
PHYLOGENY OF
CAMPANULACEAE S. STR.
INFERRED FROM ITS
SEQUENCES OF NUCLEAR
RIBOSOMAL DNA¹

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ABSTRACT

Ninety-three taxa comprising thirty-two genera (plus four outgroups from Lobeliaceae) were used to estimate a phylogeny of the Campanulaceae based on ITS sequences of nuclear ribosomal DNA. From 2629 most parsimonious trees, a strict consensus tree with bootstrap values was constructed, in addition to a phylogram showing branch lengths. The topologies of these two trees are discussed in relation to the pollen and capsule morphology within the family, in addition to chromosome number and geographical distribution. The results show that there is a major dichotomy between the colpate/colporate pollen alliance (platycodonoid taxa) and the porate pollen alliance (wahlenbergioid and campanuloid taxa). Both these major alliances are monophyletic. Within the porate alliance there are two major clades, the wahlenbergioids and the campanuloids. The campanuloid clade is further subdivided into two major clades representing the *Rapunculus* and the *Campanula* s. str. groups of taxa, plus three smaller clades that are considered as “transitional” taxa. It is argued that the family originated in a fragmenting West Gondwanaland and that tectonic processes are responsible for the major dichotomy in the family. The colpate/colporate platycodonoids subsequently remained relatively relictual in Asia, whereas the porate taxa spread over much of the Northern and Southern Hemispheres. The campanuloid lineage spread over the Northern Hemisphere from a major evolutionary center in the Mediterranean region and is represented in North America only by the *Rapunculus* group. The wahlenbergioid lineage is widely dispersed across the southern continents and oceanic islands but has a major secondary center of diversification in southern Africa. The use of ITS provides insights for future investigations and a phylogenetic framework that can be tested with other data sets. Its limitations for phylogeny reconstruction are briefly discussed. More extensive taxon sampling and additional data sets are required to refine these results and for a new classification of the Campanulaceae to be proposed.

Key words: Campanulaceae, evolution, Gondwanaland, ITS, nuclear-ribosomal DNA, phylogeny.

Classification systems of the bellflower family (Campanulaceae s. str.) have traditionally followed the arrangements of Boissier (1875, 1888) and Schönland (1889–1894) and, together with the refinements of Charadze (1949, 1970, 1976), Fedorov (1957), and others, can ultimately be traced back to the arrangement of De Candolle (1830) who di-

vided the family into two subtribes, the Campanuleae and the Wahlenbergeae, based on the mode of capsule dehiscence (Table 1). Schönland divided the family into three subtribes, separating *Platycodon* A. DC., *Musschia* Dum., and *Microcodon* A. DC. in his subtribe Platycodinae on the basis of calyx lobe position in relation to the locules of the

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Table 1. Classification of Campanulaceae (A. P. de Candolle, 1830).

Subtribe I (Wahlenbergeae)	Subtribe II (Campanuleae)
Capsule with apical (valvate) dehiscence	Capsule with lateral (porate) dehiscence
<i>Campanumoea</i> Blume (baccate capsule)	<i>Adenophora</i> Fisch.
<i>Canarina</i> L. (baccate capsule)	<i>Campanula</i> L.
<i>Cephalostigma</i> A. DC.	<i>Merciera</i> A. DC. (indehiscent)
<i>Codonopsis</i> Wall.	<i>Michauxia</i> L'Her.
<i>Jasione</i> L.	<i>Musschia</i> Dum.
<i>Lightfootia</i> L'Her.	<i>Petromarula</i> Vent. ex Hedw. f.
<i>Microcodon</i> A. DC.	<i>Phyteuma</i> L.
<i>Platycodon</i> A. DC.	<i>Specularia</i> A. DC.
<i>Prismatocarpus</i> L'Her.	<i>Symphyandra</i> A. DC.
<i>Roella</i> L.	<i>Trachelium</i> L.
<i>Wahlenbergia</i> W. Roth	

ovary (Table 2). Such natural classifications were essentially based on morphology of the calyx (e.g., the presence or absence of appendages between the lobes) or of the mode of capsule dehiscence (e.g., whether it is apical and valvate or lateral and porate). Many authors (e.g., Hutchinson, 1969; Carolin, 1978; Cronquist, 1988; Takhtajan, 1969) considered *Cyananthus* A. DC. to be the most primitive genus within the family based on its superior ovary.

These various classifications were generally useful in floristic works, especially during the 20th century when much of the research on the Campanulaceae was of a regional, floristic nature. Frequently, various authors have used their own modified system with many nomenclatural changes, and great confusion has resulted. Considerable conflict still exists as to the number of genera recognized.

Generic distinctions in the family are often subtle, being based on a suite of characters best observed in living plants. In addition, species of the Campanulaceae appear to be prone to considerable phenotypic plasticity (Eddie, 1997; Eddie & Ingrouille, 1999) as well as ontogenetic variation, and this has led to a burgeoning of the literature with superfluous species names. The few generic monographs that have been completed, although excellent, often lacked a global perspective, and have contributed little to the establishment of a new, more generally accepted classification of the family. Reconstruction of the phylogeny of the Campanulaceae has been hindered by a lack of consensus as to what constitutes a genus and the failure to apply important character combinations (e.g., cytological and palynological characters), which could potentially

Table 2. Classification of the Campanulaceae (Schönland, 1889–1894).

Tribe Campanuleae		
Subtribe Campanulinae	Subtribe Wahlenberginae	Subtribe Platycodinae
<i>Adenophora</i> Fisch.	<i>Campanumoea</i> Blume	<i>Microdon</i> A. DC.
<i>Campanula</i> L.	<i>Cephalostigma</i> A. DC.	sect. <i>Eumicrocodon</i> A. DC.
sect. <i>Medium</i> Tourn.	<i>Codonopsis</i> Wall.	sect. <i>Caelotheca</i> A. DC.
sect. <i>Rapunculus</i> Boiss.	<i>Cyananthus</i> Wall.	<i>Musschia</i> Dum.
<i>Canarina</i> L.	<i>Githopsis</i> Nuttall	<i>Platycodon</i> A. DC.
<i>Heterocodon</i> Nuttall	<i>Hedraeanthus</i> Grisebach	
<i>Michauxia</i> L'Her.	<i>Heterochaenia</i> A. DC.	
<i>Ostrowskia</i> Regel	<i>Jasione</i> L.	
<i>Peracarpa</i> J.D. Hooker & T. Thoms.	<i>Leptocodon</i> (J. D. Hooker) Lem.	
<i>Phyteuma</i> L.	<i>Lightfootia</i> L'Her.	
sect. <i>Cylindrocarpa</i> Rgl.	<i>Merciera</i> A. DC.	
sect. <i>Hedranthum</i> G. Don	<i>Prismatocarpus</i> L'Her.	
sect. <i>Petromarula</i> A. DC.	<i>Rhigiophyllum</i> Hochst.	
sect. <i>Podanthum</i> G. Don	<i>Roella</i> L.	
sect. <i>Synotoma</i> G. Don	<i>Siphocodon</i>	
<i>Symphyandra</i> A. DC.	<i>Treichelia</i>	
<i>Trachelium</i> L.	<i>Wahlenbergia</i> W. Roth	

highlight major discontinuities at the generic, tribal, and subtribal levels. Many species have been placed, for convenience, in *Campanula* L., *Asyneuma* Grisebach & Schenk, and *Wahlenbergia* Schrad. ex W. Roth, and this has further complicated our understanding of phylogenetic relationships. Indeed, some of the intrageneric taxa in these large genera are probably more deserving of generic status than some of the currently recognized segregate genera. The so-called satellite genera of *Campanula* do not appear to be any closer to each other than they do to *Campanula*, and there is no evidence to suggest that *Campanula*, despite its numerical superiority, is ancestral to them. It is thus often easier to define what *Campanula* is not rather than what its actual boundaries are. Thus, to some extent, the genus *Campanula* is conceptually useless and its continued use as a "core" genus may be misleading. The same is probably true for *Asyneuma* and *Wahlenbergia*.

Knowledge of inter- and intrageneric relationships within the family has steadily increased during the latter half of the 20th century. Cytological studies, beginning with the seminal investigations of Gadella (1962, 1963, 1964, 1966, 1967), Contandriopoulos (1964, 1966, 1970, 1971, 1972, 1976, 1980a, b, 1984), Contandriopoulos et al. (1972, 1974, 1984), Damboldt (1965a, b, 1966, 1968, 1969, 1970, 1975, 1976, 1978a, b), Phitos (1963a, b, 1964a, b, 1965), and Podlech and Damboldt (1964) have vastly increased our knowledge of intrageneric relationships, particularly of the genus *Campanula*. The most common chromosome number in the Campanulaceae is $n = 17$, and this appears to have evolved independently several times in relatively unrelated genera (e.g., in *Campanula*, *Nesocodon* M. Thulin, *Ostrowskia* Regel, and *Canarina* L.). Forty-two percent of the published chromosome counts of the family Campanulaceae s.l. have this number (Lammers, 1992). The base number in the family has been suggested to be $x = 8$ (Böcher, 1964; Contandriopoulos, 1984), but Raven (1975) suggested that $x = 7$ is the ancestral number. An ancestral base number of $x = 7$ is supported by counts for *Cyananthus* (Kumar & Chauhan, 1975; Hong & Ma, 1991).

