

Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: Molecular signal overshadows morphology

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Abstract

Relationships within the subfamily Lobelioideae in Campanulaceae are inferred from DNA sequence variation in the *rbcL* and *ndhF* genes, and the *trnL*-F region including the *trnL* intron and the *trnL*-F intergenic spacer. Results derived from Bayesian and parsimony analyses provide evidence for the long-suspected paraphyly of the genus *Lobelia*, comprising over 400 species as presently circumscribed. The perennial dwarf herbs belonging to the Andean genus *Lysipomia* are sister to a group comprising the Neotropical shrubs *Burmeistera*, *Centropogon*, and *Siphocampylus*. Giant lobelioids from the Hawaiian Islands, Brazil, Africa, and Sri Lanka form a strongly supported group. Character optimizations on the phylogenetic tree reveal that shifts in fruit types and lignification have occurred much more commonly than generally assumed. The main clades in the subfamily are outlined, which largely contradict previous classifications based on morphology.

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1. Introduction

The Campanulaceae Jussieu is a well-known group of plants comprising 84 genera and nearly 2400 species (Lammers, 2007). The family has a cosmopolitan distribution and is present in a wide array of habitats, from tropical rain forest to tundra. The variety of life forms ranges from dwarf herbs shorter than 2 cm to trees up to 15 m tall (Fig. 1). There are at least three synapomorphies that characterize the family: laticifers producing milky sap, stamens attached to a disk at the apex of the ovary, and (usually) epigynous flowers (Judd et al., 2002). Other useful features that make the family easy to identify in the field include estipulate, simple, alternate, and entire leaves; flowers with secondary pollen presentation; 5-lobed, synsepalous calyces that form a hypanthium; 5-lobed, sympetalous, and often blue or violet corollas; solitary styles; and small and numerous seeds (Lammers, 2007).

As currently circumscribed (Lammers, 1998b), the family is divided into five subfamilies: Campanuloideae Burnett, Lobelioideae Burnett, Nemacladoideae Lammers, Cyphioideae (A. DC.) Walp., and Cyphocarpoideae Miers. Of these, Lobelioideae is the largest subfamily, comprising about 1200 species, half of which are native to South America. It is distinguished by having resupinate flowers with zygomorphic corollas and connate anthers (for a three-dimensional model of a typical lobelioid flower, see the on-line version of this article). The only taxonomic treatment covering all species in the subfamily is that of Wimmer (1953, 1968), who recognized two tribes, eleven subtribes, and five rami (Table 1). The delimitations of these groups were mainly based on reproductive characters, such as floral shape, juxtaposition of the ovary, fruit type, and number and mode of dehiscence of the capsules, but also habit and geographic distribution were sometimes employed. Still largely based on Wimmer's monography, Lammers (2007) recognizes 29 genera in the Lobelioideae (reduced to 28 by Koopman and Ayers, 2005) (Table 2). It is

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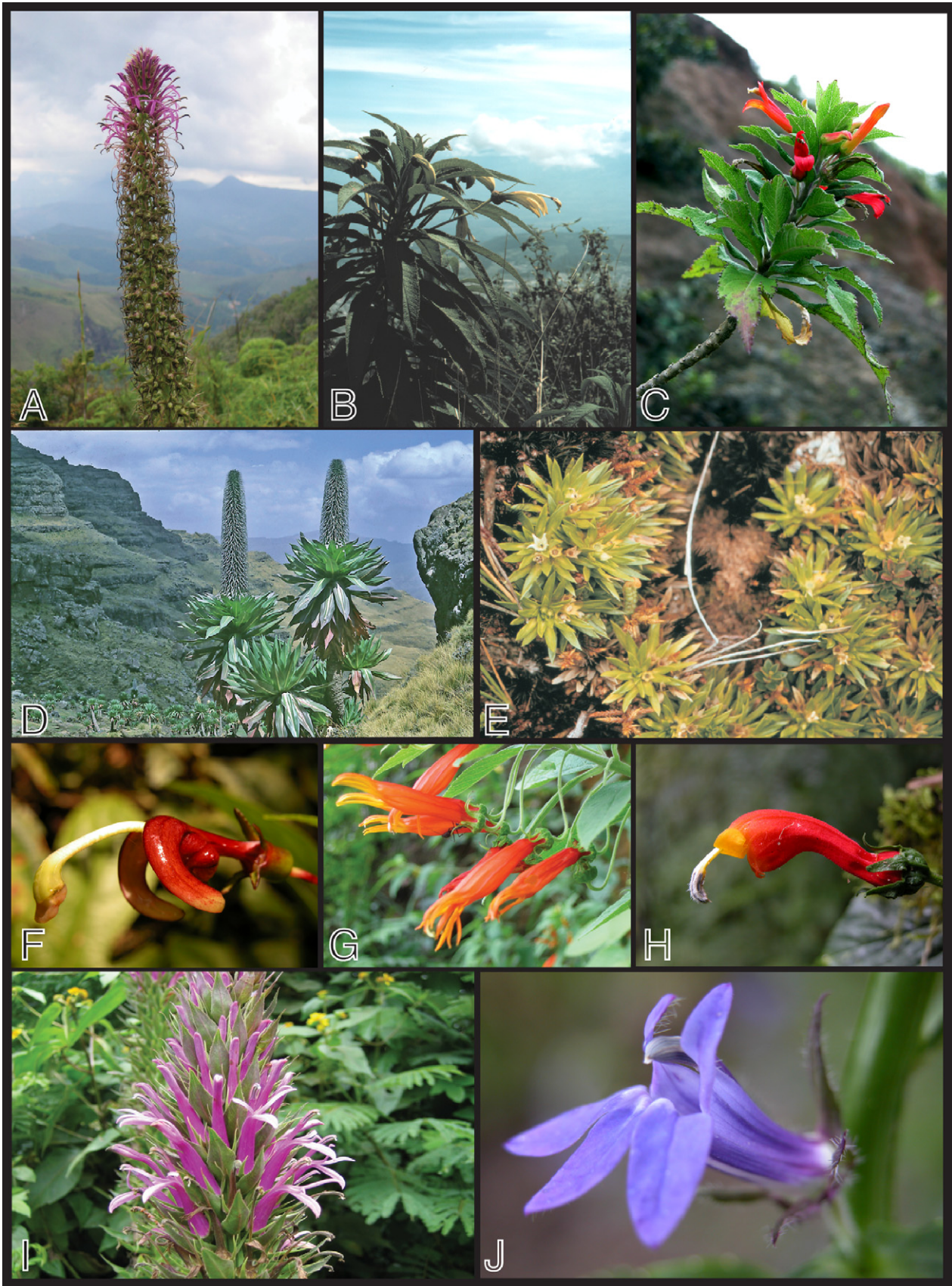


Fig. 1. Variation in floral morphology and habit within Campanulaceae subfam. Lobelioideae. (A) *Lobelia thapsoides*, (B) *Siphocampylus giganteus*, (C) *Lobelia laxiflora*, (D) *Lobelia rhynchopetalum*, (E) *Lysipomia* sp., (F) *Burmeistera cyclostigmata*, (G) *Siphocampylus macropodus*, (H) *Centropogon granulosus*, (I) *Lobelia fistulosa*, (J) *Lobelia siphylitica*. Photos by Alexandre Antonelli (A, C, F, G, H, I, and J), Lennart Andersson (B and E), and Christian Puff (D).

Table 1

Wimmer's (1953, 1968) classification of Campanulaceae subfam. Lobelioideae

1. Tribe Delisseae	{Fruits indehiscent}
1.1. Subtribe Cyaneinae	{Endemic to the Hawaiian islands}
1.1.1. Genera <i>Delissea</i> , <i>Cyanea</i> , <i>Clermontia</i> , (<i>Rollandia</i>)	
1.2. Subtribe Burmeisterinae	{Not endemic to the Hawaiian islands}
1.2.1. Genera <i>Hypsela</i> , <i>Burmeistera</i> , <i>Centropogon</i> , (<i>Cyrtandroidea</i>), (<i>Pratia</i>)	
2. Tribe Lobeliaeae	{Fruits dehiscent}
2.1. Subtribe Siphocampylinae	{Gynoecium bilocular, ovary inferior}
2.1.1. Ramus Eusiphon	{Corolla tube entire}
2.1.1.1. Genera <i>Siphocampylus</i> , <i>Diastatea</i> , (<i>Laurentia</i>)	
2.1.2. Ramus Schizosiphon	{Corolla tube split dorsally}
2.1.2.1. Genera <i>Lobelia</i> , <i>Grammatotheca</i> , <i>Monopsis</i> , (<i>Heterotoma</i>)	
2.1.3. Ramus Cherosiphon	{Without a conspicuous corolla tube}
2.1.3.1. Genus <i>Dialypetalum</i>	
2.2. Subtribe Phyllocharinae	{Gynoecium bilocular, ovary not inferior}
2.2.1. Genus (<i>Phyllocharis</i>)	
2.3. Subtribe Legenerinae	{Gynoecium unilocular, capsule dehiscent apically}
2.3.1. Genus <i>Legenera</i>	
2.4. Subtribe Unigeninae	{Gynoecium unilocular, capsule dehiscent from apex to base, one-seeded}
2.4.1. Genus <i>Unigenes</i>	
2.5. Subtribe Apetahiinae	{Gynoecium unilocular, capsule dehiscent from apex to base, many-seeded}
2.5.1. Genus <i>Apetahia</i>	
2.6. Subtribe Brighamiiinae	{Fruit membranous or papery, opening by longitudinal splits; thick, efoliate stems}
2.6.1. Genus <i>Brighamia</i>	
2.7. Subtribe Howelliinae	{Fruit membranous or papery, opening by longitudinal splits; slender, foliate stems}
2.7.1. Ramus Calanthes	{Flowers solitary}
2.7.1.1. Genera <i>Howellia</i> , <i>Downingia</i>	
2.7.2. Ramus Phoebigena	{Flowers clustered}
2.7.2.1. Genus <i>Dielsantha</i>	
2.8. Subtribe Lysipomiinae	{Fruit membranous or papery, furnished with a lid}
2.8.1. Genera <i>Lysipomia</i> , (<i>Dominella</i>)	
2.9. Subtribe Sclerothecinae	{Fruit thick-walled}
2.9.1. Genera <i>Sclerotheca</i> , <i>Trematolobelia</i>	

Curled brackets indicate the main diagnostic features for a particular group. Generic names within common brackets are no longer recognized. Lower categories have been omitted.

noteworthy that the six largest genera contain almost 80% of the species, and that eight genera are monotypic.

