

Phylogeny of Southern African and Australasian Wahlenbergioids (Campanulaceae) based on ITS and *trnL-F* sequence data: implications for a reclassification

Christopher N. Cupido , Jessica M. Prebble , and William M. M. Eddie

Abstract

The Campanulaceae: Wahlenbergioideae currently comprises 15 genera, one of which, *Wahlenbergia*, is widespread over the southern continents. Southern Africa is the region with maximum wahlenbergioid diversity with 12 genera and approximately 252 species. A second center is Australasia with 38 *Wahlenbergia* species. This study used a broad sample of wahlenbergioid diversity from South Africa, Australia, and New Zealand to reconstruct a phylogeny based on chloroplast *trnL-F* and nuclear ITS sequences. Data were analyzed separately and in combination using parsimony and Bayesian methods. The results suggest that for the wahlenbergioids to be monophyletic *Wahlenbergia hederacea* has to be excluded and that none of the South African, Australian or New Zealand lineages are strictly monophyletic. There are five species assemblages that are in some disagreement with current classification in the family. *Wahlenbergia*, *Prismatocarpus* and *Roella* are shown to be non-monophyletic and implications for a reclassification are presented. Careful consideration of morphological characters is suggested before the adjustment of generic circumscriptions can be accomplished.

Recent family-wide molecular phylogenetic studies have supported the view that the Campanulaceae s.s. can be divided into two major groups on the basis of their pollen morphology: the colpate/colporate platycodonoids and the porate wahlenbergioids and campanuloids (Eddie et al. 2003; Haberle et al. 2009). The two porate groups can be separated mostly by capsule morphology and geographical distribution. The wahlenbergioid group is essentially a southern hemisphere group that comprises 15 genera: *Wahlenbergia* Schrad. ex Roth; *Berenice* Tul.; *Craterocapsa* Hilliard & B.L.Burt; *Gunillaea* Thulin; *Heterochaenia* A.DC.; *Namacodon* Thulin; *Nesocodon* Thulin; *Prismatocarpus* L'Hé r.; *Rhigiophyllum* Hochst.; *Roella* L.; *Merciera* A.DC.; *Microcodon* A.DC.; *Siphocodon* Turcz.; *Theilera* E.Phillips; and *Treichelia* Vatke. The porate type of pollen characteristic of the wahlenbergioid taxa is shared with the predominantly northern hemisphere campanuloids (Eddie et al. 2003). Within the wahlenbergioids, pollen morphology has proved useful at the generic level (Hong and Pan 1998) and was primarily used to place *Rhigiophyllum* and *Siphocodon* in a separate tribe, the Rhigiophylleae Eddie & Cupido (Eddie et al. 2010).

In the southern hemisphere, the present day distribution suggests two centers of wahlenbergioid diversity. Southern Africa is described by Hong (1995) as one of three global centers of diversity and has 252 species assigned to 12 genera of which eight are endemic to this region (Cupido et al. 2011). A second center is Australasia with 38 *Wahlenbergia* species (Australia 28 species, 27 indigenous and one introduced from South Africa (Smith 1992; Plunkett et al. 2009); New Zealand 10 species (Pettersen 1997).

Wahlenbergia (including *Lightfootia* L'Hé r.), the largest and most widely distributed of the wahlenbergioid genera, consists of 170 species in South Africa (Welman and Cupido 2003; Cupido and Conrad 1999) that occur in the south-western Cape, KwaZulu-Natal, Eastern Cape, Mpumalanga, and Limpopo. *Wahlenbergia* has an enormous distribution, mainly in the southern hemisphere from Africa to India and south-east Asia, and from Australia, New Guinea, New Zealand, and the western Pacific Region to South America, where it is poorly represented. Several endemic species occur in the Juan Fernandez Islands and on St. Helena, with two species already extinct in the latter. It extends marginally beyond Africa into the western Mediterranean region of Europe, Macaronesia and Arabia, where just one or two species occur. In warm-temperate and subtropical Asia a single species of *Wahlenbergia* extends north to Japan and to the Bonin Islands, and has also been introduced to Hawaii. The remainder of the wahlenbergioid genera are confined either to southern Africa and Madagascar or the Mascarene Islands. In South Africa this genus comprises annuals, perennial herbs, and shrubby types, most of the latter occurring in the fynbos of the Cape region.

The Australian *Wahlenbergia* species are mostly annual or perennial herbs, although a few are partially shrubby at their stem bases. Although widely distributed in all states, the majority of species and the greatest diversity occurs in the south-eastern zone of the country (Smith 1992; Eddie and Cupido, in prep.).

All the New Zealand species are perennial herbs with either a rhizomatous or a radicate growth form. They occur throughout New Zealand, ranging from alpine to coastal habitats (Pettersen 1997).

The 29 species of small shrubs, perennial herbs and two annual species that belong to the genus *Prismatocarpus* occur in the south-western Cape and Eastern Cape. *Roella* is a genus of 25 small shrubs and herbs that is found mainly in the south-western Cape, with one species extending into the Eastern Cape and KwaZulu-Natal. The genus *Microcodon* is found only in the south-western Cape and comprises four species, all of which are annuals. *Merciera* comprises six perennial species that are also restricted to the south-western Cape. In the vegetative state they look very similar to *Roella*

ciliata L. *Craterocapsa* consisting of five species of prostrate, often mat-forming perennial herbs, is the only genus in South Africa that has no members in the south-western Cape. It occurs in KwaZulu-Natal, Eastern Cape, Free State, Northern Province, and Gauteng Province (and as an outlier in the Chimanimani mountains of Zimbabwe). *Siphocodon*, with two

divergent species, is restricted to the south-western Cape. They are slender, wiry perennials, one species, *S. debilis* Schltr., being exceptionally divaricating, often entangling both itself and other plants. *Rhigiophyllum* consists of one species that is found only in the south-western Cape. This rigid, erect shrublet is easily recognized by its stiff leaves, densely arranged on the stems and by the deep blue, tubular flowers that are borne in terminal heads. Like *Siphocodon*, *Treichelia* comprises two species that occur in the south-western Cape. These dwarf coarse herbs bear their flowers in dense terminal heads with long bracts in between the flowers. The two species of *Theilera* occur in the south-western Cape and Eastern Cape. They are erect shrublets with slender branches, cylindrical, somewhat asymmetrical corollas, and are found mainly inland.

Two additional genera found in southern Africa, *Gunillaea*, with two species ranging from Angola to Mozambique and on Madagascar, and the monotypic *Namacodon* from Namibia are not formally part of this study although morphologically interesting and of overall importance in an understanding of wahlenbergioid phylogeny as a whole.

The increasing number of molecular phylogenetic studies published over the last 10 years has allowed us to visualize an emerging picture of relationships within the Campanulaceae and to plug the gaps in our understanding of its classification. However, an unintended shortcoming of recent family-wide studies was the under-sampling of wahlenbergioid taxa across its distribution range (e.g. Eddie et al. 2003; Cosner et al. 2004; Haberle et al. 2009). Prebble et al. (2011) used a broader sampling of *Wahlenbergia* species to study the relationship within the genus across its main centers of radiation in the southern hemisphere, but still did not place the genus in the broader context of wahlenbergioid diversity, particularly with respect to endemic taxa in the Juan Fernandez Islands, the Mascarenes and St. Helena. Ideally a study on the wahlenbergioids should include all southern hemisphere groups.

In addition to the sampling dilemma, agreement on generic circumscriptions in the Campanulaceae remains largely unsettled. Typically, 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 wahlenbergioids are *Theilera*, *Microcodon* and *Craterocapsa* p.p. from *Wahlenbergia*, *Treichelia* from *Microcodon* and *Merciera* from *Trachelium* and *Roella*. Due to the removal of small genera, the monophyly of the larger genera such as *Wahlenbergia* is 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). Many genera are also recognized by their possession of a suite of characters, none of which is unique to the genus in question.