It was Avetisian (1948, 1967, 1973, 1986) who first drew attention to the different pollen morphologies within the family and gave a schematic presentation of pollen evolution based on aperture types. She further pointed out that pollen with colpate and colpate apertures are typical of those taxa found in the tropics, whereas those with porate apertures are typical of taxa from temperate regions. Dunbar (1973a, b, c, 1975a, b, 1981, 1984)

and Dunbar and Wallentinus (1976) extended Avetisian's work by providing excellent surveys of pollen from numerous genera of the Campanulaceae, and this has been augmented by Morin (1987), Nowicke et al. (1992), and Morris and Lammers (1997). Several of these studies suggest that some of the genera are artificially grouped together in De Candolle's and in Schönland's arrangements because of the limited criteria used as the basis for their classification systems.

Seed morphology has been examined for a number of taxa, principally those of North America (Shetler & Morin, 1982, 1986) and Eurasia (Belyaev, 1984a, b, 1985, 1986; Oganessian, 1985). Life-form in the Campanulaceae has been studied intensively by Shulkina (1974, 1975a, b, 1977, 1978, 1979, 1980a, b, c, 1986a, b, 1988) and Shulkina and Zykov (1980), but these data have not been incorporated into a cladistic analysis. Serological studies have been done on the tribe Phyteumatae (Gudkova & Borshchenko, 1991), while Gorovoi et al. (1971) conducted a limited chemotaxonomic survey of Russian Far-Eastern taxa. Kolakovsky (1980, 1982, 1986a, 1986b, 1987), Kolakovsky and Serdyukova (1980), and Lakoba (1986) did some pioneering carpological investigations of the family, but so far this work has not been corroborated and it remains to be seen whether their segregate genera will be accepted.

Few molecular phylogenetic studies of the Campanulaceae have been undertaken. Cosner (1993) and Cosner et al. (2004) used chloroplast DNA (cpDNA) structural rearrangements to establish a phylogeny of the family based on 18 genera, while Cosner et al. (1994) determined *rbcL* sequences for several genera as part of a study of interfamilial relationships of the Campanulales. Eddie (1997, and unpublished data), using cladistic and phenetic methodologies, investigated the morphology of most of the genera of the Campanulaceae, in addition to molecular variation of 23 to 29 taxa using internal transcribed spacers (ITS) and *matK/trnK*-intron sequence data from nuclear ribosomal (nrDNA) and cpDNA, respectively. For molecular variation within and between genera, ITS sequences have been used by Ge et al. (1997) for *Adenophora* Fisch. and by Kim et al. (1999) for *Hanabusaya* Nakai. Haberle (1998) examined relationships among the families Campanulaceae, Cyphiaceae, Nemacladaceae, Cyphocarpaceae, and Lobeliaceae using ITS sequence data.

This study is an attempt to reconstruct the phylogeny of the Campanulaceae s. str. using nrDNA ITS sequences and to compare the results with certain characters that have traditionally been used in

the classification of the Campanulaceae (i.e., capsule morphology and presence/absence of calyx appendages in addition to chromosome numbers, pollen, and geographical distribution). It is the first time that molecular methods have been applied to a broad sample of taxa (93 species in 32 genera) within the family. This study is also the first part of more extensive investigations of the Campanulaceae using a variety of molecular markers, including the sequences of chloroplast genes *matK* and *rbcL*, as well as chloroplast genome rearrangements and morphological data. Ultimately these studies should lead to a new comprehensive classification of the Campanulaceae.

MATERIALS AND METHODS

TAXA SAMPLED AND SOURCES OF PLANT MATERIAL

ITS sequences for 93 taxa of the Campanulaceae were used, including a number of which were previously published and available from Genbank (Fu et al., 1999; Ge et al., 1997; Kim et al., 1999; Schultheis, 2001; K. Dotti, unpublished data) (see Appendix 1). Many of the samples represent taxa that are commonly accepted as genera or sections within genera because of their obvious morphological discontinuities and that provide a broad sample of the family. The nomenclature used in this study is based on the classification system used by Fedorov (1957), but the names given to the major groups or clades are merely for convenience and not based on any particular classification system. Added to the data set were four outgroups from the Lobeliaceae (*Downingia bacigalupii* Weiler, *Lobelia aberdarica* R. E. Fries & T. C. E. Fries, *L. tenera* Kunth, and *L. tupa* L.), bringing the total number of taxa in the data set to 97. There is overwhelming evidence from both morphological (Lammers, 1992; Gustafsson & Bremer, 1995) and molecular (Cosner et al., 1994; Gustafsson et al., 1996; Jansen & Kim, 1996; Albach et al., 2001) studies that the Lobeliaceae are an appropriate outgroup for the Campanulaceae sensu stricto. DNA samples were obtained from living plants cultivated at The Institute of Cell and Molecular Biology (ICMB), University of Edinburgh, Scotland, U.K., The Royal Botanic Garden Edinburgh (RBGE), Scotland, U.K., The University of Texas at Austin (UT), U.S.A., and the Missouri Botanical Garden (MO), St. Louis, U.S.A. For sources of material and location of vouchers, see Appendix 1.

DNA ISOLATION, AMPLIFICATION, AND SEQUENCING

Genomic DNA was extracted following the CTAB protocol of Doyle and Doyle (1987) or with minor

modifications such as the addition of PVP-40 and/or BSA. Double-stranded DNA from the ITS and the intervening 5.8S subunit of the 18S–26S nr DNA was amplified using standard PCR procedures (Kim & Jansen, 1994). The basic primer sequences were those of White et al. (1990) or the modifications by Yokota et al. (1989). Purification of the PCR products was by means of Qiagen QIAquick spin columns (Qiagen Corp.), and sequences were obtained from ABI Prism 377 Automatic DNA Sequencers (Perkin Elmer, Applied Biosystems Division). For each taxon, forward and reverse sequences were obtained, and the results were saved as electropherograms and edited using the programs SEQUENCHER, vers. 3.0 and 4.1.2 (Gene Codes Corp.), EDITVIEW, ver. 1.0.1, and SEQUENCE NAVIGATOR, ver. 1.0.1 (Perkin Elmer, Applied Biosystems Division).

SEQUENCE ALIGNMENT

The boundaries for the ITS region were determined by comparisons with published ITS sequences of *Nicotiana rustica* L. (Solanaceae, Venkateswarlu & Nazar, 1991), *Krigia* Schreb. (Asteraceae, Kim & Jansen, 1994), *Madiinae* Benth. (Asteraceae, Baldwin, 1992), and *Gentiana* L. (Gentianaceae, Yuan et al., 1996). Alignment of ITS proved to be problematic, particularly at the 3' end of the ITS2 region close to the 26S subunit. Due to a high level of ambiguity, this region was deleted at 205 bases downstream from the start of the ITS2 region. The highly conserved 5.8 subunit was not available for all taxa and therefore was not included in phylogenetic analyses. The multiple alignment used in this study was created by CLUSTALX (ver. 1.64b; Thompson et al., 1997) in several stages using the Slow/Accurate dynamic programming option. Divergent sequences (> 40%) were delayed in the alignment procedure. Insertions from individual taxa, which created gaps and had no apparent homology with the rest of the taxa, were removed, and another round of alignment was initiated. A range of gap penalties from 5.00 to 20.00 and gap extension penalties from 3.00 to 8.00 were initially tried with various combinations until a consistent alignment was established using a gap penalty of 8.00 and a gap extension penalty of 5.00. Minor final adjustments to the alignment were done manually. The alignment is available at: <<http://www.biosci.utexas.edu/IB/faculty/jansen/lab/personnel/eddie.htm>>. All new sequences have been submitted to Genbank.

Table 3. Base composition and nucleotide divergence in the aligned partial sequences of ITS1 and ITS2 regions of nr DNA in the Campanulaceae.

Sequence parameter	ITS1 + partial ITS2			
Aligned length	497			
Constant sites	81			
Variable sites	416 (75 uninformative)			
Informative sites	345			
G + C content (%)	59.8			
Unambig. transitions	627			
Unambig. transversions	500			
Ts/Tv ratio	1.254			
Avg. base frequencies*	A = 20.8	C = 30.6	G = 29.2	T = 19.3

* Missing data and gaps excluded.

PHYLOGENETIC ANALYSES

A search for the most parsimonious tree was initiated using the PARSIMONY option of PAUP 4.068 (Swofford, 2001) with ACCTRAN, MULTREES, TBR, and COLLAPSE ZERO LENGTH BRANCHES options. All characters were given equal weight and were unordered. Gaps were treated as missing data. The HEURISTIC search algorithm was chosen, with 1000 random addition replicates and with a limit of 2000 trees saved per replicate. The amount of support for monophyletic groups was evaluated by 1000 bootstrap replicates and a 50% cut-off value for the bootstrap consensus tree (Felsenstein, 1985). Consistency Indices (CI) (Kluge & Farris, 1969) were also computed. The Retention Index (RI) and the g1 statistic (Hillis & Huelsenbeck, 1992) were also computed, the latter after computing the tree-length distribution of 100,000 random parsimony trees by means of the RANDOM TREES command.

RESULTS AND DISCUSSION

The total aligned length of the ITS1 and partial ITS2 (including gaps) was 497 bp. There were 81 constant characters, 71 variable characters that were parsimoniously uninformative, and 345 parsimoniously informative characters (Table 3). Parsimony analyses generated 2629 trees with 2130 steps, a CI of 0.3703 (excluding uninformative characters), and RI of 0.7583 (Figs. 1, 2). The g1 statistic for 100,000 trees randomly sampled was -0.327694 indicating that the ITS data set is significantly skewed from random and contains considerable phylogenetic information (Hillis & Huelsenbeck, 1992). For other statistics of the aligned sequences see Table 3. Multiple ITS types were not detected, and in one case there were two separate samples of the same species (*Adenophora divaricata* Franch. & Sav.) that did not come out together. The

branch lengths are very short for the *Adenophora* clade overall, which indicates that most of the species have very similar ITS sequences. The differences between the two samples of *A. divaricata* suggest either misidentification of the original sample or population differences in the ITS sequences.

