–16.0 mm long. Flowering from October to February.

Distribution and habitat: this species is distributed from the Groenlandberg northwards to Tulbagh. It is found on sandy or stony soil at altitudes between 450 to 1 500 m.

Merciera leptoloba A.DC.
=*M. brevifolia* A.DC. var. *leptoloba* (A.DC.) Sond.

Decumbent. Leaves scattered to crowded, lower leaves more than 8 mm long, glabrous to hairy on abaxial surface, with axillary cluster of smaller leaves. Flowers white; hypanthium hispid with trichomes clavate; corolla tube 3.0–5.5 mm long. Flowering from November to March.

Distribution and habitat: *M. leptoloba* is a common species of the Cape southeast coast, from Kogelberg to Bredasdorp. This species is found on sandy or stony flats and hills at altitudes between sea level and 400 m.

Merciera tenuifolia (L.f.) A.DC
=*Merciera tenuifolia* (L.f.) A.DC. var. *candolleana* Sond.
=*Merciera tenuifolia* (L.f.) A.DC. var. *thunbergiana* Sond.

Sub-erect, sparsely or profusely branched. Leaves crowded, ascending, hairy on abaxial surface, axillary cluster of smaller leaves occasionally present. Flowers violet-blue, rarely white; hypanthium hispid with clavate trichomes; corolla tube narrow, 10–25.5 mm long. Flowering from December to January.

Distribution and habitat*:* the distribution of *M. tenuifolia* is limited to Bot River, Houwhoek and Kogelberg where it is found on stony soil at altitudes between 110 and 600 m.

Merciera tetraloba Cupido

Decumbent or suberect and slender. Leaves scattered, ascending, the older spreading, glabrous on abaxial surface; axillary cluster of smaller leaves present. Flowers tetramerous, white, occasionally with purple tips, or very rarely pale blue; hypanthium hispid with uncinate or circinate trichomes; corolla tube 4–6 mm long. Flowering from November to January.

Distribution and habitat: this species is found in Faure, Gordon's Bay, Sir Lowry's Pass, Somerset West, Strand, Dal Josaphat, Du Toitskloof, Stellenbosch, Hermon and Malmesbury on flats and lower mountain slopes at altitudes between 30 and 350 m. It grows in open clayey soil, often in disturbed habitats.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Summary of main findings

The phylogeny presented in this study, based on molecules and morphology, disagrees with existing generic circumscriptions in the South African Campanulaceae. This disagreement is evident under parsimony and Bayesian criteria, despite the different theoretical background of the two approaches. Molecular data from the *trn*L-F and the ITS regions, provided relatively few parsimony informative characters compared to the overall number of characters in these data sets, but the tree produced by the *trn*L-F data is relatively well resolved. However, under the Bayesian criterion node support for the major clades in the ITS tree is lacking. The incorporation of ITS sequences into a published Campanulaceae matrix revealed a monophyletic South African (wahlenbergioid) clade that was estimated to be 28 myr old. It appears that the wahlenbergioids are mainly restricted to the southern hemisphere. In contrast to the molecules the unweighted morphological characters produced a poorly resolved tree under parsimony whereas under the Bayesian criterion better resolution was obtained. The best resolved tree was produced when all available data were analysed simultaneously using successive approximations weighting.

On the basis of these analyses, five species assemblages are revealed, none of which corresponds to any of the 10 genera currently recognized. Several of the smaller genera like *Theilera* and *Microcodon* are nested within a paraphyletic *Wahlenbergia*. The enigma of the classification of the Malmesbury plant is solved and its sister relationship with *Treichelia longibracteata* was established. Adamson's notion of the close relationship among *Roella*, *Prismatocarpus* and *Merciera* is supported by this study. Of these three genera only *Merciera* is monophyletic, but is nested within the clade including members of the other two genera. Surprisingly, *Prismatocarpus crispus* is not associated with other members of the genus, but rather with

Wahlenbergia. *W. krebsii* appears isolated in the family even though its *Wahlenbergia*-like nature seems obvious. The close relationship between *Rhigiophyllum* and *Siphocodon* that was revealed by the phylogenetic analyses is supported by the recent discovery of a unique pollen morphology shared by these genera. The taxonomic implications of these findings are discussed in more detail below.