In recent years, virtually every systematic study dealing with members of the Lobelioideae has shown conflicts with Wimmer's (1953, 1968) original classification. Lammers (1990a, 1993) and Lammers and Hensold (1992) listed the chromosome numbers of 153 species in 21 genera, showing that presumably natural groups can be distinguished by a combination of chromosome number, ecological preference, and habit. Although these groups were in conflict with Wimmer's, the patterns found did not suffice to present a new classification. Murata (1992, 1995) went a step further and, based on a comparison of the seed coat morphology of 61 species, proposed several new recombinations and infrageneric circumscriptions (mainly in the genus *Lobelia* L. and the formerly recognized genus *Pratia* Gaudich). Knox et al. (1993) assessed the phylogenetic relationships among 18 species (of which 16 species were *Lobelia*) by mapping restriction sites and structural rearrangements in the chloroplast genome. Their results provided new interpretations of some morphological traits, but the small sample was not enough to give a clear picture

of the morphological evolution of the subfamily as a whole. In a similar study using restriction site variation among Hawaiian taxa, Givnish et al. (1995) showed that Wimmer's division of *Cyanea* Gaudich. into three sections is unnatural. Later, Pepper et al. (1997) were the first to use molecular sequence data to investigate the relationships of lobelioid taxa. Although based on a very limited taxon sampling, their preliminary results indicated that neither *Siphocampylus* Pohl nor *Centropogon* C. Presl are monophyletic. Also using DNA sequence data, Schultheis (2001) concluded that although the genus *Downingia* Torr. is monophyletic, its infrageneric groups are not. Finally, Batterman and Lammers' (2004) morphological study showed that branched foliar trichomes, a character used by Wimmer to distinguish infrageneric taxa in *Centropogon*, have evolved several times in the genus (corroborating the works by McVaugh, 1949, and Lammers, 1998a). Table 3 summarizes the various classifications of the species treated in this work.

Facing the inconsistency of the current classification and the lack of an inclusive molecular study of the group, the main goals of this work are to: (i) reconstruct a higher level

Table 2
Genera currently recognized in Campanulaceae subfam. Lobelioideae

	Genus	No. of species	Distribution
1	<i>Lobelia</i> L.	400+	Cosmopolitan
2	<i>Siphocampylus</i> Pohl	230+	Central, South America, Greater Antilles
3	<i>Centropogon</i> C. Presl	220+	Central, South America, Lesser Antilles
4	<i>Burmeistera</i> Triana	100+	Central America, Andean South America
5	<i>Cyanea</i> Gaudich.	77	Hawaiian Islands
6	<i>Lysipomia</i> Kunth	30	Andean South America
7	<i>Clermontia</i> Gaudich.	22	Hawaiian Islands
8	<i>Monopsis</i> Salisb.	15	Africa
9	<i>Isotoma</i> (R. Br.) Lindl.	14	Australia, New Zealand
10	<i>Downingia</i> Torr.	13	Western North America
11	<i>Delissea</i> Gaudich.	10	Hawaiian Islands
12	<i>Wimmerella</i> L. Serra M. B. Crespo & Lammers	10	South Africa
13	<i>Sclerotheca</i> A. DC.	6	Polynesia
14	<i>Solenopsis</i> C. Presl	6	Mediterranean region
15	<i>Dialypetalum</i> Benth.	5	Madagascar
16	<i>Diastatea</i> Scheidw.	5	Central, South America
17	<i>Apetahia</i> Baill.	4	Polynesia
18	<i>Ruthiella</i> Steenis	4	New Guinea
19	<i>Trematolobelia</i> Zahlbr.	4	Hawaiian Islands
20	<i>Brighamia</i> A. Gray	2	Hawaiian Islands
21	<i>Dielsantha</i> E. Wimm.	1	West Africa
22	<i>Grammatotheca</i> C. Presl	1	South Africa, Australia
23	<i>Hippobroma</i> G. Don	1	Pantropical
24	<i>Howellia</i> A. Gray	1	Western North America
25	<i>Legenere</i> McVaugh	1	Western North America, Southern South America
26	<i>Palmerella</i> A. Gray	1	Western North America
27	<i>Porterella</i> Torr.	1	Western North America
28	<i>Unigenes</i> E. Wimm.	1	South Africa

The number of genera, species, and distributions follow Lammers (2007), except for the exclusion here of the monotypic genus *Heterotoma* Zucc., recently included in *Lobelia* by Koopman and Ayers (2005).

phylogeny for the subfamily Lobelioideae, identifying the major clades in the group; (ii) evaluate the taxonomic implications of the phylogeny obtained by comparing the results with earlier works; and (iii) trace the evolution of some taxonomically important morphological features in order to assess their value in revealing phylogenetic affinities.

2. Materials and methods

2.1. Taxon sampling

Helianthus L. in Asteraceae was chosen as an outgroup since it has been shown (Bremer et al., 2002, 2004) not to belong to the Campanulaceae, but is still not too distantly related to it. The ingroup taxa were chosen in order to include representatives for as many taxonomic groups as possible within the subfamily Lobelioideae. Type species for genera and other subdivisions were included whenever material was available. The remaining taxa were chosen largely depending on availability in GenBank. In addition, six taxa from the subfamily Campanuloideae and one from Rousseeae A. DC. were added to test the monophyly of Lobelioideae. In total 76 species were used. Table 4 provides a list of species, their origin, and GenBank accession numbers.

2.2. Choice of markers

In order to obtain phylogenetic resolution at different levels of the ingroup, rather conservative markers were needed together with more fast-evolving regions. After some pilot studies, a combination of markers was selected which comprised the *trnL*-F region, including the *trnL* intron and the *trnL*-*trnF* intergenic spacer, and the *rbcL* and *ndhF* genes, all plastid DNA. The *rbcL* gene is one of the most frequently used segments of DNA in plant systematics for inferring phylogenies at the family level and above (Soltis and Soltis, 1998). It has proved in earlier studies to offer information for resolving the major relationships within the order Campanulales and the asterids (e.g., Cosner et al., 1994; Gustafsson et al., 1996). However, because its low evolutionary rate typically limits its applicability down to the generic level, there is a substantial advantage in combining it with other plastid sequences (Soltis and Soltis, 1998; Chase and Albert, 1998). The *trnL*-F intergenic spacer has been shown in many previous studies to provide sufficient information for supported resolution within tribes and genera of other plant families (e.g., Andersson and Antonelli, 2005; Rova et al., 2002). The *ndhF* gene is 1.5 times longer than *rbcL* and may contain three times more phylogenetic information (Kim and Jansen, 1995). It has been used at different taxonomic