The taxonomic categories used in classifications are unequivocal and the amount of molecular divergence (and hence phylogenetic signal) within and between taxa at each level in the taxonomic hierarchy varies. For a family such as the Campanulaceae, which has numerous monophyletic genera and sections, the use of ITS sequences is justified by the phylogenetic signal obtained, but there may be substantial trade-off due to problems with alignments. The difficulties associated with sampling across a wide spectrum of taxa in the Campanulaceae should lessen as we are able to refine our molecular analyses at different levels in the taxonomic hierarchy, in conjunction with other sources of data. Due to high ambiguity at the generic level in the Campanulaceae, ITS sequence data may be approaching the limits of usefulness for phylogenetic reconstruction, whereas at the species level, there may not be enough signal, and many species may be spuriously placed with each other. For extensive discussion of the utility and limitations of the ITS region in the reconstruction of angiosperm phylogeny, see Baldwin et al. (1995), Coleman (2003), and Goertzen et al. (2003).

MAJOR CLADES IN THE ITS TREE

The topology of the strict consensus tree (Fig. 1) shows that there are two major clades of the Campanulaceae. This major dichotomy in the family is supported by pollen data. For convenience, these two major clades are referred to as alliances and are named on the basis of their pollen types. The taxa in the smaller of these two alliances comprise

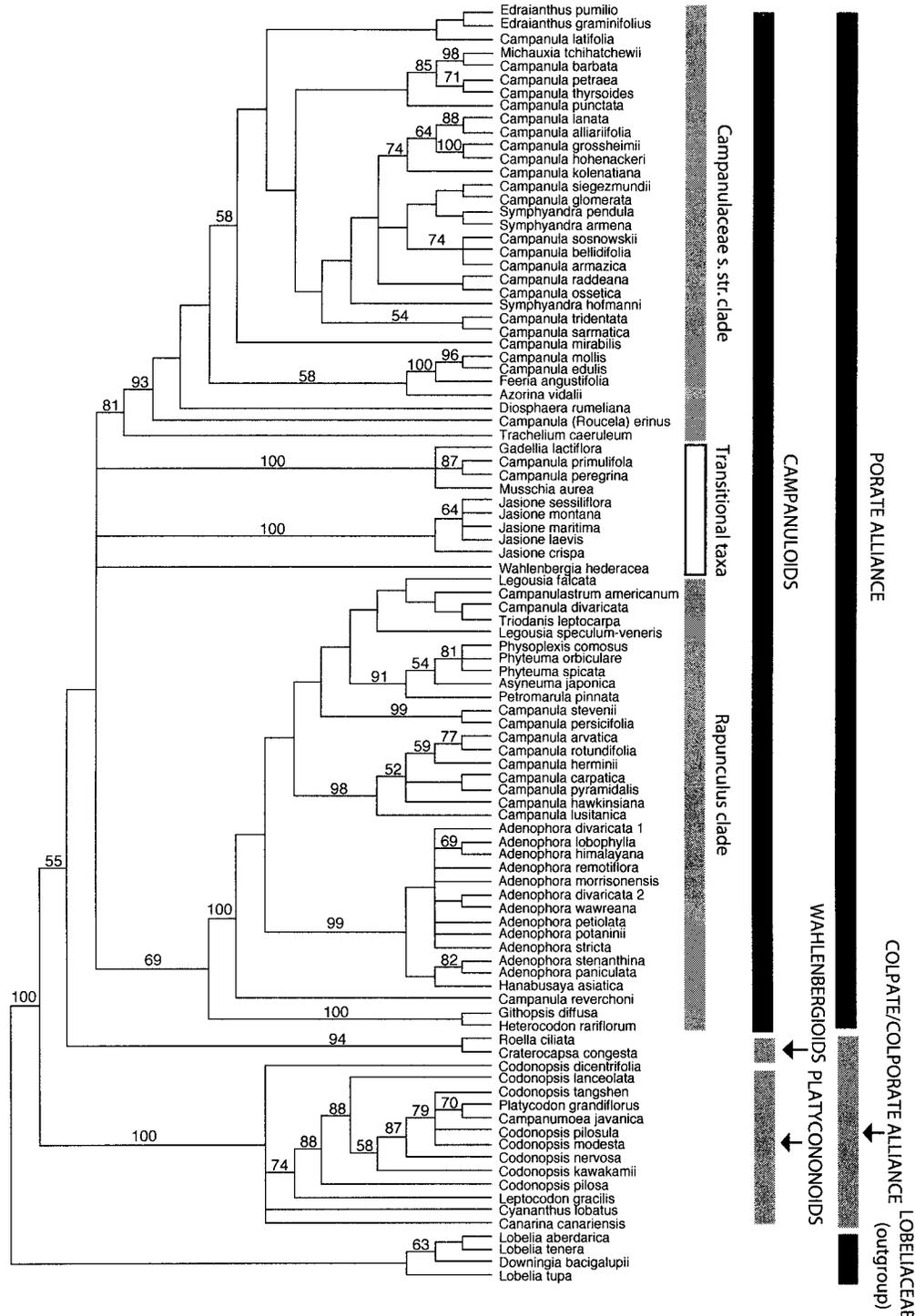


Figure 1. Strict consensus of 2629 most parsimonious trees with 2130 steps for 93 taxa of the Campanulaceae and 4 outgroups of the Lobeliaceae based on parsimony analysis of the combined ITS1 and ITS2 sequence data. The numbers above the nodes are bootstrap percentages of 1000 replicates. [CI = 0.3703 (excluding uninformative characters), RI = 0.7583.] Nodes without bootstrap values had less than 50% support.

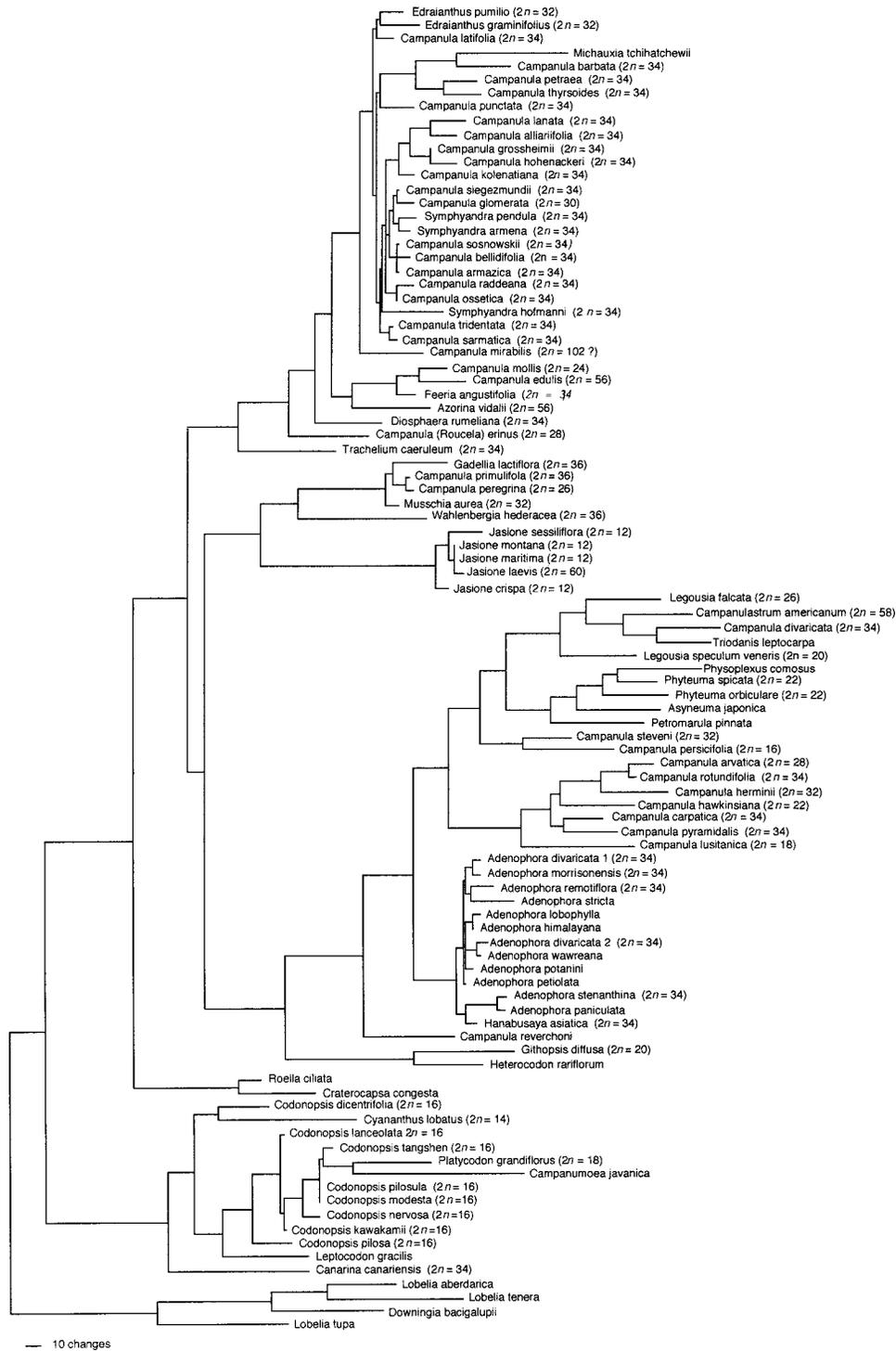


Figure 2. Phylogram of one of the 2629 equally parsimonious trees for 93 taxa of the Campanulaceae and 4 outgroups of the Lobeliaceae based on parsimony analysis of the combined ITS1 and partial ITS2 sequence data. A scale bar representing 10 changes is shown on bottom left corner.