The diversity in capsule structure and particularly the mode of dehiscence has been used to separate genera in the Campanulaceae (Hilliard and Burt 1973; Thulin 1975). This

character is not always homogeneous within the existing South African genera. In all species of *Wahlenbergia*, except *W. acaulis* E. Mey. and *W. suffruticosa* Cupido, the capsule characteristically dehisces by erect apical valves, which are formed by the dome-shaped epigynous disc. In *W. acaulis* dehiscence takes place via protruding intercalyx folds, while *W. suffruticosa* has also departed from the typical *Wahlenbergia* pattern by evolving a unique mode of dehiscence in which slits develop between the depressed apical valves and extend longitudinally, separating the capsule into five segments (Cupido 2011). Hilliard and Burt (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, which are more characteristic of *Prismatocarpus*.

Prismatocarpus schinzianus Markgraf was transferred to a new genus *Namacodon* (Thulin 1974) because it differs from *Prismatocarpus* in its unique mode of septicidal dehiscence, 3-locular ovary and pollen grains released in tetrads. Similarly, Thulin erected the genus *Gunillaea* for certain former species of *Prismatocarpus* and *Wahlenbergia* which have indehiscent capsules that open slowly by irregular decomposition of the pericarp and 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 Thulin reported it as opening 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 and its possession of fascicled leaves, which would be unique in the species of *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 incorrectly that the dehiscence takes place by slits between the ribs of the capsule. With both of these genera we can see how genera may be perceived as distinct (their overall gestalt) through suites of characters.

Craterocapsa (Hilliard and Burt 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* now 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 Burt 1973).

Apart from a few studies that could be described as merely incidental, for example Phillips's (1927) treatment of *Theilera*, no study has ever attempted to re-assess generic circumscriptions in South African Campanulaceae. Schönland's review, which is more than 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 wahlenbergioids is crucial to resolve the numerous alpha taxonomic problems that exist in the family as well as for making informed conservation decisions.

In this study we used a broad sample of wahlenbergioid genera to address the following questions: 1. With increased sampling are the wahlenbergioids monophyletic? 2. Are the current generic circumscriptions credible and what are the options for generic revision based on this phylogeny? 3. What are the relationships between taxa from the two largest centers of wahlenbergioid diversity (South Africa and Australasia)?

Materials and Methods

Taxon Sampling—Taxa were selected to include at least one representative from each South African wahlenbergioid genus, to maximize morphological and geographical diversity, and to sample all growth forms in South Africa. Our study does not include *Wahlenbergia* samples from the Mascarene Islands, Juan Fernandez, and St. Helena. Although this is largely outside the scope of this paper we acknowledge these limitations and will address them in a future study. 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 group 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 was sampled. The South African 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. The unnamed specimens do not necessarily represent undescribed taxa but rather ambiguity in the current taxonomy. The New Zealand and Australian samples are a subset of the endemic rhizomatous and radicate species included in Prebble et al. (2011).

The ingroup comprises DNA sequences from the chloroplast *trnL-F* and nuclear ribosomal ITS regions obtained from 96 and 87 taxa, respectively (Appendix 1). Of these 89 are South African, six New Zealand *Wahlenbergia*, seven Australian *Wahlenbergia*, one European *Wahlenbergia* and one mainly tropical and northern African *Wahlenbergia* samples. Every attempt was made to have the same number of taxa for each gene region, but for some taxa it was impossible to obtain ITS sequences. All taxa were field collected and DNA was isolated from silica dried 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.

Outgroup taxa were selected from the mainly East Asian platy-codonoids, the predominantly northern hemisphere campanuloids (Eddie et al. 2003) and the closely related families of Lobeliaceae and Cyphiaceae (Cronquist 1981; Lammers 1992; Gustafsson and Bremer 1995; APG 2003; Cosner et al. 2004).

DNA Extraction, Amplification and Sequencing—Extractions for all samples followed a modified CTAB method (Doyle and Doyle (1987, 1990) outlined in detail in Prebble et al. (2011). Amplification of the nuclear ribosomal ITS region was conducted using primers AB101F and AB102R (Baldwin 1992) for the South African samples, whereas the Australasian samples were amplified using primers ITS5 and ITS28cc (Wagstaff and Garnock-Jones 1998). The chloroplast region was amplified for all samples using *trnL-Ff* and *trnL-Fc* (Taberlet et al. 1991). 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.

In South Africa 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, Foster City, California). Each cycle consisted of 96^o C denaturation for 10 sec, 50^o C annealing for five seconds and 60^o C 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., Ann Arbor, Michigan).

In New Zealand the PCR products were sequenced on an ABI3730 Genetic Analyzer by the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand). Reverse compliments were sequenced to confirm the reads for a number of samples.

Sequence Alignment—Sequences were aligned by eye. Gaps that resulted from the alignment of unequal sequences were coded as missing data and not scored for inclusion in the analyses. ITS sequences proved difficult to align and the program MEGA version 3.1 (Kumar et al. 2004) was used to provide an initial alignment.

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.

Combined trnL-F and ITS Data Set—The data was concatenated following Nixon and Carpenter (1996). The concatenated molecular data set of 92 taxa consisted of 83 ingroup and 10 outgroup taxa. Only taxa common to both the individual data sets were used in the combined analysis.

Phylogenetic Analyses—The data matrices for each of the two gene regions were analyzed separately and as a combined data matrix using parsimony and Bayesian Inference. Parsimony analyses were performed using PAUP* 4.0b10 (Swofford 2003) with all characters weighted equally (Fitch parsimony; Fitch 1971). One thousand heuristic replicated searches were performed using random stepwise taxon addition with branch-swapping by tree-bisection reconnection (TBR), saving five trees per replicate. Support for each clade was assessed by bootstrap analysis (Felsenstein 1985), using 1000 simple taxon addition

replicates and TBR branch-swapping. Bootstrap values were interpreted as weak (50–74%), moderate (75–89%) and strong (90–100%).

Consistency (CI) (Kluge and Farris 1969) and retention (RI) (Farris 1989) indices were calculated for each. For Bayesian analyses models of molecular evolution that best fit the data were determined in Modeltest (version 3.06; Posada and Crandall 1998) using the Akaike information criterion (Akaike 1974) for each of these data sets. For the combined analysis, parameters applying to more than one partition were unlinked to allow values to differ among partitions. One million generations were run with four independent chains (Markov chain Monte Carlo) and were sampled every hundred generations, resulting in an overall sampling of 10,000 trees.

The Bayesian analyses were conducted using MrBayes 3.1 (Huelsenbeck and Ronquist 2001) applying the substitution models listed in Table 1. 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 Bayesian analysis, stationarity was reached after 17,400, for the second after 59,300, and for the third after 76,200 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 above the branches. PP-values of ≥ 0.95 are considered evidence of significant support for a group (Miller et al. 2004).

The partition homogeneity test (Farris et al. 1995) as implemented in PAUP* 4.0b10 (Swofford 2003) was used to assess topological congruence between the *trnL-F* and ITS data sets. One hundred partition homogeneity replicates were used with 100 replicates of random addition sequence, TBR branch swapping, saving 10 trees per replicate.

TABLE 1. Statistics for DNA sequence regions used in the phylogenetic analysis.

	<i>trnL-F</i>	ITS	Combined
Characters			
Number included	882	529	1,411
Parsimony-informative	297 (34%)	289 (55%)	553 (39%)
Variable, parsimony-uninformative	173 (20%)	99 (19%)	274 (19%)
Constant	412	141	584
Phylogenetic Analyses			
Number of Trees	334	212	8
Tree length	1,020	1,493	2,371
Consistency Index	0.679	0.494	0.568
Retention Index	0.868	0.750	0.782
Model of DNA substitution	TVM + G	GTR + I + G	Separate models for each partition

Results

The characteristics of the sequences for each analysis performed are summarized in Table 1.

Combined *trnL-F* and *ITS* Analysis—The 50% majority rule consensus tree inferred from the Bayesian analysis (Fig. 1) resolved similar clades as the maximum parsimony analysis, but is better resolved and supported.