Table 3
Classifications of the ingroup taxa

Clade	Current name	Native occurrence	Wimmer (1953, 1968)									Other authors				
			Tribe	Subtribe	Ramus	Genus	Subgenus	Section	Subsection	Grex	Subgrex	Series	Subgenus	Section		
C 1	<i>Lobelia physaloides</i>	New Zealand	Delisseeae	Burmeisterinae		<i>Pratia</i>			Colensoa						Tupa (h)	Colensoa (h)
C 1	<i>Lobelia coronopifolia</i>	South Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Holopogon	Delostemon	Genistoides						
C 1	<i>Lobelia tomentosa</i>	South Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Holopogon	Delostemon	Genistoides						
C 2	<i>Grammatotheca bergiana</i>	South Africa, Australia	Lobelieae	Siphocampylinae	Schizosiphon											
C 2	<i>Lobelia aquatica</i>	Antilles, South America	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Holopogon	Delostemon	Gratioloides					Lobelia (h)	Delostemon (h)
—	<i>Lobelia sonderiana</i> (a)	South Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Mezleria	Eumezleria							Mezleria (h)	Pratia (h)
C 3	<i>Lobelia graniticola</i>	Tropical Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Leiospermae	Eriniformes	Subgrandiflores					
C 3	<i>Lobelia erinus</i>	South Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Leiospermae	Eriniformes	Latifoliae				Lobelia (h)	Heyneana (h)
—	<i>Lobelia anceps</i>	Tropical Africa, Southeast Brazil	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Leiospermae	Pterocaulinae						
C 4	<i>Lobelia leschenaultiana</i>	Southeast India, Sri Lanka	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Haynaldianae	Imberbes					Tupa (h)	Colensoa (h)
C 4	<i>Lobelia gibberoa</i>	East Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Rhynchopetalum	Cereiformes					Gibberoa		
C 4	<i>Lobelia gregoriana</i> (b)	Tropical Africa	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Rhynchopetalum	Cereiformes					Deckenii		
C 4	<i>Trematolobelia macrostachys</i>	Hawaii	Lobelieae	Sclerothecinae	Multiporosae	<i>Trematolobelia</i>										
C 4	<i>Lobelia yuccoides</i>	Hawaii	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Revolutella	Yuccoides							
C 4	<i>Lobelia hypoleuca</i>	Hawaii	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Revolutella	Yuccoides							
C 4	<i>Lobelia stricklandiae</i>	Zambia	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Rhynchopetalum	Ramosae					Ramosae		
C 4	<i>Lobelia exaltata</i>	East Brazil	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Haynaldianae	Barbiferae	Longipedicellatae				Tupa (h)	Colensoa (h)
C 4	<i>Lobelia fistulosa</i>	East Brazil	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Haynaldianae	Barbiferae	Longipedicellatae				Tupa (h)	Colensoa (h)
C 4	<i>Brighamia insignis</i>	Hawaii	Lobelieae	Brighamiinae		<i>Brighamia</i>										
C 4	<i>Delissea undulata</i>	Hawaii	Delisseeae	Cyaneinae		<i>Delissea</i>		Micranthae								
C 4	<i>Cyanea angustifolia</i>	Hawaii	Delisseeae	Cyaneinae		<i>Cyanea</i>		Delissoideae								
C 4	<i>Clermontia kakeana</i>	Hawaii	Delisseeae	Cyaneinae		<i>Clermontia</i>		Genuinae								
C 4	<i>Cyanea koolauensis</i>	Hawaii	Delisseeae	Cyaneinae		<i>Rollandia</i>										
C 5	<i>Diastatea micrantha</i>	Mexico to Bolivia	Lobelieae	Siphocampylinae	Eusiphon	<i>Diastatea</i>										
C 5	<i>Solenopsis laurentia</i>	North Africa, Mediterranean	Lobelieae	Siphocampylinae	Eusiphon	<i>Laurentia</i>		Solenopsis								
C 5	<i>Downingia insignis</i>	Southwest USA	Lobelieae	Howelliinae	Calanthes	<i>Downingia</i>										
C 5	<i>Downingia bacigalupii</i>	North America	Lobelieae	Howelliinae	Calanthes	<i>Downingia</i>										
C 5	<i>Lobelia kalmii</i>	North America	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Trachyspermae	Coelestes	Kalmia				Lobelia (h)	Lobelia (h)
C 5	<i>Lobelia rotundifolia</i>	Antilles	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species antillanae						
C 5	<i>Lobelia cardinalis</i>	North and Central America	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Trachyspermae	Cardinales					Lobelia (h)	Lobelia (h)
C 5	<i>Lobelia dortmanna</i>	North America and Europe	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Trachyspermae	Coelestes	Dortmannia				Lobelia (h)	Lobelia (h)
C 5	<i>Lobelia cordifolia</i>	Mexico	Lobelieae	Siphocampylinae	Schizosiphon	<i>Heterotoma</i>		Microcentron							Lagotis (i)	Hemipogon (i)
C 5	<i>Lobelia xalapensis</i>	Mexico to Peru, Antilles	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Hemipogon	Leiospermae	Eriniformes	Latifoliae					
C 5	<i>Lobelia irasuenis</i>	Central America	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Lagotis	Holopogon	Cryptostemon							
C 5	<i>Lobelia aguana</i>	Central America	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Homochilus							Tupa (h)	Homochilus (h)
C 5	<i>Lobelia laxiflora</i>	Mexico to Colombia	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Homochilus							Tupa (h)	Homochilus (h)
C 5	<i>Lobelia martagon</i>	Jamaica	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species antillanae (g)					Tupa (j)	Tylomium (j)
C 5	<i>Hippobroma longiflora</i>	Jamaica	Lobelieae	Siphocampylinae	Eusiphon	<i>Laurentia</i>		Isotoma								
C 5	<i>Lobelia kraussii</i> (c)	Dominica and Martinique	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species antillanae (g)					Tupa (j)	Tylomium (j)
C 5	<i>Lobelia stricta</i> (d)	Lesser Antilles	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species antillanae (g)					Tupa (j)	Tylomium (j)
C 5	<i>Lobelia portoricensis</i>	Puerto Rico	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species antillanae (g)					Tupa (j)	Tylomium (j)
C 6	<i>Lobelia polyphylla</i>	Chile	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species chilenses (g)					Tupa (h)	Tupa (h)
C 6	<i>Lobelia tupa</i>	Chile	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species chilenses (g)					Tupa (h)	Tupa (h)
C 6	<i>Lobelia excelsa</i>	Chile	Lobelieae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Eutupa	Primanae	Species chilenses (g)					Tupa (h)	Tupa (h)

(continued on next page)

Table 3 (continued)

Clade	Current name	Native occurrence	Wimmer (1953, 1968)									Other authors		
			Tribe	Subtribe	Ramus	Genus	Subgenus	Section	Subsection	Grex	Subgrex	Series	Subgenus	Section
C 7	<i>Lobelia oligophylla</i> (e)	From Ecuador to Chile	Delisseeae	Burmeisterinae		<i>Hypsela</i>								
C 7	<i>Lobelia nummularia</i>	Southeast Asia	Delisseeae	Burmeisterinae		<i>Pratia</i>		Eupratia					Mezleria (h)	Pratia (h)
C 7	<i>Lobelia roughii</i>	New Zealand	Lobeliae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Mezleria	Paramezleria					Mezleria (h)	Paramezleria (h)
C 7	<i>Lobelia purpurascens</i>	Australia	Delisseeae	Burmeisterinae		<i>Pratia</i>		Eupratia					Mezleria (h)	Dioica (h)
C 7	<i>Isotoma fluviatilis</i>	Australia	Lobeliae	Siphocampylinae	Eusiphon	<i>Laurentia</i>								
C 7	<i>Lobelia chinensis</i>	East Asia	Lobeliae	Siphocampylinae	Schizosiphon	<i>Lobelia</i>	Tupa	Isolobus					Mezleria (h)	Isolobus (h)
C 7	<i>Lobelia arenaria</i> (f)	Auckland islands	Delisseeae	Burmeisterinae		<i>Pratia</i>		Eupratia					Mezleria (h)	Pratia (h)
C 7	<i>Lobelia angulata</i>	New Zealand	Delisseeae	Burmeisterinae		<i>Pratia</i>		Eupratia					Mezleria (h)	Pratia (h)
C 8	<i>Lysipomia sphagnophila</i>	Ecuador, Peru	Lobeliae	Lysipomiinae		<i>Lysipomia</i>	Eulysipomia							
C 8	<i>Lysipomia cuspidata</i>	Ecuador	Lobeliae	Lysipomiinae		<i>Lysipomia</i>								
C 8	<i>Siphocampylus fulgens</i>	Southeast Brazil	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Macrosiphon	Eusiphocampylus	Dissitiflori	Pyriformes			
C 8	<i>Siphocampylus affinis</i>	Ecuador	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Macrosiphon	Byrsanthes					
C 8	<i>Siphocampylus brevicalyx</i>	Ecuador	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Macrosiphon	Eusiphocampylus	Dissitiflori	Elegantes			
C 8	<i>Siphocampylus scandens</i>	Ecuador	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Macrosiphon	Eusiphocampylus	Botryoides	Bracteato-racemosi			
C 8	<i>Siphocampylus macropodus</i>	Southeast Brazil	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Macrosiphon	Eusiphocampylus	Dissitiflori	Sphaeroides			
C 8	<i>Centropogon cornutus</i>	Central America to Bolivia	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Eucentropogon	Axillares				Centropogon (k)	Centropogon (k)
C 8	<i>Centropogon granulatus</i>	Ecuador	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Eucentropogon	Corymboides	Campylobotrys			Centropogon (k)	Campylobotrys (k)
C 8	<i>Centropogon gamosepalus</i>	Ecuador, Peru	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Eucentropogon	Corymboides	Amplifolii			Centropogon (k)	Amplifolii (k)
C 8	<i>Centropogon dissectus</i>	Ecuador	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Siphocampyloides	Macranthi	Lehmannioides				
C 8	<i>Siphocampylus giganteus</i>	Colombia, Ecuador	Lobeliae	Siphocampylinae	Eusiphon	<i>Siphocampylus</i>		Brachysiphon	Megastomi					
C 8	<i>Centropogon trichodes</i>	Colombia	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Siphocampyloides	Brevilimbati	Formosi				
C 8	<i>Centropogon luteus</i>	Colombia, Ecuador, Peru	Delisseeae	Burmeisterinae		<i>Centropogon</i>		Centropogon	Siphocampyloides	Brevilimbati	Stellato-tomentosi			
C 8	<i>Burmeistera cyclostigmata</i>	Costa Rica	Delisseeae	Burmeisterinae		<i>Burmeistera</i>		Barbatae						
C 8	<i>Burmeistera domingensis</i>	Ecuador	Delisseeae	Burmeisterinae		<i>Burmeistera</i>								

The species are sorted by order of appearance in Fig. 2

Comments: (a) treated by Wimmer as a variety of *Lobelia depressa*; (b) treated by Wimmer as *L. keniensis*; (c) treated by Wimmer as *L. persicifolia*; (d) treated by Wimmer as *L. infesta*; (e) treated by Wimmer as *Hypsela reniformis*; (f) treated by Wimmer as a variety of *Pratia angulata*; (g) this group was only informally recognized. Authors: (h) Murata (1995); (i) Ayers (1990); (j) Lammers (2007); (k) Stein (1987). Classifications earlier than 1953 are not listed.