genera such as *Codonopsis*, *Platycodon*, *Canarina*, etc., which are all distinguished by their possession of either colpate or colporate pollen (Avetisian, 1948, 1967, 1973, 1986; Dunbar, 1973a, b, c, 1975a, b, 1981, 1984) and are also referred to as the platycodonoid group in this paper. The colpate/colporate alliance is strongly supported with a 100% bootstrap value and is the only clade with taxa that have baccate fruits (*Canarina*, *Campanumoea* Blume, and *Cyclocodon* W. Griff.), although the majority have dry capsules. Geographically, the colpate/colporate alliance is mostly distributed in the tropics or subtropics, from Southeast Asia and the western Himalayas to Ussuriland, Korea, and Japan, and from Indonesia and the Philippines to New Guinea. The genus *Canarina* is unique within this alliance in being essentially African, but it is disjunct, with one species in Macaronesia and two species in the mountains of East Africa. The taxa in the larger alliance comprise the remainder of the Campanulaceae, and they are distinguished by their porate pollen. The porate alliance has only weak support with a 55% bootstrap value. It is far larger numerically than the colpate/colporate alliance and is divided into two major groups, the wahlenbergioids and the campanuloids. This huge alliance is distributed mostly in the temperate regions of the world, although a few wahlenbergioid and campanuloid taxa extend to the tropics. All taxa within these two groups have capsules that are predominantly dry and dehiscent. In the discussion that follows, we describe the major groups in the two alliances and how they compare with data from morphology, chromosome number, and geography.

THE COLPATE/COLPORATE ALLIANCE (THE PLATYCODONOID GROUP)

There is strong support (100%) for the monophyly of the colpate/colporate alliance, although the major clades within this alliance are only partially resolved. *Canarina*, *Cyananthus*, and *Codonopsis* Wall. subg. *Obconicapsula* D. Y. Hong form a polytomy with the remainder of taxa, including *Codonopsis*, *Leptocodon* (J. D. Hooker) Lem., *Platycodon*, and *Campanumoea javanica* Blume. *Codonopsis* subg. *Obconicapsula* is somewhat isolated morphologically and, to a lesser extent, geographically (central Himalayas) from the rest of *Codonopsis*. It has an ovary that bulges upward above the level of the calyx lobes and an incomplete nectar dome. These features, together with the overall appearance of the flower, recall *Platycodon*. *Cyananthus* comprises highly adapted perennial and annual species of very high elevations in the moun-

tains of southern Asia. Because of its superior ovary and low chromosome number of $2n = 14$, it has traditionally been considered the most ancestral genus of the Campanulaceae (Hutchinson, 1969; Cronquist, 1988; Takhtajan, 1969). However, it also has specialized ecological characters such as deep taproots and prostrate lateral branching, both of which are characteristic of alpine plants. The isolated position of *Canarina* is supported by both geography and chromosome number. *Canarina canariensis* (L.) Vatke has $2n = 34$, while the remainder of the platycodonoids have $2n = 16$ or 18. Bootstrap support for the clade comprising *Leptocodon*, the remainder of *Codonopsis*, plus *Campanumoea javanica* and *Platycodon* is moderate (74%). Support for the minor clade containing the bulk of *Codonopsis* plus *Platycodon* and *C. javanica* is strong (88%), but the clade with only the latter two genera is moderately supported (70%). The taxa of *Codonopsis* are morphologically less divergent from each other, whereas *C. javanica* and *Platycodon* are considerably divergent. Hong and Pan (1998), on the basis of pollen morphology, seed coat, and gross morphology, restored the genus *Cyclocodon*, which was formerly included in *Campanumoea* s.l. as *C. celebica* Blume and *C. lancifolia* (Roxb.) Merr. They considered *Cyclocodon* to be more closely related to *Platycodon* than to *Campanumoea* s. str. (i.e., *C. javanica* Blume and *C. inflata* (Hook. f.) C. B. Clarke). *Campanumoea* and *Cyclocodon* have baccate fruits but would appear to be rather distant from *Canarina*.

THE PORATE ALLIANCE (THE WAHLENBERGIOID AND CAMPANULOID GROUPS)

The monophyly of the porate alliance is weakly supported (55%) and it comprises two very unequal clades, the wahlenbergioids and the campanuloids. This is undoubtedly an artifact of the undersampling of wahlenbergioid taxa.

The wahlenbergioid group. The sister relationship of the two wahlenbergioid taxa, *Craterocapsa* Hilliard & B. L. Burt and *Roella* L., has strong bootstrap support (94%). These two genera have traditionally been considered closely related (Hilliard & Burt, 1973). Both are from southern Africa, although *Craterocapsa* ranges north to the mountains of eastern Zimbabwe. Since only three traditionally accepted wahlenbergioid genera were available for molecular analysis, the discussion of the results for this group is relatively straightforward, but caution should be observed for such a small sample. *Wahlenbergia hederacea* L. falls within the campanuloid group and is therefore distant from the other two

wahlenbergioid genera. This is surprising because this species has traditionally been considered as typically wahlenbergioid. It has a chromosome number of $2n = 36$, which is not particularly unusual, but it is isolated in western Europe, and has a vegetative morphology that is rather atypical for the wahlenbergioids. Although all modern European workers have never questioned the wahlenbergioid nature of *W. hederacea*, this species was recognized as a separate genus by some early workers (*Schultesia* Roth, *Valvinterlobus* Dulac, *Aikinia* Salisb. ex A. DC.) and it was assigned to *Roucelia* by Dumortier (1827). The majority of species of *Wahlenbergia* are distributed in the Southern Hemisphere. Some species (e.g., *W. trichogyna* Stearn) have $2n = 36$, but the majority have $2n = 18$ (see also Petterson et al., 1995; Crawford et al., 1994; Anderson et al., 2000). In the study of Cosner et al. (2004), the Australian species, *W. gloriosa* Lothian (not sampled), was found to be in the same clade as *Roella ciliata* L.

The campanuloid group (*Campanula* s. str., “transitional” taxa, and *Rapunculus* clades). This huge group forms an unresolved polytomy consisting of two major clades and three smaller ones. This basic division is partially in agreement with mode of capsule dehiscence (there are exceptions such as *Edraianthus* in the *Campanula* s. str. clade and *Adenophora* and subsection *Heterophylla* in the *Rapunculus* clade) and presence or absence of calyx appendages, two characters that have traditionally been used in intrageneric classifications of *Campanula* (Boissier, 1875, 1888; Fedorov, 1957). One large, well-supported clade (81%) comprises those taxa centered around *Campanula* s. str. (i.e., mostly those taxa belonging to the sect. *Medium* DC.), but also genera such as *Trachelium*, *Diosphaera*, *Azorina*, etc. The second large clade has moderate support (69%) and comprises those taxa centered around *Campanula* sect. *Rapunculus* (Fourr.) Boiss. (the *Rapunculus* clade). Two smaller clades have strong support (100%) and consist of several transitional genera such as *Jasione* L., *Musschia*, and *Gadellia* Shulkina, while the third small clade comprises *Wahlenbergia hederacea* alone.

THE *CAMPANULA* S. STR. CLADE

The *Campanula* s. str. clade includes a small number of mostly monotypic genera that are considerably more divergent than the majority of taxa in this clade. Some have upright flowers (e.g., *Trachelium caeruleum* L., *Diosphaera rumeliana* (Hampe) Bornm., *Feeria angustifolia* (Schousb.) Buser, *Campanula* [subg. *Roucelia* (Dumort.) J.

Damboldt] *erinus* L., *Campanula mollis* L., and *Campanula edulis* Forssk.), but *Azorina vidalii* (Wats.) Feer, with its nodding flowers, is a conspicuous exception. With *Trachelium* removed, bootstrap support for this clade is 93%. *Campanula* (subg. *Roucelia*) *erinus* ($2n = 28$) belongs to a rather distinct group of annual campanuloids of the Mediterranean, which superficially resemble *C. mollis* and *C. edulis*, but its capsules are discoid and the calyx appendages are absent. The corolla approaches the hypercrateriform shape of *Trachelium* corollas to some extent. The flowers of *Diosphaera* Buser are quite similar to those of *Trachelium* and it has the same chromosome number ($2n = 34$), but there are conspicuous differences between the two genera, both vegetatively and in the form of the inflorescence. The two genera are often united, but they are disjunct geographically in the Mediterranean. Calyx appendages are absent in both genera.

Azorina Feer is quite isolated morphologically (vegetatively and in branching pattern), but its vague resemblance to *Campanula bravensis* Bolle and *C. jacobaea* C. Smith of the Cape Verde Islands, together with its chromosome number of $2n = 56$, may link it rather tenuously to *Campanula* subsect. *Oreocodon* Fed. (but see also Thulin, 1976: 354). Support for the clade that comprises *Azorina*, *Feeria*, *Campanula mollis*, and *C. edulis* is weak (58%), but when *Azorina* is removed support for the remaining taxa is 100%. *Feeria angustifolia* has traditionally been associated with *Trachelium*, but morphologically it is quite distinct. In some respects, particularly the globular, more compact shape of the inflorescence, and the valvate apical dehiscence, it approaches *Jasione* L., but the chromosome number for *Feeria angustifolia* is $2n = 34$ (vs. $2n = 12$ for *Jasione*). The similarity of its ITS sequences with those of both *Campanula mollis* and *C. edulis* does not accord with its morphology. *Campanula mollis* and *C. edulis* are probably closely related to each other, and this relationship is strongly supported in the ITS tree (96%). These two species belong to a group of annual and perennial campanuloids ($2n = 24, 28, 54, 56$), which range from Macaronesia, North Africa, and the Iberian Peninsula south to the equator in Tanzania. They have basal dehiscence and appendages between the calyx lobes (Maire, 1929; Quézel, 1953; Thulin, 1976). This group probably links up with *Campanula* subsect. *Oreocodon* of the western Himalayas and south-central Asia, which is characterized by species such as *C. incanescens* Boiss., *C. cashmeriana* Royle, and *C. colorata* Wall.