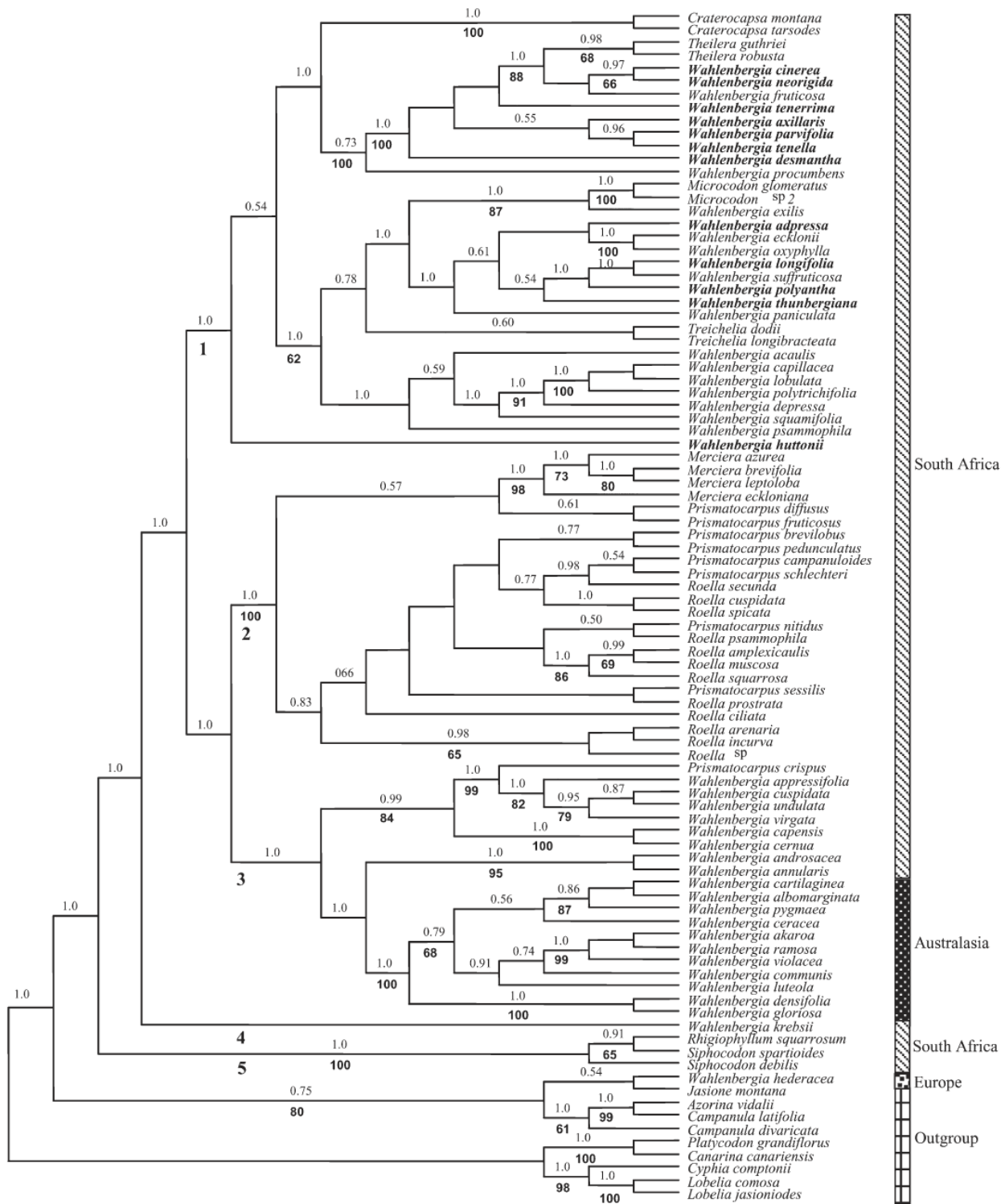


FIG. 1. Bayesian 50% majority rule consensus tree obtained for the combined *trnL-F* and *ITS* datasets for southern African and Australasian wahlenbergioids and outgroup. Taxa previously classified in *Lightfootia* nom. illeg. are in bold. Numbers above branches indicate Bayesian posterior probability (PP) values; numbers below indicate Parsimony bootstrap (BS) values. Geographic distribution of taxa is indicated in the right margin and the major clades are labeled.

The ingroup is split into two main groups, supported by a posterior probability (PP) value of 1.0. The first included the single *species* of *Rhigiophyllum* and the two species of *Siphocodon* and is well supported with a posterior probability (PP) value of 1.0. The second of the two groups includes all remaining samples, comprising representatives of eight genera.

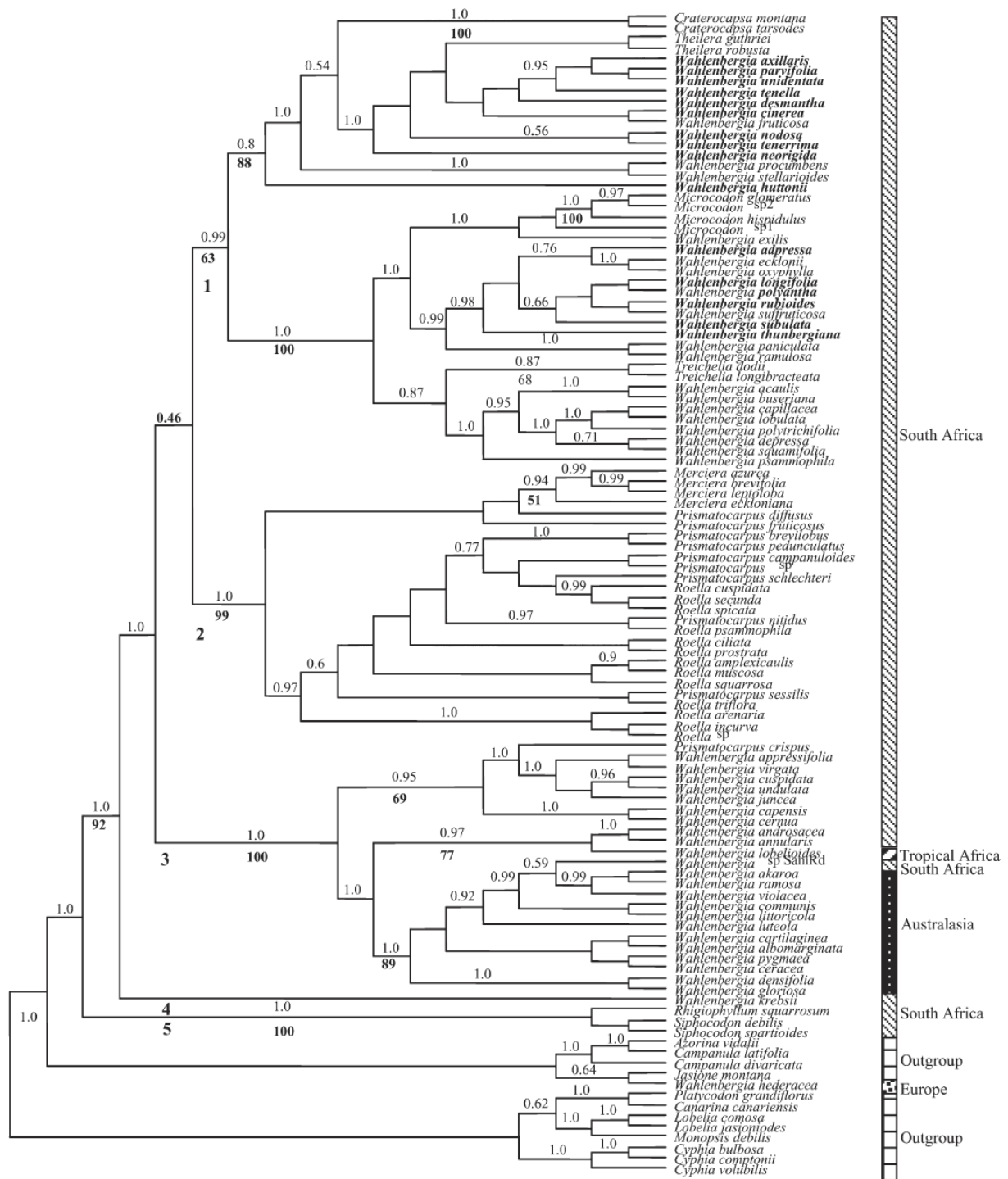


FIG. 2. Bayesian 50% majority rule consensus tree obtained from the *trnL-F* dataset for southern African and Australasian wahlenbergioids and outgroup. Taxa previously classified in *Lightfootia* nom. illeg. are in bold. Numbers above branches indicate Bayesian posterior probability (PP) values; numbers below indicate Parsimony bootstrap (BS) values. The major clades are labeled, as are the geographic distribution of taxa.