Table 4

List of sequences used in the phylogenetic analyses, together with data on origin and GenBank accession numbers

Taxon	Origin	Voucher	<i>rbcL</i>	<i>trnL-trnF</i>	<i>ndhF</i>
<i>Brighamia insignis</i> A. Gray	Cultivated	Antonelli 251 (GB)	AF042664*	DQ356189*	—
<i>Burmeistera cyclostigmata</i> Donn. Sm.	Ecuador	Andersson & Nilsson 2451 (GB)	DQ356147*	DQ356213*	DQ356097*
<i>Burmeistera domingensis</i> Jeppesen	Ecuador	Harling & Andersson 23154 (GB)	DQ356148*	DQ356214*	—
<i>Campanula asperuloides</i> (Boiss. & Orph.) Engl.	Greece	Antonelli 250 (GB)	DQ356117*	DQ356170*	DQ356090*
<i>Campanula latifolia</i> L.	Sweden	Antonelli 252 (GB)	EF141027*	DQ356169*	DQ356089*
<i>Campanula trachelium</i> L.	Sweden	Antonelli 253 (GB)	DQ356118*	DQ356171*	DQ356091*
<i>Canarina canariensis</i> (L.) Vatke	Gran Canaria	Andersson 2394 (GB)	DQ356115*	DQ356167*	DQ356087*
<i>Centropogon cornutus</i> (L.) Druce	Guyana	Jansen-Jacobs et al. 4955 (GB)	DQ356158*	DQ356226*	DQ356106*
<i>Centropogon dissectus</i> E. Wimm.	Ecuador	Luteyn et al. 5678 (GB)	EF141026*	DQ356215*	—
<i>Centropogon gamosepalus</i> Zahlbr.	Ecuador	Harling & Andersson 24342 (GB)	DQ356157*	DQ356225*	DQ356105*
<i>Centropogon granulosus</i> C. Presl. subsp. <i>Granulosus</i>	Ecuador	Andersson & Nilsson 2477 (GB)	DQ356152*	DQ356220*	DQ356101*
<i>Centropogon luteus</i> E. Wimm.	Ecuador	Andersson & Nilsson 2411 (GB)	DQ356151*	DQ356219*	DQ356100*
<i>Centropogon trichodes</i> E. Wimm.	Ecuador	Andersson & Nilsson 2546 (GB)	DQ356149*	DQ356217*	DQ356098*
<i>Clermontia kakeana</i> Meyen	Hawaii	Takeuchi Koolau 56a (GB)	L18789*	DQ356172*	DQ356092*
<i>Cyanea angustifolia</i> (Cham.) Hillebr.	Hawaii	Takeuchi et al. 1802 (GB)	DQ356119*	DQ356173*	—
<i>Cyanea koolauensis</i> Lammers, T. J. Givnish & K. J. Sytsma	Hawaii	Takeuchi & Pyle 2284 (GB)	DQ356128*	DQ356193*	—
<i>Delissea undulata</i> Gaudich subsp. <i>Undulate</i>	Hawaii	Skottsberg 691 (GB)	—	DQ356188*	—
<i>Diastatea micranta</i> (Kunth) McVaugh	Ecuador	Lejtnant & Molau 15184 (GB)	DQ356138*	DQ356203*	DQ356095*
<i>Downingia bacigalupii</i> Weiler	USA	Mason 14384 (S)	EF141031*	DQ356183*	—
<i>Downingia insignis</i> Greene	USA	Tiehm 12143 (S)	EF141030*	DQ356185*	—
<i>Grammatotheca bergiana</i> (Cham.) C. Presl.	South Africa	Bean & Viviers 2628 (GB)	DQ356116*	DQ356168*	DQ356088*
<i>Helianthus annuus</i> L.	—	—	AF097517*	AJ430967*	L39383*
<i>Hippobroma longiflora</i> (L.) G. Don	Ecuador	Andersson & Nilsson 2492 (GB)	DQ356140*	DQ356206*	DQ356096*
<i>Isotoma fluviatilis</i> (R.Br.) F. Muell. ex Benth. subsp. <i>fluviatilis</i>	Cultivated	Antonelli 238 (GB)	DQ356161*	DQ356230*	DQ356108*
<i>Jasione montana</i> L.	Sweden	Andersson 2562 (GB)	DQ356120*	DQ356174*	—
<i>Legousia hybrida</i> (L.) Delarbre	Sweden	Corneliusson s.n. (GB #152920)	DQ356163*	DQ356234*	DQ356111*
<i>Lobelia aguana</i> E. Wimm.	Mexico	Koch 82120 (NY)	DQ356122*	DQ356176*	—
<i>Lobelia anceps</i> L. f.	Chile	Skottsberg 4536 (GB)	DQ356124*	DQ356184*	—
<i>Lobelia angulata</i> G. Forst.	—	—	—	AY568754+AY568744	—
<i>Lobelia aquatica</i> Cham.	Guyana	Jansen-Jacobs et al. 4080 (GB)	EF141029*	DQ356182*	—
<i>Lobelia arenaria</i> Salzm. ex A. DC.	—	—	—	AY568756+AY568737	—
<i>Lobelia cardinalis</i> L.	USA	Vincent 4377 (GB)	AF042659*	DQ356231*	AF130187*
<i>Lobelia chinensis</i> Lour.	Japan	National Science Museum 409 (GB)	—	DQ356228*	—
<i>Lobelia cordifolia</i> Hook. & Arn.	Mexico	Mexia 8780 (S)	—	DQ356204*	—
<i>Lobelia coronopifolia</i> L.	South Africa	Dahlstrand 1084 (GB)	EF141025*	DQ356181*	—
<i>Lobelia dortmanna</i> L.	Sweden	Andersson 2561 (GB)	DQ356162*	DQ356232*	DQ356109*
<i>Lobelia erinus</i> L.	Cultivated	Andersson 2570 (GB)	L01931*	DQ356233*	DQ356110*
<i>Lobelia exaltata</i> Pohl	Brazil	Antonelli 335 (GB)	DQ356135*	DQ356200*	DQ356093*
<i>Lobelia excelsa</i> Leschen.	Chile	Lammers et al. 6357 (GB)	DQ356146*	DQ356212*	—
<i>Lobelia fistulosa</i> Vellozo	Brazil	Antonelli & Andersson 279 (GB)	DQ356136*	DQ356201*	DQ356094*
<i>Lobelia giberroa</i> Hemsl.	Kenya	Jaasund s.n. (GB 1.1.67)	DQ356127*	DQ356192*	—
<i>Lobelia graniticola</i> E. Wimm.	Tanzania	Thulin & Mhoro 3210 (C)	DQ356129*	DQ356194*	—
<i>Lobelia gregoriana</i> Baker f. subsp. <i>sattimae</i> (R. E. Fr. & T. C. E. Fr.) E. B. Knox	Kenya	Hedberg 1608 (S)	—	DQ356187*	—
<i>Lobelia hypoleuca</i> Hillebr.	Hawaii	Selling & Skottsberg 3194 (GB)	DQ356126*	DQ356191*	—

(continued on next page)

Table 4 (continued)

Taxon	Origin	Voucher	<i>rbcL</i>	<i>trnL–trnF</i>	<i>ndhF</i>
<i>Lobelia irasuensis</i> Planch. & Oerst. subsp. <i>irasuensis</i>	Costa Rica	Burger & Burger 8151 (GB)	DQ356121*	DQ356175*	—
<i>Lobelia kalmii</i> L.	USA	Boufford 7292 (GB)	DQ356166*	EF126736*	DQ356114*
<i>Lobelia kraussii</i> Graham	Dominica	Hill 25677 (NY)	EF141024*	DQ356179*	—
<i>Lobelia laxiflora</i> Kunth subsp. <i>laxiflora</i>	Costa Rica	Santamaria S-985 (GB)	DQ356143*	DQ356209*	—
<i>Lobelia leschenaultiana</i> (C. Presl) Skottsberg	Ceylon	Skottsberg s.n. (GB 11.XII.1926)	DQ356131*	DQ356196*	—
<i>Lobelia martagon</i> (Griseb.) A. S. Hitchc.	Jamaica	Judd 5375 (NY)	DQ356139*	DQ356205*	—
<i>Lobelia nummularia</i> Lam.	Cultivated	Andersson 2571 (GB)	DQ356164*	DQ356235*	DQ356112*
<i>Lobelia oligophylla</i> (Wedd.) Lammers	Ecuador	Harling et al. 6807 (GB)	DQ356159*	DQ356227*	—
<i>Lobelia physaloides</i> A. Cunn.	—	—	—	AY568757+AY568745	—
<i>Lobelia polyphylla</i> Hook. & Arn	Chile	Lammers et al. 6331 (GB)	DQ356123*	DQ356177*	—
<i>Lobelia portoricensis</i> (Vatke) Urb.	Puerto Rico	Boom 10069 (NY)	DQ356142*	DQ356208*	—
<i>Lobelia purpurascens</i> R. Br.	Australia	Egeröd s.n. (GB#0176064)	DQ356160*	DQ356229*	DQ356107*
<i>Lobelia rotundifolia</i> Juss. ex A. DC.	Dominican Republic	Ståhl & Lindström 120 (GB)	—	DQ356178*	—
<i>Lobelia roughii</i> Hook. f.	New Zealand	Skottsberg s.n. (GB#8694)	DQ356165*	EF126737*	DQ356113*
<i>Lobelia sonderiana</i> (Kuntze) Lammers	Namibia	Volk 1095 (S)	DQ356130*	DQ356195*	—
<i>Lobelia stricklandiae</i> Gilliland	South Rhodesia	Fisher & Schweicherdt 291 (S)	—	DQ356186*	—
<i>Lobelia stricta</i> Sw.	Dominica	Wilbur et al. 7849 (NY)	DQ356141*	DQ356207*	—
<i>Lobelia tomentosa</i> L. f.	South Africa	Dahlstrand 2600 (GB)	EF141028*	DQ356180*	—
<i>Lobelia tupa</i> L.	Chile	Lammers & Rodriguez 6329 (GB)	DQ356145*	DQ356211*	—
<i>Lobelia xalapensis</i> H. B. & K.	Costa Rica	Santamaria S-1009 (GB)	DQ356144*	DQ356210*	—
<i>Lobelia yuccoides</i> Hillebr.	Hawaii	Cranwell et al. 2909 (GB)	DQ356125*	DQ356190*	—
<i>Lysipomia cuspidata</i> McVaugh	Ecuador	Andersson & Nilsson 2559 (GB)	DQ356133*	DQ356198*	—
<i>Lysipomia sphagnophila</i> Griseb. ex Wedd. subsp. <i>angelensis</i> Jeppesen	Ecuador	Andersson & Nilsson 2444 (GB)	DQ356132*	DQ356197*	—
<i>Roussea simplex</i> Sm.	—	—	AF084477*	AJ430977*	AJ277384*
<i>Siphocampylus affinis</i> (Mirb.) McVaugh	Ecuador	Løjtnant & Molau 11426 (GB)	DQ356155*	DQ356223*	DQ356104*
<i>Siphocampylus brevicalyx</i> E. Wimm.	Ecuador	Løjtnant et al 11826 (GB)	DQ356156*	DQ356224*	—
<i>Siphocampylus fulgens</i> Dombroin	Brazil	Hatschbach 44995 (GB)	EF141032*	DQ356216*	—
<i>Siphocampylus giganteus</i> (Cav) G. Don	Ecuador	Andersson & Nilsson 2432 (GB)	DQ356154*	DQ356222*	DQ356103*
<i>Siphocampylus macropodus</i> (Thunb.) G. Don	Brazil	Antonelli 334 (GB)	DQ356153*	DQ356221*	DQ356102*
<i>Siphocampylus scandens</i> (Kunth) G. Don	Ecuador	Andersson & Nilsson 2515 (GB)	DQ356150*	DQ356218*	DQ356099*
<i>Solenopsis laurentia</i> (L.) C. Presl	Spain	Dept.Syst.Bot. 381 (GB)	DQ356134*	DQ356199*	—
<i>Trematolobelia macrostachys</i> (Hook. & Arn.) Zahlbr. ex Rock	Hawaii	Fagerlind 6872 (S)	DQ356137*	DQ356202*	—