The remaining taxa in the *Campanula* s. str. clade are weakly supported (58%) as a monophy-

letic group. They are mostly Eurasian and North African, although at least one species in this alliance occurs as far east as the Aleutian Islands (*C. chamissonis* Fed. subsect. *Scapiflorae* (Boiss.) Fed., not sampled), and another south to the equator in northern Tanzania (*C. keniensis* Thulin, also not sampled). The isolated species *C. mirabilis* Albov (subsect. *Spinulosae* (Fom.) Fed.) is the sister taxon to all the others. The small clade formed by *Edraianthus pumilio* (Schultes) A. DC., *E. graminifolius* (L.) A. DC., and *C. latifolia* L. is weakly supported (< 50%). The two species of *Edraianthus* (A. DC.) DC. are confined to the mountains of southeastern Europe, and are rather dissimilar morphologically. *Edraianthus pumilio* has solitary flowers on multiple inflorescence stems, whereas *E. graminifolius* has a glomerulate inflorescence. Morphologically, *E. pumilio* may be closer to *Campanula* (*Petkovia* Stefanoff) *orphanidea* Boiss. (not sampled), which has a similar mode of capsule dehiscence (Hartvig, 1991) and similar corollas (*C. orphanidea* has $2n = 26$). *Edraianthus* was formerly considered to be wahlenbergioid because of the apical rupture of its capsule, but its overall morphology is very similar to *Campanula* and its chromosome number ($2n = 32$) is more typical of campanuloid taxa. *Campanula latifolia* is rather isolated in the *Campanula* s. str. clade. It belongs to a distinct group of tall mesophytic species from Eurasia that lack appendages and have nodding flowers on long spicate inflorescences (e.g., *C. trachelium* L., *C. bononiensis*, *C. rapunculoides* L., etc.). In general morphology this group (subsect. *Eucodon* (A. DC.) Fed.) resembles *Adenophora*.

Several other minor groups within the *Campanula* s. str. clade have moderate to strong support. *Michauxia tchihatchewii* Fisch. & C. A. Meyer and *C. barbata* L. have a bootstrap value of 98%. This relationship is surprising since the morphology of these two species is very divergent. The monophyly of the two, yellow-flowered species from the European Alps, *C. thyrsoides* L. and *C. petraea* L., is moderately supported (71%). Collectively, these four taxa form a strongly supported clade (85%). The long branches (Fig. 2) show clearly that these four taxa are all very divergent from each other. In some cases, relationships in the *Campanula* s. str. clade are in accord with classification of Fedorov (1957), whereas in other instances there is conflict. For example, *C. armazica* Kharadze, *C. sosnowskii* Kharadze, and *C. bellidifolia* Adam have a support value of 74%, which agrees with their placement in section *Scapiflorae* (Boiss.) Fed. In contrast, *C. hohenackeri* Fisch. & C. A. Mey. (subsect. *Triloculares* Boiss.) and *C. grossheimii* Kharadze (sub-

sect. *Eucodon*) have bootstrap support of 100%, but their relationship conflicts with Fedorov's arrangement.

THE "TRANSITIONAL" TAXA

The clade comprising *Musschia*, *Gadellia*, and the two species of *Campanula* sect. *Pterophyllum* Damboldt (*C. peregrina* L. and *C. primulifolia* L.) is strongly supported (100%). *Musschia aurea* Dumort. is an endemic of Madeira together with its congener, *M. wollastoni* Lowe, whereas *C. peregrina* and *C. primulifolia* are disjunct between the eastern Mediterranean region and the western Iberian Peninsula, respectively. *Gadellia lactiflora* (M. Bieb.) Shulkina is endemic to the Caucasus region. Morphologically, *Musschia* is different from the other three taxa except for a vague similarity of form, robustness, and disposition of the stigmatic lobes. Its capsule is 5-loculed, prismatic, and opens with numerous transverse slits. Its chromosome number is $2n = 32$. *Gadellia* was erected by Shulkina (1979) for *Campanula lactiflora* M. Bieb. based on its distinct growth form and chromosome number ($2n = 36$). It has open, upright flowers and dehisces somewhat medially/apically. *Campanula primulifolia* was placed in the genus *Echinocodon* (= *Echinocodonia* Kolak.) by Kolakovsky (1986b). *Campanula peregrina* was acknowledged to be very close to *C. primulifolia* by Damboldt (1978b) and was placed in the section *Pterophyllum*. Bootstrap support for a close relationship between these two species is 87%. Despite their strong resemblances, the chromosome number for *C. primulifolia* is $2n = 36$, while *C. peregrina* is recorded as $2n = 26$ (Gadella, 1964). However, Marchal (1920) recorded the former also as $2n = 26$, so these findings require clarification.

The genus *Jasione* L. is strongly supported as a monophyletic group (100%). Within the genus, *J. crispa* (Pourr.) Samp. is sister to all the others sampled, but the clade formed by them is weakly supported (64%) and relationships among species within the group are unresolved. The relationship of *Jasione* to other taxa of Campanulaceae is unresolved in the ITS tree. *Jasione* has most frequently been associated with the wahlenbergioid alliance, although it does bear some resemblance to *Feeria* Buser with which it shares a similar mode of capsule dehiscence, but it has a chromosome number of $2n = 12$ (vs. $2n = 34$ for *Feeria*).

THE RAPUNCULUS CLADE

The *Rapunculus* clade has moderate support (69%) and has a number of smaller clades that are

all relatively divergent from each other morphologically. In terms of branch length, the taxa within the *Rapunculus* clade are much more divergent overall than the taxa within the *Campanula* s. str. clade (Fig. 2). *Githopsis* Nuttall and *Heterocodon* Nuttall are rather divergent in morphology from each other, particularly that of the capsule (see McVaugh, 1945), but are probably closely related and have strong bootstrap support (100%). They are sister to the remaining members of the *Rapunculus* clade. Most of these taxa are either Mediterranean or North American in distribution. The majority of taxa within this clade have open, upright flowers that are rather stellate in form, and the capsule opens apically or medially by a pore, but there are conspicuous exceptions (see below). None of the taxa in the *Rapunculus* clade has calyx appendages. The irregular rupture of the capsule apex in *Githopsis* may represent a derived condition, but this is not to imply that its ancestral state was lateral (e.g., it may be derived from an apical valvate condition similar to that present in the wahlenbergioid alliance). In *Adenophora*, *Hanabusaya*, and *Campanula rotundifolia* L. (the sole representative of the harebell group sampled, *Campanula* subsect. *Heterophylla* Fed.), the flowers are campanulate and nodding and the capsule opens basally. The inclusion of these taxa within the *Rapunculus* clade is surprising. Morphologically these taxa seem to be more closely allied to groups within the *Campanula* s. str. clade (e.g., *C. latifolia* and its allies in sect. *Eucodon*).

When *Githopsis* and *Heterocodon* are removed, the remaining taxa of the *Rapunculus* clade have 100% bootstrap support. Within this clade the Texan endemic annual *Campanula reverchonii* A. Gray is sister to all the remaining taxa, although support for this group is weak (< 50%). Within this clade there are several small groups with moderate to strong support. The clade comprising *Adenophora* and *Hanabusaya* is strongly supported (99%), although species relationships are largely unresolved. This confirms the close relationship between *Hanabusaya* and *Adenophora* suggested previously by Eddie (1997) and by Kim et al. (1999), and it tentatively suggests that *Hanabusaya* is closest to the two species *A. stenanthina* (Ledeb.) Kitagawa and *A. paniculata* Nannf. (sect. *Thyrsanthe* (Borb.) Fed.). Support for the clade uniting these three taxa is weak (< 50%). The remaining species of *Adenophora* form an unresolved polytomy, although there is weak support for a group consisting of *A. himalayana* Feer (sect. *Pachydiscus* Fed.) and *A. lobophylla* D. Y. Hong (sect. *Microdiscus* Fed.).

The sister group to the *Adenophora/Hanabusaya*

clade is only weakly supported, but it contains several well-supported smaller groups. These taxa are divergent morphologically and have a wide range of chromosome numbers. The group containing the serpentine endemic from the Balkans, *C. hawkinsiana* Hausskn. & Heldreich ($2n = 22$), and Iberian endemics *C. lusitanica* Loeffl. ($2n = 18$), *C. herminii* Hoffmanns & Link. ($2n = 32$), and *C. arvatica* Lag. ($2n = 28$), is strongly supported (98%), while the clade with *C. stevenii* M. Bieb. ($2n = 32$) and *C. persicifolia* L. ($2n = 16, 18$) has a support value of 99%. The two morphologically divergent species, *C. arvatica* and *C. rotundifolia* ($2n = 34$), are sister species with 77% bootstrap support. *Campanula carpatica* Jacq. (subsect. *Rotula* Fed.) does not appear to be as close to *C. pyramidalis* L. ($2n = 34$), but it does resemble *C. herminii* from the Iberian Peninsula. *Campanula pyramidalis* is part of the “isophylloid” group of species (e.g., *C. isophylla* Moretti, *C. garganica* Tenore, *C. versicolor* Andrews [not sampled], etc.), which is centered in Italy and the western Balkan Peninsula and is somewhat intermediate between the *Phyteuma* L./*Asyneuma* alliance and those species that could be considered as typically rapunculoid (e.g., *Campanula carpatica*, etc.) (see also Damboldt, 1965a). However, many species in this group hybridize freely, and numerous hybrids involving *C. carpatica* are known in cultivation (Lewis & Lynch, 1989). Thus, the ITS data suggest that this grouping is a natural one. Broader sampling would perhaps have helped clarify the positions of the “isophylloid” and *Heterophylla* groups.