The discovery of synapomorphies to support the five species assemblages appears difficult, except in the case of the *Rhigiophyllum*- *Siphocodon* clade. Character optimization however revealed that the use of the fruit character in the systematics of the Campanulaceae is unreliable, in isolation, at higher taxonomic levels. It would appear that the diversification of the Campanulaceae in southern Africa during the Oligocene (28 mya) coincided with climatic and topographical changes in the region.

Taxonomic implications

Taxon delimitation

A key premise of systematics is that there is a pattern in nature that can be discovered. However, biological units such as species or species assemblages (genera) are not self-revealing entities of nature, but have to be constructed. For such a construct to be regarded as a suitable scientific concept it has to include a theoretical and a practical component (Henderson 2005). Several concepts have been used in classification and are briefly explored below.

The origin of the genus concept in botany is pre-Linnaean, presumably developed from the need to name distinguishable groups of plants to facilitate communication (Bartlett 1940). It is therefore not surprising that the criteria used during this time were not objective and lack a theoretical basis. But this mindset persisted. Clayton (1983) argued that a genus concept should serve classification and in essence should be a construct of convenience. Stevens (1985) found Clayton's notion of genera too subjective and unsuitable to address biological questions. He recommended that genera should not simply be recognizable but should have a phylogenetic basis. Kornet (1998)

proposed more explicit criteria for delimiting genera. She preferred genera to be monophyletic groups embracing one or more species. The concept so applied is supported by the evolutionary theory, which is in her view the most relevant theory for systematics. However one short-coming of Kornet's concept does exist. The decision at which level in the phylogenetic tree to assign the genus rank remains relatively subjective, but is guided by practical taxonomic considerations such as those advocated by Clayton (1983). Backlund and Bremer (1998) propose useful guidelines on recognizing taxa in phylogenetic studies. In the absent of a universal genus concept the principles of phylogenetic classification as proposed by them serve as strong guidelines for practicing taxonomists to transform a phylogeny to classification. These principles are in some ways a summary of the genus concepts of the previously mentioned authors and embrace theoretical and practical aspects as proposed by Henderson (2005). First, the principle of monophyly is fundamental to phylogenetic classification, followed by the secondary principles of maximum stability, phylogenetic information, support for monophyly and ease of identification. The utility of these principles is demonstrated in a recent study on *Phalaenopsis* by Yukaw *et al*. (2005) in which they applied these principles with great success.

The genus concept used in the Campanulaceae by De Candolle (1830) in his *Monographie* was based upon a combination of characters (reproductive and vegetative). He erected genera when they formed natural units recognizable by a unique combination of characters, which do not appear elsewhere in the family. He explained that the reason for the separation between *Platycodon* and *Microcodon* is not based on any strong characters, but their habit is so different that combining this character with others signals two distinct genera. Unfortunately, what is considered a strong character was never defined. Perhaps it is a multistate character where each state can be used to diagnose a genus. The fruit character suits such a definition. It became an important generic character in the Campanulaceae, but the present study reveals its unsuitability as a synapomorphy for maintaining most of the currently recognized genera. A similar practice in the Brassicaceae where the fruit was the only character separating genera, was shown to be unsuitable. Phylogenetic studies in this

case supported the inclusion of six genera with diverse fruit types into *Heliophila* (Al-Shehabz and Mummenhoff 2005).

McVaugh (1945) proposed generic criteria to support his decision to separate *Triodanis* from *Specularia* and *Campanula.* Although his main concern was establishing criteria that can provide the signal to segregate small genera from core genera such as *Campanula*, he provided some insight into the thinking at that time. More importantly, he highlighted the difficulty in circumscribing genera in the Campanulaceae. He emphasized the importance of strong morphological characters, which will indicate the biological unity of a genus. Whether these so-called strong characters overlap with those found in other genera is of no consequence to him. McVaugh's reasons for separating *Triodanis* were severely criticize by Fernald (1946) who considered them to be too weak to separate the genera. The genus concept as applied by De Candolle (1830) and McVaugh (1945) may have lead to the recognition of paraphyletic genera in the Campanulaceae, which is undesirable but provided recognizable taxonomic entities. In practice the recommendations proposed by Backlund and Bremer (1998) are defensible, relatively easy to apply and therefore serve best to recognize natural groups of taxa - the ultimate goal of modern systematics. These recommendations of classification are followed in this study.