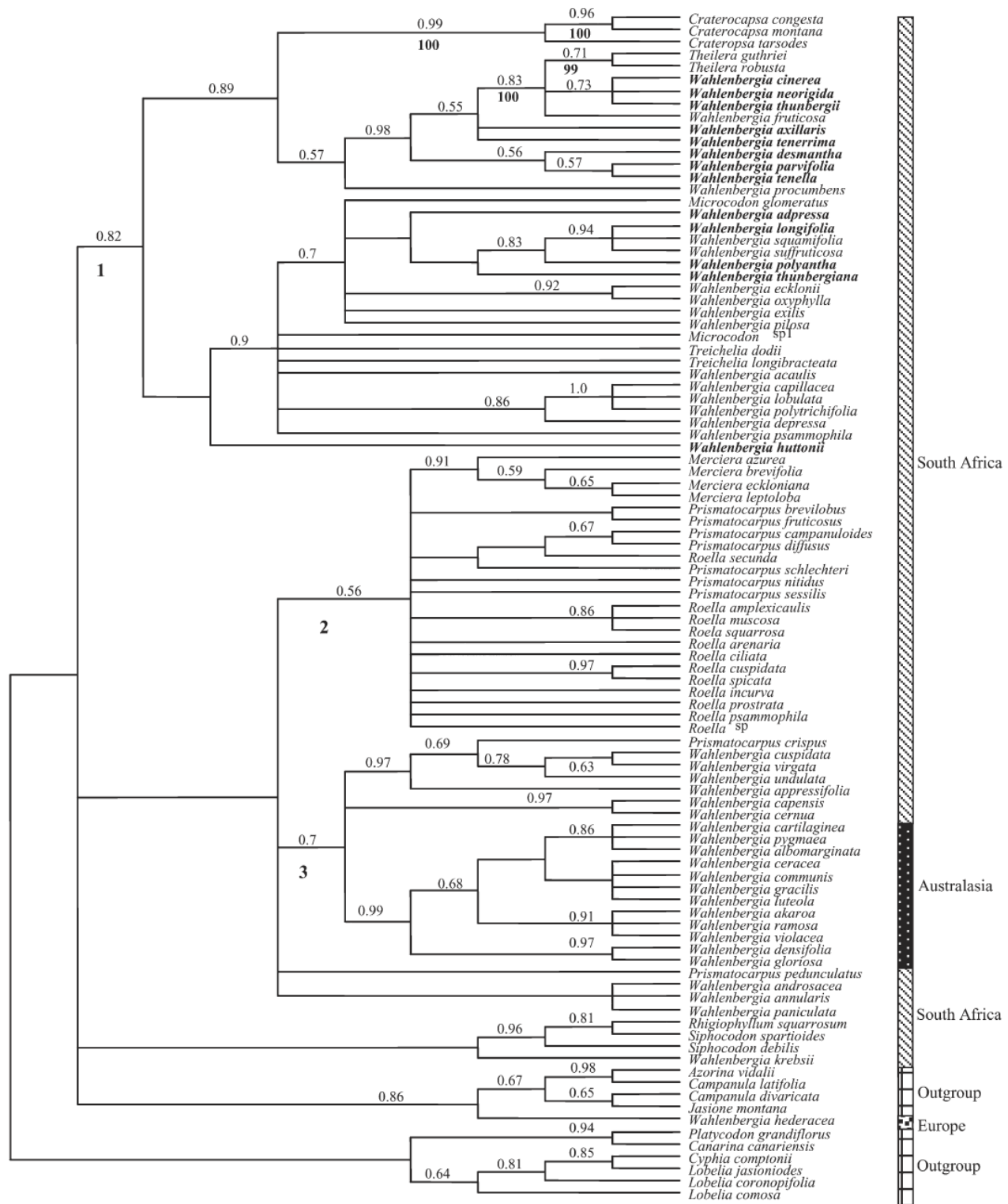


FIG. 3. Strict consensus tree for the maximum parsimony analysis for the ITS data set for southern African and Australasian wahlenbergioids and outgroup. Taxa previously classified in *Lightfootia* nom. illeg. are in bold. Numbers above branches indicate Bayesian posterior probability (PP) values; numbers below indicate Parsimony bootstrap (BS) values. The major clades are labeled, as are the geographic distribution of taxa.

Wahlenbergia krebsii is sister to the rest of the species in this group. The remainder is resolved into three clades (1, 2, and 3), among which the relationships are strongly supported. The largest of the three clades, 1, is strongly supported (PP = 1.0) and resolved into two subclades sister to *W. huttonii*. The first subclade is strongly supported

(PP = 1.0) and includes species of *Wahlenbergia*, *Theilera* and *Craterocapsa*. The *Craterocapsa* species formed a clade with PP = 1.0, while the *Theilera* species formed a clade with *Wahlenbergia* species most of which were previously classified in the genus *Lightfootia* nom. illeg. The second subclade has a PP value of 1.0, and comprises species of *Wahlenbergia*, *Microcodon* and *Treichelia*. The genus *Treichelia* is weakly supported (PP = 0.60), while the two species of *Microcodon* form a strongly supported (PP = 1.0) clade. The position of *Wahlenbergia* in Clades 1 and 3 renders this genus paraphyletic.

Clade 2 is strongly supported (PP = 1.0) and is formed by species of *Roella*, *Prismatocarpus* (except *P. crispus* in clade 3) and *Merciera*. The four species of *Merciera* resolve as monophyletic (PP = 1.0) and are sister to two *Prismatocarpus* species in a weakly supported (PP = 0.57) subclade. The remainder of the *Prismatocarpus* species are interspersed among the *Roella* species in a moderately supported (PP = 0.83) subclade.

Clade 3 is strongly supported (PP = 1.0) and is formed by species of *Wahlenbergia* with *Prismatocarpus crispus*. This clade comprises three subclades. The first group of annuals and perennial herbs which include *P. crispus* is supported by a PP value of 0.99. The second comprise the South African, *W. annularis* and the tropical and southern Africa *W. androsacea* annual species, supported by a PP value of 1.0, and the third is a strongly supported group (PP = 1.0) of Australasian *Wahlenbergia*.

The topology of this combined 50% majority rule consensus tree shows a high degree of congruence with the consensus trees of the separate *trnL-F* data set (Fig. 2). As in the *trnL-F* analysis *Wahlenbergia krebsii* appears isolated and is placed sister to the large clade that excludes *Rhigiophyllum* and *Siphocodon*. *W. huttonii*, which resolved as sister to the *Craterocapsa-Theilera-Wahlenbergia* subclade in the *trnL-F* analysis, resolves as sister to the entire clade A as in the ITS analysis (Fig. 3). The *Craterocapsa-Theilera-Wahlenbergia-Microcodon-Theilera* clade, the *Roella-Prismatocarpus-Merciera* clade, the clade involving the Australasian *Wahlenbergia* as well as the placement of *W. hederacea* among the outgroup taxa are common in all analyses.

Data Combinability—The partition homogeneity test found that the *trnL-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; Ramírez 2006). Therefore, the data sets were nonetheless combined in a total evidence approach (Kluge 1989) because of the possibility of resolving more clades, reducing the number of parsimonious trees and obtaining better supported clades.