The *Phyteuma* clade includes morphologically similar species and has strong bootstrap support (91%). *Petromarula* Vent. ex Hedw. f. is sister to all the other taxa, followed by *Asyneuma japonicum* (Miq.) Briq. The clade comprising *Physoplexis* (Endl.) Schur and *Phyteuma* has a bootstrap support of 81%, but relationships within this group are unresolved. The long branches in this clade (Fig. 2) suggest these taxa are relatively divergent. The sister group of *Phyteuma* and closely related genera includes Eurasian genera such as *Legousia* Dur. and several diverse North American taxa, such as *Triodanis* Raf., *Campanula divaricata* Michx., and *Campanulastrum americanum* (L.) Small. This clade is weakly supported with a bootstrap value of less than 50%. Apart from *Triodanis*, which is sometimes considered to be congeneric with *Legousia* (McVaugh, 1945, 1948), these taxa are all rather divergent morphologically. In *Asyneuma*, *Phyteuma*, *Petromarula*, *Physoplexis*, the “isophylloid” species such as *Campanula pyramidalis*, and the American taxa such as *Campanulastrum* and *Triod-*

anis, the capsule opens apically or medially by a more irregular pore. Morphologically, *C. divaricata* resembles *Adenophora* somewhat, and the capsule opens basally. In other respects, such as the open stellate shape and upward orientation of the flower, the majority of the other taxa in this clade are typically rapunculoid (e.g., *C. rapunculus* L., *C. patula* L., etc.).

CONCLUSIONS

Overall, there is a remarkable congruence between the ITS tree and traditional ideas on species relationships within the Campanulaceae (Eddie, 1999). The insights of early workers such as De Candolle and Boissier have proved to be remarkably clear, and their classification systems have, on the whole, been logically consistent with our findings on phylogeny. This study also supports the serological studies of Gudkova and Borshchenko (1991) and the cpDNA phylogenies of Cosner (1993) and Cosner et al. (2004).

The ITS trees indicate that the colpate/colporate alliance (the platycodonoids) is sister to the remainder of the Campanulaceae (Eddie, 1997, 1999; Shulkina & Gaskin, 1999). This is in agreement with phylogenies of the Campanulaceae based on cpDNA structural rearrangements (Cosner et al., 2004). In comparison with the porate taxa, the colpate/colporate taxa show considerably more molecular divergence, although the wahlenbergioid taxa were under-sampled. As a group, the colpate/colporate alliance has not radiated into drier, more temperate regions and its area of greatest diversity remains the region between the eastern Himalayas and southwest China. It is hypothesized that *Ostrowskia* (not sampled) represents a minor element of this alliance, which has evolved in the dry, temperate, and highly seasonal environments of Central Asia and thus displays features that parallel certain porate taxa, particularly the mode of capsule dehiscence. *Canarina* is clearly part of this alliance and was misplaced in the classifications of De Candolle (1830) and Schönland (1889–1894), although its chromosome ($2n = 34$) is anomalous within the platycodonoid group. These results also suggest that baccate fruits evolved several times in the colpate/colporate taxa (see Hong & Pan, 1998). Within this alliance there are combinations of certain morphological features that also occur in the porate taxa, e.g., valvate apical dehiscence, a nectary protected by expanded basal filaments (nectar dome), and colored pollen, and these may afford some clues about possible links between the two major alliances of the family.

The wahlenbergioids probably branched off early in the evolution of the porate alliance and constitute the only major group in the Southern Hemisphere. They have radiated most in southern Africa, although distinctive taxa occur on islands of the Atlantic, Indian, and Pacific Oceans. Several species of *Wahlenbergia* have ovaries that are almost superior, while *Nesocodon* from Mauritius has flowers that recall some species of *Codonopsis* in the colpate/colporate alliance. In contrast, the campanuloids are dominant over much of the Northern Hemisphere. The relative isolation of monotypic or small, distinctive genera within the two main campanuloid clades (e.g., the *Rapunculus* and *Campanula* s. str. clades) suggests that the group as a whole evolved in the Mediterranean Basin and spread rapidly over the Northern Hemisphere. The *Rapunculus* clade is considerably heterogeneous both cytologically and morphologically, although all taxa within this clade are exappendiculate. Many of the species were included in section *Rapunculus* (Fourr.) Boiss. (Boissier, 1875). It is the most geographically widespread clade, most diverse in the Mediterranean Basin, and the only one that has spread into North America (apart from *Campanula chamissonis* in the Aleutian Islands). The numerically small but diverse campanulaceous taxa of North America probably contain many relicts from pre-glacial times and represent several relatively independent groups derived from the main rapunculoid radiation in Eurasia (Shetler, 1979). An early radiation of the *Rapunculus* group in the Northern Hemisphere would explain the distinctiveness of subgroups (e.g., *Phyteuma*, *Petromarula*, and related genera) that are associated with the European Alpine orogenic events and fluctuating Mediterranean sea levels during the Tertiary period (Eddie, 1984; Favarger, 1972; Greuter, 1979). It would also explain the presence of endemic genera such as *Githopsis* in California and the other rather heterogeneous taxa in North America, e.g., *Heterocodon* and diverse *Campanula* annuals in California (see Morin, 1980), China, and southern Asia (e.g., *Homocodon* D. Y. Hong and *Peracarpa* J. D. Hooker & T. Thoms.). The ancestral group(s) that eventually gave rise to *Adenophora*, *Hanabusaya*, and the harebell group (subsect. *Heterophylla*) may be related to some of the North American taxa such as *C. divaricata* and *C. robinsiae* Small (not sampled), and may also have been ancestral to the predominantly appendiculate *Campanula* s. str. group, of which the mesophytic, exappendiculate species such as *C. latifolia*, *C. trachelium*, etc. (sect. *Eucodon*), may be the least morphologically modified descendants.

Species of the *Campanula* s. str. clade are mostly appendiculate, have basal dehiscence, and are cytologically more homogeneous, particularly those species in *Campanula* and *Symphyandra*. Many of them were included in *Campanula* sect. *Medium* (DC.) Boiss. (Boissier, 1875). Much of the radiation of this group is associated with the mountain-building processes of Eurasia, from the Atlas Mountains in the west to the western Himalayas. Subcenters of high diversity for the *Campanula* s. str. clade include the Balkan Peninsula, Anatolia, and the Caucasus Mountains. *Campanula*, as it is currently constituted, is clearly polyphyletic. The more divergent taxa in this clade are found mainly in the Mediterranean basin and are placed in small or monotypic genera (e.g., *Azorina*, *Diosphaera*, *Edraianthus*, *Feeria*, and *Michauxia*). Since De Candolle's monograph of 1830, *Edraianthus* has been associated with the wahlenbergioid group, but it is clearly campanuloid, although its exact relationships within the *Campanula* s. str. clade remain unclear (see also Hilliard & Burt, 1973).

Symphyandra A. DC. is now generally considered to be artificial (Greuter et al., 1984; Oganesian, 1995), and this analysis supports that conclusion. However, the four sections of the genus recognized by Fedorov (1957) are all quite distinct, and we suggest that the species formerly included in this genus should be re-examined and not necessarily included in *Campanula* without substantial evidence. The generic status of *Symphyandra odontosepala* (Boiss.) E. Esfandiari (not sampled) and the Iranian endemic genus *Zeugandra* P. H. Davis (not sampled) also need to be reassessed. *Symphyandra hofmanni* Pant. seems to be rather distant from the bulk of species in *Campanula*, whereas *S. pendula* (M. Bieb.) DC. and *S. armena* (Stev.) A. DC. are much closer.

Several genera may best be regarded as transitional between the wahlenbergioid group and the campanuloid group. *Musschia* is probably better placed with the campanuloids, but it is somewhat intermediate morphologically between the two major porate groups and shows some resemblance to wahlenbergioids such as *Heterochaenia* A. DC. from Réunion. It does not appear to be close to *Platycodon* or *Microcodon* A. DC. as in the arrangement of Schönland (1889–1894). On the basis of ITS sequence similarity to *Gadellia*, we suggest that the distinct morphological evolution of *Musschia* on Madeira was relatively rapid. *Jasione* also appears to be basal within the complex of Northern Hemisphere genera but its exact relationships remain unclear. On the whole it appears to have more affinities with campanuloid taxa. In the cpDNA tree

of Cosner et al. (2004), *Jasione* forms an unresolved polytomy with *Symphyandra*, *Edraianthus*, *Campanula*, and *Trachelium*.

Chromosome numbers (Fig. 2) are lowest overall in the colpate/colporate alliance, although the lowest recorded diploid number is for *Jasione* ($2n = 12$). Within the *Rapunculus* clade, with the exception of the clade comprising *Adenophora* and *Hanabusaya*, the chromosome numbers are diverse and are consistently lower than numbers recorded for the *Campanula* s. str. clade, which are predominantly $2n = 34$. If we accept the premise that there has been a general increase in chromosome number during the evolution of the Campanulaceae, then the platycodonoids are ancestral to all other groups and the wahlenbergioids and rapunculoids are ancestral to the campanuloids s. str. This accords well with our knowledge of pollen morphology and evolution in the family, as well as the morphology of the capsule in the different groups. However, the diploid number $2n = 34$ occurs in several unrelated lineages (*Campanula*, *Nesocodon*, *Canarina*, and *Ostrowskia*) and probably evolved independently in each of these genera.