(*): Sequences produced in this study.

levels, ranging from the intrageneric level (Källersjö and Ståhl, 2003) to studies of major lineages of asterids (Albach et al., 2001; Olmstead et al., 2000).

2.3. DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from leaf tissue dehydrated and stored in silica gel or taken from herbarium material. Extractions were made primarily using the method described by Andersson and Rova (1999), but some were made with the DNeasy® Plant Mini Kit (QIAGEN®), according to the manufacturer's instructions.

Amplification and sequencing were performed using the primers listed in Table 5. In some cases PCR was done using PuReTaq™ Ready-To-Go™ PCR beads (Amersham Biosciences) for 25 µl reactions, using 25–100 ng of tem-

plate DNA, and 20 pmol of each primer. In other cases, the MasterAmp™ PCR Optimization Kit (Epicentre® Biotechnologies) was employed, using 25 µl of MasterAmp™ 2× PCR PreMix G, 30 pmol of each primer, 1 U Thermo-prime Plus DNA Polymerase (ABgene™), 25–100 ng of template DNA, and water to fill a reaction volume of 50 µl. PCR products were visualized by electrophoresis on a 1% agarose gel (Standard Saveen Werner AB) and purified using QIAquick® PCR Purification Spin Columns (QIAGEN®). The PCR product was then quantified using GeneQuant II (Pharmacia Biotech). Sequencing was performed on a CEQ™ 8000 (Beckman Coulter®) automated sequencer. Reactions were done with the GenomeLab™ DTCS-Quick Start Kit (Beckman Coulter) according to manufacturer's instructions, except that 10 µl reactions were used, with ca. 75 fmol template and 1.6 pmol primer.

Table 5
List of primers used

Marker	Use (Amplification/Sequencing)
<i>rbcL</i> ^a	A: <i>rbcL</i> 26f/t, <i>rbcL</i> 26f/1312r S: <i>rbcL</i> 26f, <i>rbcL</i> 358f, <i>rbcL</i> 361r, <i>rbcL</i> 667r, <i>rbcL</i> 799f, <i>rbcL</i> 1010r, <i>rbcL</i> 1117f
<i>trnL</i> -F ^{a,b}	A: <i>trnc</i> 2/f2 S: <i>trnc</i> , <i>trnd</i> , <i>trne</i> , <i>trnf</i> 2
<i>ndhF</i> ^c	A: <i>ndhF</i> 1f/2110r, <i>ndhF</i> 917f/2110r, <i>ndhF</i> 1f/1600r, <i>ndhF</i> 1320f/2110r S: <i>ndhF</i> 917f, <i>ndhF</i> 1320f, <i>ndhF</i> 1318r, <i>ndhF</i> 1600r, <i>ndhF</i> 2110r
New primers described here:	
<i>rbcL</i> 1312r (5'–3')	GCA CCC GGC GCA TTA CCC CAA GGR T
<i>trnc</i> 2 (5'–3')	CTA CGG ACT TAA TTG GAT TGA GC

^a Andersson and Antonelli (2005).

^b Taberlet et al. (1991).

^c Olmstead and Sweere (1994).

Editing and compilation of sequences was done using Sequencher™ version 4.1 (Gene Codes Corporation).

2.4. Alignment and gap coding

First, sequences from all three loci were concatenated and sorted after similarity by ClustalX (Thompson et al., 1997). All gaps were then removed and the sequences completely realigned using MAFFT v. 5.64 (Katoh et al., 2005). Gaps were finally coded in accordance with the principles specified by Andersson and Chase (2001), with two modifications: (i) gap-codes were always coded as binary, as required by the phylogenetic analyses described below; and (ii) deletions involving one to two nucleotides were coded as absent, and their homologous nucleotides as present, even when over 50% of these nucleotides varied. However, the latter was only coded provided that these gaps were presumed to be homologous, which was arbitrarily established when >80% of the 10 adjacent positions on both sides of the gap did not vary within the ingroup taxa.

2.5. Phylogenetic analyses

The Partition Homogeneity Test (Farris et al., 1995) as implemented in PAUP* version 4.0b10 (Swofford, 1999) was applied to test the congruency of results produced independently from the three markers including gap codings. For this purpose, a heuristic search was performed with 3000 replicates, 10 random addition sequences, TBR branch swapping, and saving up to 50 trees per replicate. In order to test the congruency of results from different methods of phylogenetic inference, trees were reconstructed using both maximum parsimony as implemented in TNT (Goloboff et al., 2000) and PAUP*, and Bayesian inference of phylogeny as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). First, a parsimony search was performed in TNT using the New Technology method, running 10,000 replicates of random addition sequence, and using the Ratchet and Drift search algo-

rithms. A jackknife analysis using heuristic searches was then performed in PAUP*, running 5000 replicates with 37% deletion, each with 100 random addition sequence replicates, TBR branch swapping, and saving up to 50 trees per replicate. MrModelTest 2.2 (Nylander, 2004) was used to find the evolutionary model that best explained each of the three regions analyzed. Following the recommendations of recent works (Pol, 2004; Posada and Buckley, 2004), the evolutionary models chosen by the Akaike Information Criterion were then incorporated into a MrBayes block in the input file. Gap codings were analyzed as a separate partition under the Restriction Site (Binary) Model. The program performed two simultaneous runs until the average standard deviation of split frequencies became lower than 0.01. For each run, eight Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains were initiated, sampling every 1000 generations, saving branch lengths, and using the other default settings. The software Tracer (Rambaut and Drummond, 2003) was used to determine when the tree sampling stabilized.

2.6. Tests of monophyly

In order to further test the monophyly of the taxa which were not supported as monophyletic, alternative evolutionary hypotheses were investigated using the SH test (Shimodaira and Hasegawa, 1999). This test provides an appropriate (Goldman et al., 2000) probabilistic model for assessing whether competing trees are significantly less likely than a departing tree. For each of the genera and subgenera not supported as monophyletic in the Bayesian tree obtained without topological constraints, a Bayesian search was conducted enforcing the taxon in question to be monophyletic. Two parallel runs were performed, each comprising six MCMCMC chains and producing one million generations. The most likely (ML), fully dichotomous tree from each search was then computed into a tree block and the SH test performed as implemented in PAUP*. Likelihood settings were set in accordance to the results obtained from MrModelTest 2.2. The test performed 5000 bootstrap replicates using the REL algorithm and excluding gap codings.

2.7. Tracing of morphological characters

In order to analyze the evolution of morphological characters in Campanulaceae, three features were traced on the most likely Bayesian tree obtained from molecular data: (i) fruit: coded as capsule (0) or berry (1); (ii) corolla tube: coded as entire (0), totally cleft dorsally (1) or only partially cleft (2) (cleft more than 1/3 of the total length of the corolla tube, but not reaching its base); and (iii) habit, coded as small herb (0), shrub/subshrub (1) or giant herb/treetlet (2). PAUP* was used to optimize these characters on the tree and calculate their Consistency and Retention Indices (CI and RI, respectively), using both the ACC-TRAN and DELTRAN assumptions. Since currently

available reports on chromosome numbers and seed types account for only a minority of the species analyzed here, only character plotting (without optimizations) was performed.