Proposed generic re-circumscriptions

On the basis of the phylogenetic hypotheses presented in this study a few options are available to transform the phylogeny into a classification whilst adhering to the criterion of monophyly.

The first option is to recognize five genera representing each of the five species assemblages. Finding morphological characters to diagnose each genus is difficult and therefore this option is of limited practical use.

a. *Wahlenbergia* with subgenus *Wahlenbergia* (the clade with *W. capensis*).

b. *Microcodon* (oldest available name in the clade) with subgroups *Microcodon, Theilera, Craterocapsa, Treichelia* and subgroup *novum* (to accommodate the remaining *Wahlenbergia* species).

c. *Roella* with subgroups, *Roella*, *Merciera* and *Prismatocarpus*.

d. new genus to accommodate *W. krebsii*.

e. *Rhigiophyllum* with subgroups *Rhigiophyllum* and *Siphocodon*.

Option two involves splitting the five species assemblages into further units where applicable. Again it is not always possible to diagnose each proposed genus morphologically.

a. *Theilera* with subgenera *Theilera* and *Craterocapsa,* and subgroup *novum* (to accommodate the species of *Wahlenbergia* present in the clade).

b. *Microcodon* with subgroups *Microcodon, Treichelia* and subgroup *novum* (to accommodate the remaining *Wahlenbergia* species).

c. *Wahlenbergia* with subgenus *Wahlenbergia* (the clade with *W. capensis*).

d. *Prismatocarpus* (excluding *P. crispus*).

e. *Merciera*.

f. *Roella*

g. new genus to accommodate *W. krebsii*.

h. *Rhigiophyllum* with subgroups *Rhigiophyllum* and *Siphocodon*.

The third option is to recognize four genera.

a. *Microcodon* (oldest available name in the clade) with subgroups *Microcodon*, *Theilera*, *Craterocapsa*, *Treichelia* and subgroup *novum* (to accommodate the remaining *Wahlenbergia* species).

b. *Roella* with subgroups, *Roella*, *Merciera*, *Prismatocarpus* and *Wahlenbergia* (clade that includes *W. capensis*).

c. *Wahlenbergia* (monotypic genus to accommodate *W. krebsii*).

d. *Rhigiophyllum* with subgroups *Rhigiophyllum* and *Siphocodon*.

Option four is to recognize two genera.

a. *Wahlenbergia* characterized by free stamens, comprising the following subgenera, *Theilera*, *Wahlenbergia* with subgroup *Wahlenbergia* and subgroup *novum* (*Wahlenbergia* species not nested in the *W. capensis* clade), *Craterocapsa*, *Microcodon*, *Treichelia*, *Roella* with subgroups, *Roella*, *Merciera* and *Prismatocarpus*.

b. *Rhigiophyllum* characterized by epipetalous stamens with subgroups *Rhigiophyllum* and *Siphocodon*.

Appraisal of the infrafamilial classification

The infrafamiliall classification for the Campanulaceae is far from settled. Unfortunately the criteria used for tribal or subfamilial classification of genera are not explicit or logical. Several authors such as Kolakovsky (1987, 1994) and Takhtajan (1997) presented infrafamilial classifications for the Campanulaceae. The molecular data present an opportunity to examine these classification systems.

The subfamily Prismatocarpoidea (Kolakovsky 1987, 1994) the equivalent of the tribe Prismatocarpeae (Takhtajan 1997) containing *Craterocapsa, Prismatocarpus, Roella* and *Treichelia* is not fully supported by the molecular data. Only the placement of *Roella* and *Prismatocarpus* are supported by these data. The placement of *Wahlenbergia, Theilera* and *Microcodon* in the tribe Wahlenbergieae by Takhtajan (1997) or in the subfamily Wahlenbergioideae by (Kolakovsky 1987, 1994) is consistent with the molecular results. Perhaps the most consistent grouping is that of *Siphocodon* and *Rhigiophyllum* in the Siphocodoneae, which formed a well-supported monophyletic group in all analyses. The placement of *Merciera* in the Mercieреae by Takhtajan (1997), conflicts with the molecular evidence. He presumably followed the treatment of De Candolle (1839) in this regard. Kolakovsky (1987, 1994) did not classify *Siphocodon*, *Rhigiophyllum* and *Merciera*.