This analysis suggests that the ancestral home of the Campanulaceae may be in the region of eastern Asia (of current geography) (see also Hong, 1995; Cosner et al., 2004), but such an interpretation cannot be easily reconciled with the distribution of many genera within the family or with closely related families such as the Lobeliaceae, Cyphiaceae s. str., or Nemacladaceae (Eddie, 1984, 1997, 1999). Carolin (1978), citing the distribution of *Cyananthus* in India, concluded that the Campanulaceae are essentially an African family that evolved primarily in western Gondwanaland. Bremer and Gustafsson (1997), using nucleotide substitutions in *rbcL*, suggested an East Gondwanaland origin at the end of the Cretaceous for the asteraeous alliance of families, and that the group subsequently diversified and expanded to West Gondwanaland before the breakup of the supercontinent. On the basis of *atpB-rbcL* spacer sequence data, E. B. Knox (pers. comm.) has stated, “. . . The interpretation is that *Cyphia* and the Lobeliaceae originated in southern Africa because the eight ‘basal’ lineages are entirely or predominantly African, and many of these are restricted to southern Africa.” “. . . The Lobeliaceae, Cyphiaceae, and Campanulaceae go back at most 40–50 MYA, and I do not think that the biogeographic patterns can be attributed to Gondwanaland.” If the family had arisen in Asia one would have expected platycodonoids to be represented in Eurasia and in North America. The presence of the colporate genus *Canarina* in

Africa and Macaronesia suggests that the family may have been more widespread in Africa and around the Indian Ocean than now, but this additional hypothesis does not conflict with an Asian, African, or a Gondwanaland origin for the family. The major dichotomy in the family between the colpate/colporate and the porate taxa suggests that major tectonic processes in the early to mid Tertiary period are implicated in its evolutionary history. A fragmenting West Gondwanaland origin, with the Asian platycodonoid taxa as relictual in land masses that now form the region of the eastern Himalayas and western China, seems a more likely scenario, and this would accord well with the hypothesis (Eddie, 1997) that the more basal members of the wahlenbergioid group are essentially southern or oceanic in their distribution (e.g., *Nesocodon*, *Heterochaenia*, *Berenice* L. R. Tulasne, and the shrubby species of *Wahlenbergia* from New Zealand, St. Helena, and the Juan Fernandez Islands). The endemic genera of the Cape Region of South Africa probably represent a very early radiation of the wahlenbergioid group in the fynbos vegetation as the climate there cooled and became more arid during the mid to late Tertiary (Eddie & Cupido, 2001).

The ITS phylogeny does not necessarily reflect a species phylogeny (Doyle, 1992), but it does provide a basis for inferring possible relationships within and between taxa at several taxonomic levels and provides insights for future investigations. It also provides a phylogenetic framework that can be tested with other data sets. We must await more extensive taxon sampling and data from other genes (both nuclear and chloroplast), as well as intragenomic analyses and chloroplast genome rearrangement studies in order to refine these results. At the same time it must be emphasized that refined data sets of floral morphology and developmental studies are also desirable before a new classification of the Campanulaceae can be proposed.

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Appendix 1. Taxa sequenced for the ITS region of nr DNA, listed alphabetically by genera and species within the Campanulaceae and Lobeliaceae. Institutional abbreviations are: Royal Botanic Gardens Edinburgh (RBGE); Royal Botanic Gardens, Kew (RBGK); Missouri Botanical Garden (MO); Tbilisi (TBI); National *Campanula* Collection, Cambridge (NCC); University of Texas, Austin (UT); Plant Resources Center, University of Texas, Austin (TEX); and University of Edinburgh (EGHB). Accession numbers follow the abbreviations for Botanical Gardens. Material with voucher specimens is followed by collector, collection number, and herbarium acronym. Sources of material for published sequences can be found in the cited publications.

Taxon name and authority	DNA accession number and (repository)	Voucher information, botanical garden accession number, or reference for published sequences (country of origin when available)	Genbank accession number (ITS1, ITS2)
Campanulaceae			
<i>Adenophora divaricata</i> Franch. & Sav.	Eddie 96086 (UT)	RBGE 19875003	AY322005, AY331418
<i>Adenophora divaricata</i> Franch. & Sav.		Ge et al. (1997)	AF090710, AF09071
<i>Adenophora himalayana</i> Feer		Ge et al. (1997)	AF090716, AF09071
<i>Adenophora lobophylla</i> D. Y. Hong		Ge et al. (1997)	AF090706, AF09070
<i>Adenophora morrisonensis</i> Hayata		Ge et al. (1997)	AF090718, AF09071
<i>Adenophora paniculata</i> Nannf.		Ge et al. (1997)	AF090714, AF09071
<i>Adenophora petiolata</i> Pax & Hoffm.		Ge et al. (1997)	AF090700, AF09070
<i>Adenophora potaninii</i> Korsh.		Ge et al. (1997)	AF090704, AF09070
<i>Adenophora remotiflora</i> (Sieb. & Zucc.) Miq.	Eddie 96087 (UT)	RBGE 19900973 (Japan)	AY322006, AY331419
<i>Adenophora stenanthina</i> (Ledeb.) Kitagawa		Ge et al. (1997)	AF090708, AF09070
<i>Adenophora stricta</i> Miq.		Ge et al. (1997)	AF090712, AF09071
<i>Adenophora waureana</i> Zahlbr.		Ge et al. (1997)	AF090702, AF09070
<i>Asyneuma japonicum</i> (Miq.) Briq.		Kim et al. (1999)	AF183437, AF18343
<i>Azarina vidatii</i> (Wats.) Feer	Eddie 4548404814 (UT)	<i>Eddie 4548404814</i> (EGHB)	AY322007, AY331420
<i>Campanula alliarifolia</i> Willd.	Cosner s.n. (UT)	RBGK 16225	AY322008, AY331421
<i>Campanula armazica</i> Charadze	Gaskin: 463 (UT)	<i>T. Shulkina</i> s.n. (Caucasus, Georgia, MO)	AY322009, AY331422
<i>Campanula arvatica</i> Lag.	Eddie 94003 (UT)	NCC 94003 (EGHB)	AY322010, AY331423
<i>Campanula barbata</i> L.	Eddie 251.700 (UT)	<i>J. Archibald 251.700</i> (Italy, TEX)	AY322011, AY331424
<i>Campanula bellidifolia</i> Adams	Gaskin: 115 (UT)	<i>Gaskin</i> s.n. (Caucasus, Georgia, TBI)	AY322012, AY331425
<i>Campanula carpatica</i> Jacq.	Cosner s.n. (UT)	<i>Lammers 8858</i> (USA, Illinois, F)	AY322013, AY331426
<i>Campanula divaricata</i> Michx.	Haberle (UT)	<i>R. C. Haberle 150</i> (USA, Virginia, TEX)	AY322014, AY331427
<i>Campanula edulis</i> Forssk.	Eddie 96055 (UT)	<i>S. Collenette 8782</i> (Saudi Arabia, TEX)	AY322015, AY331428
<i>Campanula erinus</i> L.	Eddie 95016 (UT)	<i>Eddie 95.016</i> (Turkey, TEX)	AY322016, AY331429
<i>Campanula glomerata</i> L.	Eddie 98016 (UT)	<i>Eddie 98016</i> (EGHB)	AY322017, AY331430
<i>Campanula grossheimii</i> Kharadze	Gaskin 2084 (MO, UT)	<i>M. Merello 2084</i> (Caucasus, Georgia, MO)	AY322018, AY331431
<i>Campanula hawkinsiana</i> Hauskn. & Heldreich	Eddie 94002 (UT)	NCC 94002, <i>Eddie 94002</i> (EGHB)	AY322019, AY331432
<i>Campanula herminii</i> Hoffmans. & Link.	Neves 227 (UT)	<i>S. Neves 227</i> (Portugal, TEX)	AY322020, AY331432
<i>Campanula hohenackeri</i> Fisch. & C. A. Mey.	Gaskin 205 (UT)	<i>M. Merello 2194</i> (Caucasus, Georgia, MO)	AY322021, AY331434

Appendix 1. Continued.