A comparison of the trees obtained for the separate *trnL-F* and ITS analyses suggest a general congruence in topology. The areas of conflict involve the placement of species such as *Lobelia comosa*, *L. coronopifolia*, *L. jasionoides*, *Cyphia comptonii*, *W. huttonii*, *W. krebsii* and *P. pedunculatus*, which are nested in poorly supported clades.

Discussion

Monophyly of the Wahlenbergioid Group—Our results support previous molecular studies using ITS sequences (Eddie et al. 2003) and combined *atpB*, *matK* and *rbcL* (Haberle et al. 2009) that have shown that for the wahlenbergioids to be monophyletic, *W. hederacea* has to be excluded. Recent molecular studies on *Wahlenbergia* (Prebble et al. 2011) confirmed the distant relationship between *W. hederacea* and its congeners. This species is somewhat intermediate in morphology between typically wahlenbergioid and typically campanuloid taxa, and has an unusual geographical distribution (Lusitanian) on the western seaboard of temperate Europe. In its mode of capsule dehiscence it resembles the wahlenbergioids but its overall vegetative morphology is similar to the isophylloid species of *Campanula* (Eddie and Cupido, 2013, in review). Furthermore, the molecular results for *W. hederacea* (ITS, *matK*, *atpB*, *rbcL*, and *trnL-F*) are not equivocal. It obviously is not closely related to the wahlenbergioid genera of the southern hemisphere sampled in this and previous studies but possibly to other northern-hemisphere genera such as *Jasione* and *Feeria* although it does not bear a close resemblance to either. *Jasione* was also traditionally placed within the wahlenbergioids but studies of rearrangements of the chloroplast genome (Cosner et al. 2004) have shown that, from a molecular point of view, it is best treated as campanuloid. Subsequent studies suggest that *W. hederacea* should also be placed among the campanuloids (Eddie et al. 2003; Haberle et al. 2009).

The taxonomic history of *W. hederacea* indicates its uncertain position in classifications. It has been treated as belonging to a separate genus, e.g. *Schultesia* Roth (nom. illeg.), *Aikinia* Salisb. ex Fourr. (nom. illeg.) and even *Roucela* (Dumort). Eddie et al. (2003) referred to *W. hederacea*, *Jasione* and other taxa such as *Musschia* Dumort. in an unresolved clade as transitional (i.e. ambiguous in an evolutionary sense) between the wahlenbergioids and campanuloids. When Eddie (in Eddie et al. 2003) introduced the term ‘wahlenbergioid’, referring to the possible affinities and appearance of a taxon, he applied it principally and most appropriately to the southern hemisphere group with porate pollen, comprising about 15 genera. At the same time it was recognized that taxa with ‘wahlenbergioid’ characteristics also occurred in Europe, e.g.: ‘*Wahlenbergia*’ *hederacea*; *Edraianthus* A. DC.; *Jasione* L.); the Mediterranean region (*Feeria* Buser, *Jasione*); North America (*Githopsis* Nutt.); and south-east Asia (*Peracarpa* Hook. f. & Thomson). Similarly, within southern Africa there are formal members of the Wahlenbergioideae that have campanuloid characteristics, e.g. *Prismatocarpus*, *Namacodon* and some species of *Wahlenbergia*, e.g. *W. campanuloides* (Delile) Vatke. Within the southern hemisphere, the highest generic and species diversity of wahlenbergioids is found in southern Africa. This region also contains 12 endemic genera and is clearly the current center of maximum wahlenbergioid divergence. This diversification is probably the result of intense selection pressures present in the very diverse South African environments in addition to long periods of isolation (Cupido 2009).

The topologies discovered by the *trnL-F* and combined datasets suggest, as inferred by Eddie et al. (2003) for ITS, that the wahlenbergioids and campanuloids shared a common ancestor. The splitting of the two lineages correlates with a vicariance interpretation of the

respective groups, with the campanuloids predominantly inhabiting the northern hemisphere and the wahlenbergioids inhabiting the southern hemisphere.

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. This overlap may not have been present in the early history of the family; it is highly likely that the $2n = 28$ group of *Campanula*, which is now highly relictual in central Sahara, Cape Verde Islands and eastern Africa, spread into sub-Saharan Africa in mid-Tertiary times reaching as far south as Tanzania (Eddie and Cupido in prep.). The equivalent northward migration of wahlenbergioids resulted in the presence of species such as *Wahlenbergia lobelioides* in the Mediterranean and Macaronesia, merely fringing on Europe proper. These relatively late migrations must not be confused with the presence of Eddie's 'transitional' taxa such as *Feeria* and *Jasione* which, on the basis of their geographic distribution and their position on molecular topologies, appear to be much more ancient and relictual in the Mediterranean region (Eddie et al. 2003; Eddie and Cupido in prep.).

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 subsequent diversification in southern Africa. *Monophyly of South African and Australasian Wahlenbergioid Genera*—Evidence from all analyses suggests that neither samples from South African, Australian nor New Zealand group to form discrete clades. The New Zealand species form two clades nested within the Australian species, and the Australian and New Zealand species form a strongly supported monophyletic sister group to one of the South African clades. Although, the study of Harberle et al. (2009) lacked South African *Wahlenbergia* and *Treichelia* samples and included only a single Australian sample, they also found *Wahlenbergia* and *Prismatocarpus* to be not monophyletic. *Generic Limits*—The molecular data do not support all the current generic circumscriptions in the family, six smaller genera are recovered as monophyletic (i.e. *Craterocapsa*, *Theilera*, *Microcodon*, *Treichelia*, and *Merciera* and *Siphocodon*) but *Wahlenbergia*, *Prismatocarpus* and *Roella* are non-monophyletic. The monophyly of *Rhigiophyllum* is unclear as only a single individual was included in our study. Five species assemblages become apparent, corresponding to clades 1–5 on Fig. 1: 1. *Wahlenbergia-Theilera-Microcodon-Craterocapsa-Treichelia*, 2. *Roella-Prismatocarpus-Merciera*, 3. *Wahlenbergia-P. crispus*, 4. *Wahlenbergia krebsii*, 5. *Rhigiophyllum-Siphocodon*.

1. WAHLENBERGIA-THEILERA-MICROCODON-CRATEROCAPSA-TREICHELIA—*Wahlenbergia* is not monophyletic, with *Theilera*, *Craterocapsa*, *Microcodon* and *Treichelia* all nested within it. These genera are part of the wahlenbergioid line of diversification, and each of them has probably adapted to unique ecological conditions such as

fire, rainfall and soil type. 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 (Cupido 2009). Most of the *Wahlenbergia* species adapted to the summer-dry conditions and fire by developing or perhaps retaining an ancestral shrubby habit that allows them to die back and resprout. *Treichelia* and *Microcodon* have adapted to the same dry conditions as spring flowering annuals that survive the harsh summer as seed, whereas *Craterocapsa* and *Theilera* occupy areas where these conditions are mostly 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 separated from *Wahlenbergia* on a suite of characters, including its tubular corolla and fascicles of leaves. The corolla is slightly curved, almost zygomorphic and both anthers and stigmatic lobes are unusual. 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 mostly shrublets (except *R. muscosa*, *R. uncinata*, *R. recurvata* and *R. goodiana* which are 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, KwaZulu Natal and Zimbabwe. *Craterocapsa insizwae* (separated from *Roella*) was not sampled for this study. *Craterocapsa* and *Roella* are not found in similar positions on our tree (Fig. 1). Although the molecular data suggest that *Treichelia*, *Microcodon*, *Theilera*, and *Craterocapsa* are most probably congeneric with *Wahlenbergia* it does however support them as coherent separate groups within a larger *Wahlenbergia*, which is corroborated by the morphology and ecology of the plants.