Future research on South African Campanulaceae

This study represents the first attempt to reconstruct a phylogeny for the South African Bellflowers and employing the resultant phylogeny to reappraise its generic limits and is the basis for future investigations in the biology and evolution of the Bellflowers.

- The generic limits in the Campanulaceae remain problematic and more data are needed to help clarify delimitations. To start with a more comprehensive sampling to include more species from the summer rainfall region and those in specialized habitats and narrow distribution such as *Roella rhodantha, Prismatocarpus cordifolius, P. alpinus* would be desirable. In addition to these

species, sampling should be expanded to include non-South African wahlenbergioid taxa from e.g. Australia, New Zealand, Fernandez Island, St. Helen and Mascarene Islands. With the increased number of taxa more characters (molecular and morphological) become available for analysis.

- Detailed morphological studies are required to develop a comprehensive list of characters and states for phylogenetic analysis in an attempt to find a well-resolved tree and diagnostic characters to circumscribe clades. Up to now micro-morphological characters such as pollen and seedcoat structure have been largely overlooked. To address this oversight a survey on the seedcoat morphology of a subset of taxa included in this study is in progress. Ontogenetic research needs to investigate whether the valvate capsule of *Theilera, Wahlenbergia* and *Microcodon* are homologous. A similar investigation could test for the convergence in narrow tubular corolla in *Merciera, Theilera, Rhigiophyllum* and species of *Prismatocarpus* subgenus Afrotrachelium. The Inflorescence structure in the Campanulaceae is complex and is potentially of taxonomic significance. It ranges from the reduced type in *Merciera* to the expanded inflorescence in *P. diffusus*. In *Roella*, there is a reduction in flower number whereas, in *Merciera* many flowers are produced of which two thirds are rudimentary. A tendency towards the development of a capitulum is present in *Treichelia* and *Rhigiophyllum*.

- The gene regions use in this study showed low numbers of parsimony informative characters. Other gene region such as matK or *rps*16 could be useful for taxonomic studies at the generic level.

- This study merely hinted on bio-geogaphical patterns within the family. Investigations into the relationship between the summer and winter rainfall species and the direction of migration of the Campanulaceae (north-south or south-north) will give insight into the pattern and factors responsible for the

adaptive radiation of species. In essence this will also provide a test for the Cape to Cairo hypothesis proposed Galley *et al*. (2006).

- Little is known about the reproductive biology of the South African Bellflowers and how finely tuned it is to the diverse flower structure, pollinators, habitat and its significance in systematics.

- Species boundaries in particularly *Roella* and *Wahlenbergia* need refinement. Adamson also reported hybridization in *Roella* that remains untested. In addition the intriguing diversity in flower colour and petal markings displayed in some species of *Roella* in a single population needs to be investigated at the population genetic level. Is there a genetic basis for such diversity and does it provide an advantage in competing for pollinators? Numerous annual species of *Wahlenbergia* are based on a single specimen with no recent collections. The validity of these species requires confirmation to free the taxonomy of superfluous names. A morphometric study of species complexes was already successfully used in *Merciera* and could be employed to clarify species boundaries in these genera.

- Translation of significant Russian and Chinese papers into English is perhaps not a future research project in the Campanulaceae, but the language barrier deprives us of the insights of Kolakovsky (1986) into fruit morphology and its taxonomic significance or that of Hong (1995) into biogeographical patterns of the family.

Appearances seem misleading in the Campanulaceae. What you see is not always what you get. We have to concede that, in our endeavour to find assumed underlying patterns in nature, our efforts are limited by our methods, interpretations and understanding. Perhaps the apparent lack of pattern is due to the incompatibility between our framework and the underlying undiscovered pattern that does exist. Our contribution is simply one stepping-stone towards future breakthroughs.

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To every thing there is a season,

and time to every purpose under the heaven…

Ecclesiastes 3:1