Taxon name and authority	DNA accession number and (repository)	Voucher information, botanical garden accession number, or reference for published sequences (country of origin when available)	Genbank accession number (ITS1, ITS2)
<i>Campanula holerantiana</i> C. A. Mey.	Gaskin 466 (UT)	<i>T. Shulkina 18</i> (Caucasus, TBI)	AY322022, AY331435
<i>Campanula lanata</i> Friv.	Eddie 96051 (UT)	NCC 96092, <i>Eddie 96051</i> (EGHB)	AY322023, AY331436
<i>Campanula latifolia</i> L.	Cosner s.n. (UT)	Lammers, no voucher	AY322024, AY331437
<i>Campanula lusitanica</i> Loeffl.	Neves 226 (UT)	<i>S. Neves 226</i> (Portugal, TEX)	AY322025, AY331438
<i>Campanula mirabilis</i> Albov	Eddie 96056 (UT)	RBGE 19972042	AY322026, AY331439
<i>Campanula mollis</i> L.	Neves 230 (UT)	<i>S. Neves 230</i> (Spain, TEX)	AY322027, AY331440
<i>Campanula ossetica</i> Bieb.	Gaskin 468 (UT)	<i>T. Shulkina 58</i> (Caucasus, TBI)	AY322028, AY331441
<i>Campanula peregrina</i> L.	Eddie 95007 (UT)	<i>Eddie 95007</i> (Turkey, TEX)	AY322029, AY331442
<i>Campanula persicifolia</i> L.	Eddie 95027 (UT)	RBGE 1969372, <i>Eddie 95027</i> (EGHB)	AY322030, AY331443
<i>Campanula petraea</i> L.	Eddie s.n. (UT)	RBGE 19860223 (France)	AY322031, AY331444
<i>Campanula primulifolia</i> L.	Neves 229 (UT)	<i>S. Neves 229</i> (Portugal, TEX)	AY322032, AY331445
<i>Campanula punctata</i> Lam.	Eddie 96092 (UT)	NCC 96092, <i>Eddie 96092</i> (EGHB)	AY322033, AY331446
<i>Campanula pyramidalis</i> L.	Eddie 96089 (UT)	NCC, <i>Eddie 96089</i> (EGHB)	AY322034, AY331447
<i>Campanula raddeana</i> Trautv.	Gaskin 57 (UT)	<i>T. Shulkina s.n.</i> (Caucasus, Georgia, TBI)	AY322035, AY331448
<i>Campanula reverchoni</i> A. Gray	Eddie 00004 (UT)	<i>Eddie 00004</i> (USA, Texas, TEX)	AY322036, AY331449
<i>Campanula rotundifolia</i> L.	Cosner s.n. (UT)	<i>Lammers 8714</i> (USA, F)	AY322037, AY331450
<i>Campanula samatica</i> Ker-Gawl.	Gaskin 458 (UT)	<i>T. Shulkina s.n.</i> (Caucasus, Georgia, TBI)	AY322038, AY331451
<i>Campanula siegismundii</i> Fed.	Gaskin 462 (UT)	<i>T. Shulkina s.n.</i> (Caucasus, TBI)	AY322039, AY331452
<i>Campanula sosnoskyyi</i> Charadze	Gaskin 314 (UT)	<i>J. Gaskin 442</i> (Caucasus, Georgia, TBI)	AY322040, AY331453
<i>Campanula steveni</i> M. Bieb.	Gaskin 302 (UT)	<i>J. Gaskin 158</i> (Caucasus, Georgia, TBI)	AY322041, AY331454
<i>Campanula thyrsoides</i> L.	Eddie s.n. (UT)	NCC, <i>Eddie s.n.</i> (EGHB)	AY322042, AY331455
<i>Campanula tridentata</i> Schreb.	Gaskin 417 (MO, UT)	<i>J. Gaskin 417</i> (Caucasus, Georgia, MO)	AY322043, AY331456
<i>Campanulastrum americanum</i> (L.) Small	Eddie 96050 (UT)	NCC, <i>Eddie 96050</i> (TEX)	AY322044, AY331457
<i>Campanumoea javanica</i> Blume	Eddie 96048 (UT)	Fu et al. (1999)	AF134862
<i>Canarina canariensis</i> (L.) Vaitke	Eddie 95022 (UT)	RBGE 19770035 (Spain, Canary Islands)	AY322045, AY331458
<i>Codonopsis dicentrifolia</i> W. W. Sm.	Eddie 95022 (UT)	RBGE 19920352 (Nepal)	AY322046, AY331459
<i>Codonopsis kawakamii</i> Hayata	Cosner s.n. (UT)	<i>Lammers 8439</i> (Taiwan, F)	AY322047, AY331460
<i>Codonopsis lanceolata</i> (Sieb & Zucc.) Benth. & Hook.f.	Eddie 95023 (UT)	RBGE 19870950	AY322048, AY331461
<i>Codonopsis modesta</i> Nannf.		Fu et al. (1999)	AF134859
<i>Codonopsis nervosa</i> Nannf.		Fu et al. (1999)	AF136237
<i>Codonopsis pilosa</i> Chipp		Fu et al. (1999)	AH008217
<i>Codonopsis pilosula</i> Nannf.		Fu et al. (1999)	AF134860
<i>Codonopsis tangshen</i> Oliv.		Fu et al. (1999)	AF134861

Appendix 1. Continued.

Taxon name and authority	DNA accession number and (repository)	Voucher information, botanical garden accession number, or reference for published sequences (country of origin when available)	Genbank accession number (ITS1, ITS2)
<i>Craterocapsa congesta</i> Hilliard & B. L. Burt	Eddie 0448 (UT)	Hirst & Webster D. 448 (Lesotho, EGHB)	AY322049, AY331462
<i>Cyananthus lobatus</i> Wall. ex Benth.	Cosner s.n. (UT)	Cosner 179 (OS)	AY322050, AY331463
<i>Diosphaera rumeliana</i> (Hampe) Bomm.	Eddie 95045 (UT)	Eddie 95045 (EGHB)	AY322051, AY331464
<i>Edraianthus graminifolius</i> (L.) A. DC.	Eddie 95029 (UT)	RBGE 19860931	AY322052, AY331465
<i>Edraianthus pumilio</i> (Schultes) A. DC.	Eddie 940119 (UT)	RBGE 19940119	AY322053, AY331466
<i>Feeria angustifolia</i> (Schousb.) Buser	Eddie 98004F (UT)	S. L. Jury et al. 17429 (Morocco, TEX)	AY322054, AY331467
<i>Gadellia lactiflora</i> (M. Bieb.) Schulkina	Eddie 95009 (UT)	RBGE 19693714	AY322055, AY331468
<i>Githopsis diffusa</i> A. Gray	Cosner s.n. (UT)	Morin, no voucher	AY322056, AY331469
<i>Hanabusaya asiatica</i> Nakai	Eddie 95018 (UT)	RBGE 19872386 (South Korea)	AY322057, AY331470
<i>Heterocodon rariflorum</i> Nutt.	Haberle 149 (UT)	R. C. Haberle 149 (USA, California, TEX)	AY322058, AY331471
<i>Jasione crista</i> (Pouret.) Samp.	Eddie 95083 (UT)	Eddie 95003 (EGHB, TEX)	AY322059, AY331472
<i>Jasione laevis</i> Lam.	Eddie 95035 (UT)	Eddie 95035 (EGHB)	AY322060, AY331473
<i>Jasione maritima</i> (Duby) L. M. Dufour ex Merino	Eddie 49 (UT)	Sales & Hedge 98.49 (Spain, RBGE)	AY322061, AY331474
<i>Jasione montana</i> L.	Eddie 98 (UT)	Sales & Hedge 98.98 (Spain, RBGE)	AY322062, AY331475
<i>Jasione sessiliflora</i> Boiss. & Reut.	Eddie 13 (UT)	Sales & Hedge 98.13 (Spain, RBGE)	AY322063, AY331476
<i>Legousia falcata</i> (Ten.) Fritsch	Eddie 97017 (UT)	Eddie 97017 (EGHB, TEX)	AY322064, AY331477
<i>Legousia speculum-veneris</i> (L.) Fisch.	Eddie 95034 (UT)	Eddie 95034 (EGHB, TEX)	AY322065, AY331478
<i>Leptocodon gracilis</i> Lem.	Eddie 95021 (UT)	RBGE 198921881 (Nepal)	AY322066, AY331479
<i>Michauxia tchihatchewii</i> Fisch. & C. A. Mey.	Eddie s.n. (UT)	RBGE s.n.	AY322068, AY331480
<i>Musschia aurea</i> Dumort.	Eddie 95030 (UT)	Eddie 95030 (EGHB, TEX)	AY322067, AY331481
<i>Petromarula pinnata</i> (L.) A. DC.	Eddie 96066 (UT)	Eddie 96066 (Greece, TEX)	AY322069, AY331482
<i>Physoplexis comosa</i> (L.) Schur	Eddie 95008 (UT)	RBGE 19771648	AY322070, AY331483
<i>Phyteuma orbiculare</i> L.	Cosner s.n. (UT)	Lammers 9993 (F)	AY322071, AY331484
<i>Phyteuma spicata</i> L.	Eddie 96090 (UT)	RBGE 19782029 (Spain)	AY322072, AY331485
<i>Platycodon grandiflorus</i> (Jacq.) A. DC.	Eddie 96076 (UT)	Eddie 96076 (EGHB)	AY322073, AY331486
<i>Roella ciliata</i> L.	Cosner s.n. (UT)	T. Ayers s. n. (BH)	AY322074, AY331487
<i>Symphyanandra armena</i> (Stev.) A. DC.	Eddie 760258 (UT)	RBGE 19760258	AY322075, AY331488
<i>Symphyanandra hofmanni</i> Pant.	Eddie 750893A (UT)	RBGE 19750893	AY322076, AY331489
<i>Symphyanandra pendula</i> (Bieb.) A. DC.	Gaskin 255(UT)	T. Shulkina s.n. (Caucasus, TBI)	AY322077, AY331490
<i>Trachelium caeruleum</i> L.	Eddie 98008T (UT)	Eddie 98008T (EGHB)	AY322078, AY331491
<i>Triodanis leptocarpa</i> (Nutt.) Nieuwl.	Haberle 132 (UT)	R. C. Haberle 132 (USA, Texas, TEX)	AY322079, AY331492
<i>Wahlenbergia hederacea</i> L.	Eddie 98004W (UT)	Eddie 98004W (Scotland, TEX)	AY322080, AY331493

Appendix 1. Continued.

Taxon name and authority	DNA accession number and (repository)	Voucher information, botanical garden accession number, or reference for published sequences (country of origin when available)	Genbank accession number (ITS1, ITS2)
Lobeliaceae			
<i>Downingia bacigalupii</i> Weiler		Schultheis (2001)	AF176900
<i>Lobelia aberdarica</i> R. E. Fries & T. C. E. Fries		Schultheis (2001)	AF163435
<i>Lobelia tupa</i> L.		Schultheis (2001)	AF163436
<i>Lobelia tenera</i> Kunth		Dotti (1998)	AF054938