3. Results

The aligned matrix comprised a total of 3823 characters, of which 1402 derived from the *rbcL* gene, 1167 from the *trnL-F* region, 1231 from the *ndhF* gene, and 23 were gap codings. Out of the 1454 variable characters, 888 were parsimony informative, attributed in decreasing order of importance to *trnL-F* (41.7% of all information), *ndhF* (29.8%), *rbcL* (25.9%), and gap codings (2.59%). In addition to the three taxa for which no plant material was available (only GenBank sequences; Table 4), six *rbcL* sequences and forty-two *ndhF* sequences could not be obtained due to amplification or sequencing problems. The Partition Homogeneity Test did not approach significance for the rejection of congruency among the data partitions ($p = 0.86$; see Dolphin et al., 2000; Darlu and Lecointre, 2002, and Hipp et al., 2004 for discussions of the test). The Akaike Information Criterion implemented in MrModelTest chose the GTR + I + G evolutionary model for all three markers. The Bayesian analysis was stopped after 3 million generations, i.e. after the two parallel runs had converged. The burn-in value was set to 300 samplings (reflecting 300,000 generations), long after the analysis had stabilized.

The analysis in TNT found 138 most parsimonious trees (tree length = 3127, CI = 0.65, RI = 0.73). There was no conflict between the trees yielded by the Bayesian and the maximum parsimony analyses, in the sense that there were no clades that were strongly supported in one tree (or present in the strict consensus tree of the TNT analysis) but contradicted in the other. Fig. 2 shows the majority-rule consensus tree estimated using Bayesian inference. In cases when a clade was also present in the majority-rule consensus tree of the jackknife analysis in PAUP*, the jackknife support value is shown together with the posterior probability of the clade. The Bayesian, jackknife, and strict consensus trees, together with the data set used to generate them, can be downloaded from <http://www.treebase.org>, Study No. S1868, Matrix No. M3435. In the following, support will be referred to as strong for a posterior probability ≥ 0.91 or a jackknife support $\geq 88\%$. These values have been shown to represent minimal values required for a 95% confidence interval of a node under certain circumstances (Zander, 2004). Other arbitrarily defined intervals of jackknife support values will be referred to as moderate (76–87% jackknife support, 0.85–0.90 Bayesian posterior probability), weak (63–75% jackknife support, 0.75–0.84 Bayesian posterior probability), and ambiguous (<63% jackknife support, <0.75 Bayesian posterior probability).

The Bayesian consensus tree (Fig. 2) is composed of two major clades, with Campanuloideae as sister to Lobelioideae, both strongly supported as monophyletic. The basal

portion of Lobelioideae is a ladder composed mainly of *Lobelia* species, some aggregated into distinct clades (Clades 1–3 in Fig. 2). The remaining species are nested within five strongly supported clades (Clades 4–8).

Table 6 shows the results from the SH test, listing the length of each ML Bayesian tree inferred under enforcement of a taxon constraint, its likelihood and p -values. Since this is a one-tailed test, p -values ≥ 0.05 indicate significance using a 95% confidence interval. While the ML Bayesian trees in which *Lobelia* and its subgenera are monophyletic are all significantly less likely than the ML tree inferred without constraints, the test shows that the monophyly of *Centropogon*, *Siphocampylus*, and *Cyanea* cannot be rejected.

The character optimizations using the ACCTRAN and DELTRAN assumptions yielded exactly the same results. Fig. 3 shows the most parsimonious optimization for the evolution of fruit type (11 steps, CI = 0.09, RI = 0.44), corolla tube (14 steps, CI = 0.14, RI = 0.67), and habit (7 steps, CI = 0.29, RI = 0.86). The figure also shows all known reports on chromosome numbers (Lammers, 1993; Ruas et al., 2001; Stace and James, 1996; Index to Plant Chromosome Numbers, <http://www.mobot.org>) and type of seed coat (Murata, 1992, 1995; Buss et al., 2001). Table 7 gives a short description of the seed types plotted on the tree.

4. Discussion

4.1. Phylogenetic analyses (Fig. 2)

4.1.1. Clade 1

This is a strongly supported group that is sister to the rest of the Lobelioideae, composed of *L. physaloides*, *L. coronopifolia*, and *L. tomentosa*. This result agrees with Wimmer's (1953) placement of *L. tomentosa* and *L. coronopifolia* within grex *Genistoides* E. Wimm. However, *L. physaloides* also belongs here, although it was placed by Wimmer (1953) in a totally different genus, viz. *Pratia*. The position of *L. physaloides* also indicates that section *Colensoa* (Hook f.) J. Murata, as circumscribed by Murata (1995) is paraphyletic, since all other members of this section included in this study (*L. exaltata*, *L. fistulosa*, and *L. leschenaultiana*) are nested in Clade 4. The species in Clade 1 are distinguished by having clearly pedicellate flowers, but this feature may be plesiomorphic rather than synapomorphic (Lammers, 1993).

4.1.2. Clade 2

This clade is composed of *Grammatotheca bergiana* and *L. aquatica*. There is no evidence supporting Wimmer's (1953) placement of these species in separate genera. Moreover, the presence of *L. aquatica* in this clade indicates that *Lobelia* section *Holopogon* Benth. is paraphyletic as circumscribed by Wimmer (1953), since the other species from this section included in this study (*L. tomentosa*, *L. coronopifolia*, and *L. irasuensis*) pertain to other clades.

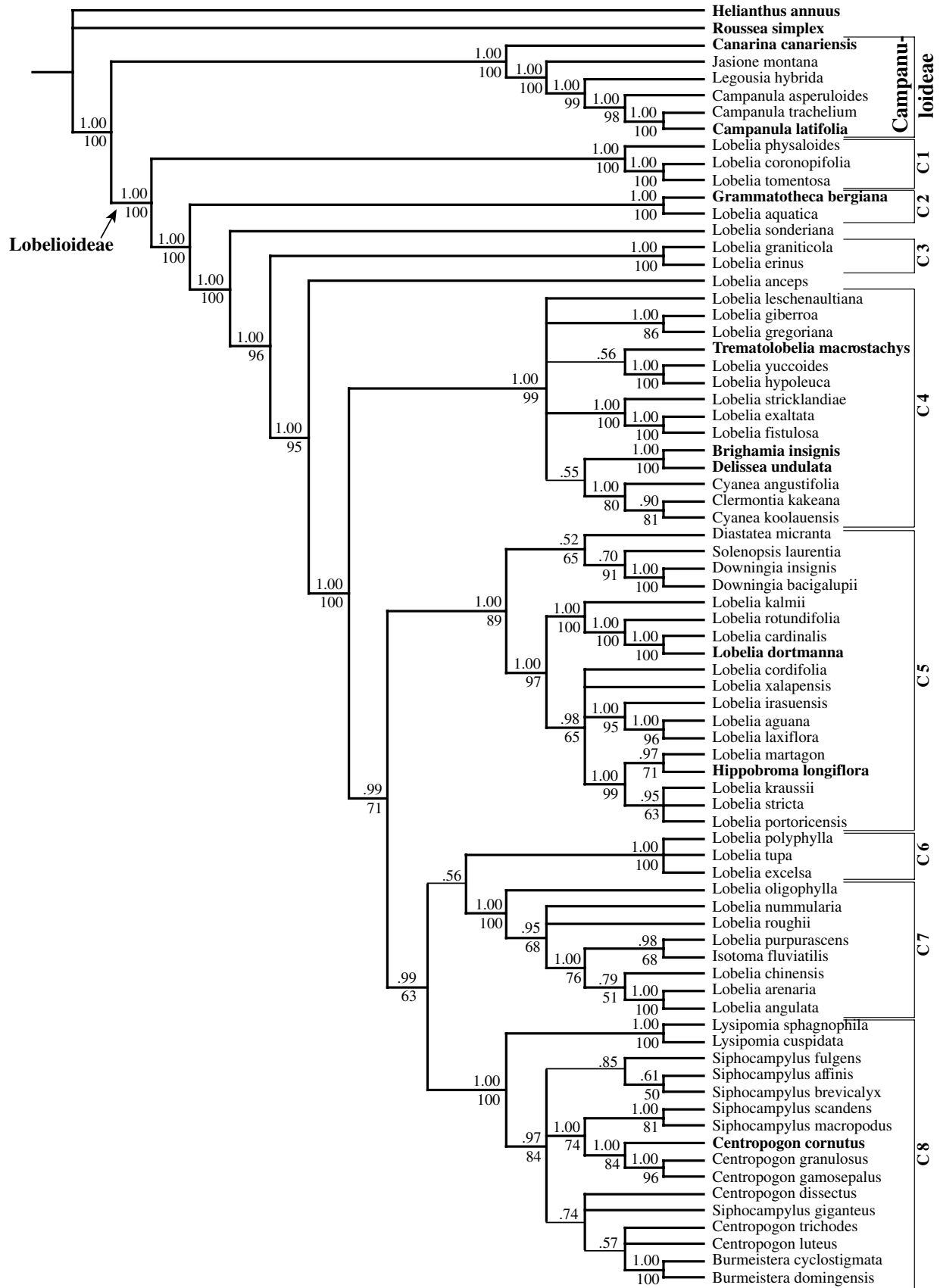


Fig. 2. The most likely phylogenetic tree obtained using Bayesian inference. A thick-lined branch indicates that the branch was also present in the majority-rule consensus tree of the jackknife analysis. Numbers above branches indicate the posterior probability of the clade. Numbers below branches show jackknife support values, whenever applicable. Major clades are enumerated “C1–C8”. Names in bold indicate type species for genera.