The original concept of *Lightfootia* as a distinct genus from *Wahlenbergia* is not supported by these data, giving some support to Thulin's (1975) union of the two or at least some species of *Lightfootia* with *Wahlenbergia*. However, it is partially supported by the fact that some of the shrubby species formerly treated as *Lightfootia* do cluster together and are recognizably distinct. 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 on their own. 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 closely related to *Wahlenbergia*. *Theilera* is unique among wahlenbergioids in its flowers, habitat and geographical distribution.

2. ROELLA-PRISMATOCARPUS-MERCIERA—The close relationship between *Roella*, *Prismatocarpus* and *Merciera*, as suggested by Adamson (1952, 1955), 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. However, in our analyses the relationships of *Merciera* are poorly supported, though it is shown to be sister to a clade containing most species of *Prismatocarpus* and all species of *Roella* sampled for this study. 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 two genera into five series and two sub-genera respectively. Subgenus *Prismatocarpus* is further subdivided into three series. Little support for the subgeneric classification of Adamson (1952) is evident, except that in the *trnL-F* topology *Roella* series *Spicatae*, is discovered with strong support ($P = 0.99$), formed by *R. cuspidata*, *R. secunda* and *R. spicata*. Also *P. diffusus*, the type species of the *Prismatocarpus* subgenus *Afrotrachelium* is sister to *Merciera* with which it shares a narrow tubular corolla. The paraphyletic nature of these two genera casts doubt on the value of the single fruit character to indicate generic limits. Only species of *Merciera* formed a monophyletic group in all analyses.

2. WAHLENBERGIA-P. CRISPUS—*Prismatocarpus crispus*, one of two herbaceous (annual) species in *Prismatocarpus* is nested within a strongly supported clade comprising several South African 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 (*Esterhysen 1422*). The type was probably destroyed in Berlin during WWII 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: *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 ancient flora of southern Africa was tropical (Linder and Hardy 2004), but when the climate became drier the tropical flora was largely decimated, leaving behind relics such as *Prionium*, *Metrosideros*, *Brabejum* and most likely a shrubby wahlenbergioid flora that would later occupy the fynbos. The nesting of species (*W. androsacea*, *W. virgata*, *W. undulata* and *W. lobelioides* (*trnL-F* analysis only)) shared with tropical Africa in this clade corroborates the affinity between the two floras and perhaps suggests a northward migration of these species. Furthermore, the close relationship between the complex species *W. lobelioides* and *W. androsacea*, and with species in Australia as suggested by Thulin (1975) is confirmed, at least, by the *trnL-F* data. The argument for the affinity between African and Australian species is further supported by the association of the unidentified South African species, *W.* sp (Sani Rd) with the Australasian species. It is possible that this species is related to the mainly Asian *W. marginata* complex which is no longer recognized in mainland Australia. It is recorded from New Guinea but may be easily confused with other similar species (Smith 1992; Petterson 1997; Lammers 2007). The possibility that the unidentified taxon was introduced to South Africa is not excluded as suggested by Prebble et al. (2011) and will be discussed in a separate article.

The monophyletic relationship of the Australasian *Wahlenbergia* (plus the unidentified *W.* sp (Sani Rd) was also recovered in Prebble et al. (2011). The species affinities within this clade also match previously identified groups e.g. *W. cartilaginea*, *W. pygmaea* and *W. albomarginata* are all species with the rhizomatous growth form endemic to New Zealand. The relationships within the Australasian *Wahlenbergia* are discussed further in Prebble et al. (2012).

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 *trnL-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). In a recent seed coat study for the South African taxa Cupido et al. (2011) described the seed coat pattern of

W. krebsii as distinctly reticulate with keeled and verrucate radial walls, which was unique among the species sampled. However, it remains unclear whether this species could be the type for the formation of a new genus.

5. RHIGIOPHYLLUM-SIPHOCODON—The most obvious morphological similarity between these two genera is their 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 (1955) 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 molecular results together with the unique pollen morphology in *Rhigiophyllum* and *Siphocodon* have allowed us recognize the distinctiveness of these genera and to place them in a separate tribe, the Rhigiophylleae Eddie & Cupido (Eddie et al. 2010). *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.

Implications for a Reclassification—For most of the 20th century, the classification of southern African wahlenbergioids has served well enough from an end-users perspective. With the publication of additional genera the classification still remained stable and workable. However, with the advent of molecular and cladistic techniques, the search for phylogenetic relations among the various taxa has shown that the situation is far from clear-cut. The molecular evidence of how evolution has proceeded within the group presented here is clearly in some disagreement with the current classification in the family based on morphological criteria, and raises issues regarding generic classification.

In order to translate the results of this study into a generic classification based on the principle of monophyly, following for example the guidelines proposed by Backlund and Bremer (1998) of maximum stability, phylogenetic information, support for monophyly, and ease of identification, there are three main options. Option 1 is to recognize all in-group taxa as a single enlarged genus with the possibility of mono-phyletic lineages at subgeneric level. This would mean merging clades 1, 2, 3, 4, and 5 into a single genus, for which the oldest valid name, without conservation of *Wahlenbergia*, is *Roella*. Option 2 is to recognize two genera with the possibility of monophyletic lineages at subgeneric level. This would be achieved by merging clades 1, 2, 3, and 4 into a single large genus for which the earliest valid name would be *Roella*. The second genus corresponds to clade 5 for which the earliest available name is *Rhigiophyllum*. These two genera would be distinguishable from each other by the difference in stamen fusion which is free in clades 1–4 and epipetalous in clade 5. Option 3 is to recognize the clades 1, 2, 3, 4, and 5, or reciprocally monophyletic groups within these clades, at the genus level. However, no morphological characters are currently

known with which to base genera comprising clade 1, 2 or 3 on – i.e. there are no known morphological synapomorphies for these clades, which makes this option undesirable at present. A similar situation in *Veronica* has been resolved recently by collapsing the nested genera into an enlarged *Veronica* (Albach et al. 2004; Garnock-Jones et al. 2007) (as in Option 2) but that has not been without controversy (Brummitt 2006; Gardner 2007; Garnock-Jones and Albach 2008).

This is a divisive issue in taxonomy today, and it is difficult to reconcile (A) the preference to see classifications based on monophyletic groups, as the best way to make taxonomy a falsifiable and therefore science based field, and as a method for generating a stable classification scheme that reflects evolution; with (B) the desirability of using morphological criteria based on recognition of gestalt (i.e. the totality of appearance through suites of characters) as the basis for a workable classification that is both expedient and generally reflective of phylogenetic relations. This study has shown that a small number of taxa present considerable ambiguity in their placement, for example *Prismatocarpus crispus*, *P. pedunculatus*, *Wahlenbergia krebsii*, *W. acaulis*, *W. suffruticosa*, and *W. hederacea*, and it is to these problems that future taxonomic research should be addressed.

At this stage, we recommend that only minor adjustments are appropriate for the classification of South African wahlenbergioids. This allows us to recognize the evolutionary uniqueness (ecological and/or morphological) of taxa without losing sight of their context in phylogeny. In a forthcoming paper we will explore various taxonomic options and the associated problems with each of them in a revised generic classification.

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Appendix 1. Taxa, voucher/source (locality, collector and collecting number, herbarium where specimen is housed or references in the case of published sequences) and GenBank accession numbers (trnL-F, ITS) for sequences used in this study, -indicates a sequence not available, * indicates no accession number allocated as the sequence is less than 200 bp.