Table 6
SH tests of monophyly for currently recognized taxa

Taxon constraint	λ	$-\ln L$	p -value
(None)	3127	22900	—
Genus <i>Lobelia</i>	3310	23543	0.0000*
<i>Lobelia</i> subgen. <i>Lobelia</i>	3252	23276	0.0000*
<i>Lobelia</i> subgen. <i>Mezleria</i> (<i>Isolobus</i>)	3179	23032	0.0030*
<i>Lobelia</i> subgen. <i>Tupa</i>	3218	23231	0.0000*
Genus <i>Centropogon</i>	3133	22924	0.5640
Genus <i>Siphocampylus</i>	3132	22919	0.6346
Genus <i>Cyanea</i>	3128	22901	0.9256

λ , tree length; L, likelihood. An asterisk indicates that the ML Bayesian tree inferred under enforcement of a specific constraint was not significantly less likely than the tree inferred without constraints—meaning that the monophyly of the taxon cannot be rejected using a 95% confidence interval.

L. aquatica occurs in South America and the Antilles, whereas the monotypic genus *Grammatotheca* C. Presl has a disjunct distribution in South Africa and Australia. Although they differ in a few morphological characteristics, such as sessile (*Grammatotheca*) vs. pedicellate (*L. aquatica*) flowers, they share a number of features: both are slender annual herbs, and the dorsal corolla lobes are smaller than the ventral ones. However, these characters seem to be plesiomorphic rather than synapomorphic.

Lobelia sonderiana, a South African herbaceous species, is sister to the remaining lobelioids. This species represents the small section *Eumezleria* E. Wimm., a group of slender procumbent herbs with tiny, solitary flowers bearing two conspicuous aristae on the apex of the two inferior anthers.

4.1.3. Clade 3

This clade comprises *L. granitica* and *L. erinus*, and is strongly supported as monophyletic. This result is consistent with their placement in grex *Eriniformes* E. Wimm., in which they represent subreges *Latifoliae* and *Subgrandiflores*, respectively. There are no evident synapomorphies uniting all species attributed by Wimmer (1953) to this grex, which is morphologically very heterogeneous.

Lobelia anceps is a circumaustral herb that in this analysis is sister to the Clades 4–8. Since Wimmer (1953) placed it together with *L. erinus* and *L. granitica* in subsection *Leiospermae* E. Wimm., this makes the subsection paraphyletic, as well as section *Hemipogon* Benth. and subgenus *Lagotis* E. Wimm. *Lobelia anceps* represents grex *Pterocaulinae* E. Wimm., a group of decumbent herbs with conspicuously winged stems, mainly confined to tropical Africa, although a couple of widespread species reach South America and southeast Asia.

4.1.4. Clade 4

This clade is composed of species from the genus *Lobelia*, intercalated with representatives of the endemic Hawaiian genera *Trematolobelia* Zahlbr., *Delissea* Gaudich., *Brighamia* A. Gray, *Clermontia* Gaudich., and *Cyanea*. Although this clade comprises some well-defined and strongly supported subclades, its basal relationships are

unresolved. There is a strong Bayesian (and moderate jackknife) support indicating that *Cyanea* is paraphyletic without the inclusion of *Clermontia*. The paraphyly of *Cyanea* agrees with Lammers' (1990b) observation that the genus is “arguably unnatural, with infrageneric groups that seem as close to other genera as to each other”. However, as shown by the SH test (Table 6), the monophyly of the genus cannot be rejected when alternative phylogenetic trees are taken into consideration. *L. exaltata*, *L. fistulosa*, and *L. stricklandiae* form a strongly supported group, with *L. stricklandiae* as sister to the other two. While *L. exaltata* and *L. fistulosa* are giant herbs confined to eastern Brazil, *L. stricklandiae* is a treelet occurring in Zambia. This close relationship between *Lobelia* species from tropical Africa and east Brazil was first proposed by Knox et al. (1993), although this relationship only received poor support in their analysis. The position of the other species from tropical Africa included here (*L. giberroa* and *L. gregoriana*) is uncertain, although these two species are sisters. *Delissea undulata* and *Brighamia insignis* are closely related, a result consistent with earlier studies based on molecular (Givnish et al., 1995) and micromorphological (Murata, 1995; Buss et al., 2001) data. The Hawaiian endemics *L. hypoleuca* and *L. yuccoides* are closely related. The internal positions of the remaining taxa are uncertain; these include *L. leschenaultiana*, a giant herb/treelet found in southeast India and Sri Lanka, and *Trematolobelia macrostachys*, a treelet endemic to the Hawaiian Islands. However, since *Trematolobelia* does not form a group together with *L. exaltata* and *L. fistulosa*, this provides additional evidence that section *Colensoa* as circumscribed by Murata (1995) is paraphyletic. The results from this clade alone render the following groups artificial: tribes Delisseae Rchb. and Lobelieae C. Presl, subtribes Cyaneinae E. Wimm. and Siphocampylinae E. Wimm., ramus Schizosiphon E. Wimm., subgenus *Tupa* (G. Don) E. Wimm., sections *Eutupa* (A. DC.) E. Wimm. and *Rhynchoptalum* (Fresen.) Benth. On the other hand, it corroborates subsection *Cereiiformes* (represented by *L. giberroa* and *L. gregoriana*), section *Revolutella* E. Wimm. and subsection *Yuccoides* (represented by *L. hypoleuca* and *L. yuccoides*), and finally grex *Barbiferae* and subgrex *Longipedicellatae* (represented by *L. exaltata* and *L. fistulosa*).

The species in this clade are either pachycaul treelets (up to 15 m tall) or giant herbs with a varying degree of lignification, often sparsely branched. Many species are rosette plants, sometimes assuming a palm-like habit as in certain species of *Cyanea*. They all grow in montane and pre-montane habitats of tropical regions across the globe—sometimes totally dominating the landscape (Fig. 1D). The giant habit, lignification, and a basic number of 14 chromosomes seem to be synapomorphies for the group, although the two latter features are clearly homoplasious in the subfamily. Clade 4 is sister to the remaining clades.

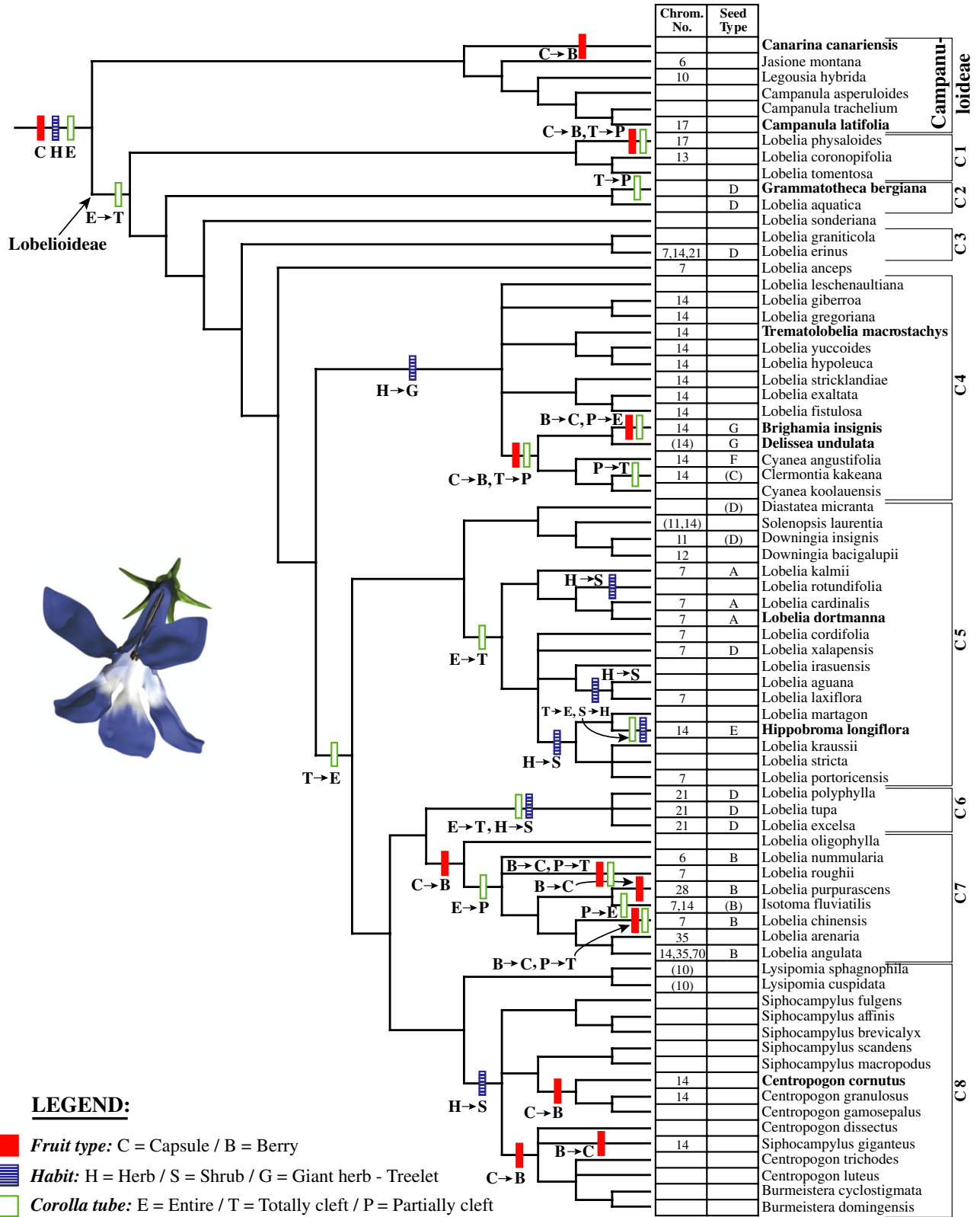


Fig. 3. Most parsimonious optimization of characters traditionally used in the taxonomy of subfamily Lobelioideae: fruit type, habit, and corolla tube. Chromosome numbers and seed types (Table 7) are plotted beside each species. Brackets indicate that the feature was reported for other species in the genus, and is only shown for genera whose monophyly is not strongly contradicted in this study. Names in bold indicate type species for genera. The illustration beside the tree depicts a flower of *Lobelia erinus*, showing the corolla tube totally cleft dorsally—the only feature that distinguishes the genus *Lobelia* from all other lobelioid taxa. An animation of the flower is available from the on-line version of this article (graphics: Olof Helje).