Ingroup—*Craterocapsa congesta* Hilliard & Burtt, South Africa, ITS Eddie et al. (2003), -, AY322049; *C. montana* (A. DC.) Hilliard & Burtt, South Africa, Eastern Cape, Keiskamahoe, *Goldblatt s. n.*, NBG, KCo13605, KCo13664; *C. tarsodes* Hilliard & Burtt, South Africa, KwaZulu-Natal, Himeville, *Cupido 306*, NBG, KCo13606, KCo13665; *Merciera azurea* Schltr., South Africa, Western Cape, Bredasdorp, *Cupido 111*, NBG, KCo13607, KCo13666; *M. brevifolia* A. DC., South Africa, Western Cape, Caledon, *Cupido 235*, NBG, KCo13608, KCo13667; *M. eckloniana* H. Buek, South Africa, Western Cape, Villiersdorp, *Cupido 76*, NBG, KCo13609, KCo13668; *M. leptoloba* A. DC., South Africa, Western Cape, Bredasdorp, *Cupido 108*, NBG, KCo13610, KCo13669; *Microcodon glomeratus* A. DC., South Africa, Western Cape, Kraaifontein, *Cupido 105*, NBG, KCo13611, KCo13670; *M. hispidulus* (L. f.) Sond., South Africa, Western Cape, Malmesbury, *Cupido 82*, NBG, KCo13614, -; *M. sp.1*, South Africa, Western Cape, Clanwilliam, *Cupido 257*, NBG, KCo13612, -; *M. sp.2* 'sparsiflorus', South Africa, Western Cape, Hopefield, *Cupido 197*, NBG, KCo13613, KCo13671; *Prismatocarpus brevilobus* A. DC., South Africa, Western Cape, Darling, *Duckitt s. n.*, NBG, KCo13615, KCo13672; *P. campanuloides* (L. f.) Sond., South Africa, Western Cape, Genadendal, *Cupido 219*, NBG, KCo13616, KCo13673; *P. crispus* L'Hé r., South Africa, Western Cape, Clanwilliam, *Manning 2651E*, NBG, KCo13617, KCo13674; *P. diffusus* (L. f.) A. DC., South Africa, Western Cape, Genadendal, *Cupido 220*, NBG, KCo13618, KCo13675; *P. fruticosus* L'Hé r., South Africa, Western Cape, Somerset West, *Cupido 118*, NBG, KCo13619, KCo13676; *P. nitidus* L'Hé r., South Africa, Western Cape, Cape Town, *Cupido 228*, . NBG, KCo13620, KCo13677; *P. pedunculatus* (P. J. Bergius) A. DC., South Africa, Western Cape, Citrusdal, *Cupido273*, NBG, KCo13621, KCo13678; *P. schlechteri* Adamson, South Africa, Western Cape, Caledon, *Cupido237*, NBG, KCo13622, KCo13679; *P. sessilis* Eckl. ex A. DC., South Africa, Western Cape, Bredasdorp, *Cupido 112*, NBG, KCo13623, KCo13680; *P. sp.* 'Vil', South Africa, Western Cape, Villiersdorp, *Cupido 241*, NBG, KCo13624, -; *Rhigiophyllum squarrosus* Hochst., South Africa, Western Cape, Napier, *Cupido 106*, NBG, KCo13625, KCo13681; *Roella amplexicaulis* Wolley-Dod, South Africa, Western Cape, Cape Town, *Cupido 122*, NBG, KCo13626, KCo13682; *R. arenaria* Schltr.; South Africa, Western Cape, Napier, *Cupido s. n.*, NBG, KCo13627, KCo13683; *R. ciliata* L., South Africa, Western Cape, Cape Town, *Cupido 213*, NBG, KCo13628, -; *R. ciliata* L., South Africa, ITS Eddie et al. (2003), -, AY322074; *R. cuspidata* Adamson, South Africa, Western Cape, Caledon, *Cupido 234*, NBG, KCo13629, KCo13684; *R. incurva* A. DC., South Africa, Western Cape, Hermanus, *Cupido 200*, NBG, KCo13630, KCo13685; *R. muscosa* L.f., South Africa, Western Cape, Cape Town, *Cupido 232*, NBG, KCo13631, KCo13686; *R. prostrata* E. Mey. ex A. DC., South Africa, Western Cape, Malmesbury, *Cupido 208*, NBG, KCo13632, KCo13687; *R. psammophila* Schltr., South Africa, Western Cape, Genadendal, *Cupido 216*, NBG, KCo13633, KCo13688; *R. secunda* H. Buek, South Africa, Eastern Cape, Joubertina, *Cupido 285*, NBG, KCo13634, KCo13689; *R. spicata* L. f., South Africa, Western Cape, Elim, *Cupido 245*, NBG, KCo13635, KCo13690; *R. squarrosa* P. J. Bergius, South Africa, Western Cape, Cape Town, *Cupido 229*, NBG, KCo13636, KCo13691; *R. triflora* (R. D. Good) Adamson, South Africa, Western Cape, Cape Town, *Cupido 226*, NBG, KCo13637, -; *R. sp.* 'Genadendal', South Africa, Western Cape, Genadendal, *Cupido 223*, NBG, KCo13638, KCo13692; *Siphocodon debilis* Schltr., South Africa, Western Cape, Napier, *Cupido 139*, NBG, KCo13639, *; S.

spartioides Turcz., South Africa, Western Cape, Villiersdorp, *Cupido* 133, NBG, KCo13640, KCo13693; *Treichelia dodii* Cupido, South Africa, Western Cape, Malmesbury, *Cupido* 83, NBG, KCo13641, KCo13694; *T. longibracteata* (H. Buek) Vatke, South Africa, Western Cape, Hermanus, *Cupido* 199, NBG, KCo13642, KCo13695; *Theilera guthriei* (L. Bolus) Phillips, South Africa, Western Cape, Prins Albert, *Cupido* 279, NBG, KCo13643, KCo13696; *T. robusta* (A. DC.) Cupido, South Africa, Eastern Cape, Willowmore, *Cupido* 317, NBG, KCo13644, KCo13697; *Wahlenbergia acaulis* E. Mey., South Africa, Northern Cape, Kamiesberg, *Cupido* 267, NBG, HQ823495, HQ823460; *W. adpressa* (Thunb.) Sond., South Africa, Western Cape, Hopefield, *Cupido* 210, NBG, HQ823496, HQ823461; *W. akoroa* J. A. Petterson, New Zealand, Banks Peninsula, Palm Gully, *Wilson* 2, WELT, HQ823497, HQ823436; *W. albomarginata* Hook. subsp. *albomarginata*, New Zealand, Canterbury, Mt. Cook, *JMP* 0994, WELT, HQ823498, HQ823437; *W. androsacea* A. DC., South Africa, Western Cape, Melkbos, *Cupido* 183, NBG, HQ823499, HQ823462; *W. annularis* A. DC., South Africa, Western Cape, Elandsbaai, *Cupido* 251, NBG, HQ823500, HQ823463; *W. appressifolia* Hilliard & Burt, South Africa, KwaZulu-Natal, Himeville, Cobhan, *Cupido* 358, NBG, KCo13645, KCo13698; *W. axillaris* Sond., South Africa, Western Cape, Bredasdorp, *Cupido* 107, NBG, HQ823501, HQ823464; *W. buseriana* Schltr. & Brehmer, South Africa, Northern Cape, Platbakkies, *Cupido* 263, NBG, KCo13646, -; *W. capensis* (L.) A. DC., South Africa, Western Cape, Malmesbury, *Cupido* 184, NBG, HQ823502, HQ823465; *W. capillacea* (L. f.) A. DC., South Africa, *Cupido* 313, Western Cape, Uniondale, NBG, HQ823503, HQ823466; *W. cartilaginea* Hook. f., New Zealand, Hanmer Springs, Island Saddle, *JMP* 09117c, WELT, HQ823504, HQ823438; *W. ceracea* Loth., Australia, NSW, Kosciuszko NP, summit Mt Kosciuszko, *JMP* 0944, WELT, HQ823505, HQ823439; *W. cernua* (Thunb.) A. DC., South Africa, Western Cape, Cape Town, *Cupido* 188, NBG, HQ823506, HQ823467; *W. cinerea* (L. f.) Sond., South Africa, Western Cape, Genadendal, *Cupido* 222, NBG, HQ823507, HQ823468; *W. communis* Carolin, Australia, NSW, Cooma, *JMP* 0964, WELT, HQ823508, HQ823440; *W. cuspidata* Brehmer, South Africa, KwaZulu-Natal, Himeville, *Cupido* 302, NBG, HQ823509, HQ823469; *W. densifolia* Loth., Australia, NSW, Kosciuszko NP, Rennix walk, *JMP* 0934, WELT, HQ823510, HQ823441; *W. depressa* J. M. Wood & M. S. Evans, South Africa, Free State, Baker's Kop, *Roux* 3350, NBG, HQ823511, HQ823470; *W. desmantha* Lammers, South Africa, Western Cape, Albertinia, *Cupido* 310, NBG, HQ823512, HQ823471; *W. ecklonii* H. Buek, South Africa, Western Cape, Paarl, *Cupido* 206, NBG, HQ823514, HQ823472; *W. exilis* A. DC., South Africa, Western Cape, Malmesbury, *Cupido* 81, NBG, HQ823515, HQ823473; *W. fruticosa* Brehmer, South Africa, Western Cape, Riversdale, *Cupido* 311, NBG, HQ823517, HQ823474; *W. gracilis* (Forster. f.) A. DC., Australia, NSW, Blue Mts, *JMP* 0902, WELT, HQ823519, HQ823445; *W. gloriosa* Loth., Australia, NSW, Kosciuszko NP, Diggers Ck, *JMP* 0939, WELT, HQ823518, HQ823444; *W. hederacea* L., Europe, *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088792, AY322080; *W. huttonii* (Sond.) Thulin, South Africa, KwaZulu-Natal, Himeville, *Cupido* 304, NBG, KCo13647, KCo13699; *W. juncea* (H. Buek) Lammers, South Africa, Eastern Cape, Sterkstroom, *Cupido* 296, NBG, KCo13648, -; *W. krebsii* Cham., South Africa, Eastern Cape, Hogsback, *Cupido* 294, NBG, HQ823521, HQ823475; *W. littoricola* P. J. Smith, Australia, Victoria, Mt Buffalo, *Petterson* 1, WELT, HQ823523, -; *W. lobelioides* (L. f.) Link, Europe/Africa. *trnL-F* Roquet et al.

(2008), EF088793, -; *W. lobulata* Brehmer, South Africa, KwaZulu-Natal, Himeville, Sani Pass, *Cupido* 352, NBG, KCo13649, KCo13700; *W. longifolia* A. DC., South Africa, Western Cape, Darling, *Cupido* 212, NBG, HQ823524, HQ823476; *W. luteola* P. J. Smith, Australia, NSW, Abercrombie Caves, *JMP* 0911, WELT, HQ823525, HQ823448; *W. neoridiga* Lammers, South Africa, Western Cape, Prins Albert, *Cupido* 278, NBG, HQ823527, HQ823477; *W. nodosa* H. Buek, South Africa, Western Cape, Worcester, *Cupido* 144, NBG, KCo13650, -; *W. oxyphylla* A. DC., South Africa, Western Cape, Vanrhynsdorp, *Cupido* 259, NBG, HQ823529, HQ823478; *W. paniculata* (Thunb.) A. DC., South Africa, Western Cape, Yzerfontein, *Cupido* 181, NBG, HQ823530, HQ823479; *W. parvifolia* (P. J. Bergius) Adamson, South Africa, Western Cape, Cape Town, *Cupido* 119, NBG, HQ823531, HQ823480; *W. pilosa* H. Buek, South Africa, Northern Cape, Calvinia, *Cupido* 272, NBG, -, KCo13701; *W. polyantha* Lammers, South Africa, Western Cape, Albertinia, *Cupido* 287, NBG, HQ823532, HQ823481; *W. polytrichifolia* Schltr., Lesotho, Top of Sani Pass, *Cupido* 349, NBG, KCo13651, KCo13702; *W. procumbens* (Thunb.) A. DC., South Africa, Western Cape, Napier, *Cupido* 244, NBG, HQ823533, HQ823482; *W. psammophila* Schltr., South Africa, Western Cape, Vanrhynsdorp, *Cupido* 260, NBG, HQ823534, HQ823483; *W. pymaea* Colenso subsp. *pygmaea*, New Zealand, Central Plateau, Mt. Ruapehu, Ohakune Rd, *PGJ* 2770a, WELTU, HQ823535, HQ823451; *W. ramosa* G. Simpson, New Zealand, Wellington, Pukerua Bay, *JMP* 09105a, WELT, HQ823536, HQ823452; *W. ramulosa* E. Mey., South Africa, Western Cape, Clanwilliam, *Cupido* 256, NBG, KCo13652, -; *W. rubioides* A. DC., South Africa, Western Cape, Genadendal, *Cupido* 215, NBG, KCo13653, -; *W. stellarioides* Cham. & Schltl., South Africa, Eastern Cape, Sterkstroom, *Cupido* 295, NBG, KCo13654, -; *W. squamifolia* Brehmer, South Africa, Free State, Harrismith, Bezuidenhouts Pass, *Cupido* 342, NBG, KCo13655, KCo13703; *W. subulata* (L'Hér.) Lammers, South Africa, Western Cape, Somerset West, *Cupido* 207, NBG, HQ823541, HQ823484; *W. suffruticosa* *Cupido*, South Africa, Western Cape, Malmesbury, *Cupido* 209, NBG, KCo13656, KCo13704; *W. tenella* (L. f.) Lammers, South Africa, Western Cape, Cape Town, *Cupido* 194, NBG, HQ823544, HQ823485; *W. tenerrima* H. Buek, South Africa, Western Cape, Prins Albert, *Cupido* 277, NBG, HQ823545, HQ823486; *W. thunbergiana* H. Buek, South Africa, Western Cape, Elandsbaai, *Cupido* 250, NBG, HQ823546, HQ823487; *W. thunbergii* (Schult.) B. Nordenstam, South Africa, Eastern Cape, Port Elizabeth, *Forest* s. n., NBG, -, KCo13705; *W. undulata* (L. f.) A. DC., South Africa, Eastern Cape, Hogsback, *Cupido* s. n., NBG, HQ823547, HQ823488; *W. unidentata* (Thunb.) A. DC., South Africa, Western Cape, Caledon, *Cupido* 274, NBG, KCo13657, -; *W. violacea* J. A. Pettersen, New Zealand, Nelson area, Dun Mts, *JMP* 0972, WELT, HQ823549, HQ823459; *W. virgata* Engl., South Africa, KwaZulu-Natal, Himeville, *Cupido* 299, NBG, HQ823550, HQ823489; *W. sp.* 'Sani Rd', South Africa, KwaZulu-Natal, Sani Road, *Cupido* 309, NBG, HQ823539, -.

Outgroup—*Azorina vidalii* (Wats.) Feer, *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088696, AY322007; *Campanula divaricata* Michx., *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088718, AY322014; *C. latifolia* L., *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088732, AY322024; *Canarina canariensis* (L.) Vatke, *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088777, AY322045; *Cyphia bulbosa* (L.) P. J. Bergius, South Africa, Western Cape, Cape Town, *Cupido* s. n., NBG, KCo13658, -;

C. comptonii Bond, South Africa, Western Cape, Katbakkies, *Manning s. n.*, NBG, KCo13659, KCo13706; *C. volubilis* (Burm. f.) Willd., South Africa, Western Cape, Paarl, *Cupido 249*, NBG, KCo13660, -; *Jasione montana* L., *trnL-F* Antonelli (2008), ITS Park et al. (2006), DQ356174, DQ304566; *Lobelia comosa* L., South Africa, Western Cape, Cape Town, *Cupido s. n.*, NBG, KCo13661, KCo13707; *L. coronopifolia* L., South Africa, Western Cape, Villiersdorp, *Mannie s. n.*, NBG, -, KCo13709; *L. jasionoides* (A. DC.) E. Wimm., South Africa, Western Cape, Cape Town, *Cupido 120*, NBG, KCo13662, KCo13708; *Monopsis debilis* (L. f.) C.Presl, South Africa, Western Cape, Stellenbosch, *Cupido s. n.*, NBG, KCo13663, -; *Platycodon grandiflorus* (Jacq.) A. DC., *trnL-F* Roquet et al. (2008), ITS Eddie et al. (2003), EF088788, AY322073.