Table 7
Morphological characterization of the seed types discussed in this study

Seed type	Diagnosis
A	Seed coat with pronounced longitudinal ridges
B	Reticulate surface, sometimes verruculate on the reticulum
C	Striate or striate-verruculate testa
D	Wavy-striped or beaded testa
E	As in B but lack a cuticle
F	As in C, but without verruculae and with the radial wall at opposite ends of the areole swollen into knob-like protuberances
G	As in C, but with irregularly wrinkled seed coat and rounded ridges perpendicular to the long axis of the seed

Adapted from Murata (1992, 1995) and Buss et al. (2001).

4.1.5. Clade 5

This strongly supported clade includes several species of *Lobelia*, clustered together with the monotypic genus *Hippobroma* G. Don, *Downingia*, *Solenopsis* C. Presl, and *Diastatea* Scheidw. Four *Lobelia* species form a strongly supported group: *L. dortmanna*, *L. cardinalis*, *L. rotundifolia*, and *L. kalmii*. *L. dortmanna*, the type species of the genus, is sister to *L. cardinalis*. Together they are sister *L. rotundifolia*, followed by *L. kalmii*. Except for *L. rotundifolia*, all species in this group were placed by Wimmer (1953) in subsection *Trachyspermae* E. Wimm. (subgenus *Lagotis*, section *Hemipogon*). *Trachyspermae* was later synonymized as section *Lobelia* (subgenus *Lobelia*) by Murata (1995). Section *Lobelia* is a group of 22 species occurring chiefly in North America. They are all herbs, characterized by having spike-like racemes and wrinkled to scabrous seeds with a special coat structure (type A, Table 7). Wimmer (1953) subdivided *Trachyspermae* into two greges, *Cardinales* E. Wimm. comprising four robust species with red, large flowers and *Coelestes* E. Wimm. with smaller, blue flowers, and slender habit. These results suggest that *Coelestes* (here represented by *L. kalmii* and *L. dortmanna*) is paraphyletic. *L. rotundifolia* is an Antillean shrub up to 2 m tall. It was placed by Wimmer (1953) in subgenus *Tupa*, which is shown to be unnatural.

There is strong support indicating that the sister to *L. kalmii*, *L. rotundifolia*, *L. cardinalis*, and *L. dortmanna* is a group of Central American and Antillean species. This group is composed of two strongly supported subclades: one restricted to Central America (*L. irasuensis*, *L. aguana*, and *L. laxiflora*), the other to the Antilles (*L. kraussi*, *L. stricta*, *L. portoricensis*, *L. martagon*, and *Hippobroma longiflora*). The close relationship between *L. aguana* and *L. laxiflora* is in agreement with their placement in section *Homochilus* A. DC. by Wimmer (1953), which was reiterated by Lammers (2004). However, their sister-taxon relationship to *L. irasuensis* reaffirms the paraphyly of *Lobelia* section *Holopogon*. The existence of an Antillean clade indicates the monophyly of the species placed by Wimmer (1953) in the informal group “Species antillanae”, traditionally recognized as *Lobelia* section *Tylomium* (C. Presl)

Benth. Although *Hippobroma longiflora* is a widespread weed in the tropics, it is believed to have its origin in the West Indies (Lammers, 2007). There is a strong Bayesian (but weak jackknife) support indicating that *L. cordifolia* and *L. xalapensis* also belong to this group, but their internal positions are uncertain.

There is ambiguous Bayesian and moderate jackknife support placing *Solenopsis* as sister to *Downingia*, but their relationship to *Diastatea* and the remaining taxa in Clade 5 remains uncertain. *Downingia* appears monophyletic, a result consistent with Schultheis (2001). The two species included here, *D. bacigalupii* and *D. insignis*, represent the two main clades found in her phylogeny. Except for these three genera, counts of 7 chromosomes are suggested as the typical number in the rest of the clade; the only exception being *Hippobroma longiflora*, with 14 chromosomes. Since 7 is considered the basic chromosome number in the Lobelioideae (Lammers, 1993), *Hippobroma* is therefore hypothesized to be tetraploid. There is a strong Bayesian (but weak jackknife) support indicating that Clade 5 is sister to the remaining clades.

A long-debated (e.g., Lammers, 2007; Pepper et al., 1997; Murata, 1992, 1995) and crucial question in the systematics of the Lobelioideae is how the genus *Lobelia* should be circumscribed in order to become monophyletic. Given the present results, the alternative that seems best would be to restrict the genus to the species of *Lobelia* included in Clade 5, together with the species in the monophyletic groupings they represent (sections *Lobelia*, *Homochilus*, and *Tylomium*), plus the genus *Hippobroma*. In that case, only *Hippobroma longiflora*, originally described as *L. longiflora* L., would have to be re-incorporated into *Lobelia*. *Lobelia* would then comprise about 70 species. However, given the small taxon sampling in relation to the total number of species in the subfamily, a much expanded sampling may be needed in order to confidently provide the grounds for a taxonomic revision.

4.1.6. Clade 6

This is a small group comprising *L. polyphylla*, *L. tupa*, and *L. excelsa*. The monophyly of these species confirms earlier studies (e.g., Lammers and Hensold, 1992; Knox et al., 1993) and their current placement in the section *Tupa* (Lammers, 2000). This result is also in agreement with their previous assignment to their own grex (“Species chilensis”; Wimmer, 1953). As circumscribed by Lammers (2000), this group comprises four species of robust subshrubs restricted to the Andes of Chile. They all have 21 gametic chromosomes, and are considered to be hexaploids originally derived from other *Lobelia* species (Lammers and Hensold, 1992). Both the frutescent habit and the hexaploid condition seem to be synapomorphic for the clade, whereas the seed coat of type D (Table 7), shared by all species in the clade, is likely to be plesiomorphic in the Lobelioideae (Lammers, 1993). The relationship of this clade to the others remains uncertain.

4.1.7. Clade 7

This is a strongly supported clade containing several *Lobelia* species together with *Isotoma fluviatilis*. All Australasian species in this study are placed here (except *L. physaloides*, Clade 1), but the clade also includes one Neotropical species (*L. oligophylla*), which is sister to the remaining species.

Except for *L. sonderiana*, a taxon found near the base of the phylogenetic tree, this clade agrees with the subgenus *Mezleria* (Presl) E. Wimm. as circumscribed by Murata (1995) (sometimes regarded as a synonym of subgenus *Isolobus* [A. DC.] Lian). *L. arenaria* and *L. angulata* appear as closely related, which is not surprising since already Wimmer (1953) considered them as being varieties of the same species. Their close relationship to *L. chinensis* is moderately supported only in the Bayesian analysis. These three species form together with the sisters *Isotoma fluviatilis* and *L. purpuracens* a group with strong Bayesian and moderate jackknife support. Although *Lobelia nummularia* and *L. roughii* seem closely related to the other Australasian species, their position in the group is unresolved. The seed type B (Table 7) appears to be synapomorphic for this group.

4.1.8. Clade 8

This clade is strongly supported and comprises the Neotropical genera *Lysipomia* Kunth, *Burmeistera* Triana, *Centropogon*, and *Siphocampylus*. The results show that: (i) *Burmeistera*, *Centropogon*, and *Siphocampylus* together form a strongly to moderately supported group of essentially robust shrubs and scandent lianas in montane and pre-montane areas in Central and South America, and that (ii) this group is strongly supported as sister to *Lysipomia*, a genus of perennial dwarf herbs from the Andean highlands.

Lysipomia is strongly supported as monophyletic, with the dwarfish habit and the capsule dehiscent by an umbonate operculum as possible synapomorphies.

There is strong support that *Burmeistera domingensis* and *B. cyclostigmata* are sisters, a result consistent with recent studies (e.g., Luteyn, 1986; Stein, 1987a,b; Pepper et al., 1997), which all corroborate the monophyly of the genus. The interspecific phylogeny of *Burmeistera*, as well as its sister-group relationship, remains ambiguous.

There is a strong Bayesian (but only weak jackknife) support indicating that *Centropogon* is paraphyletic, since some species (but not all) are part of a clade that also comprises species of *Siphocampylus*. However, as shown by the SH test (Table 6), the monophyly of *Centropogon* cannot be fully rejected when alternative phylogenetic scenarios are considered. *Centropogon granulatus* and *C. gamosepalus* are strongly supported as sisters, corroborating Wimmer's (1953) placement of these two species in subsection *Corymboides* E. Wimm. There is also strong to moderate support for the monophyly of section *Centropogon* ("Eucentropogon", Wimmer, 1953), here represented by *C. granulatus*, *C. gamosepalus*, and the type species of the

genus, *C. cornutus*. These results are also consistent with the classification adopted by Stein (1987a).

The relationships within *Siphocampylus* are similar to those in *Centropogon*: although there is only resolution enough to reveal the positions of a few species, there is strong to moderate support indicating that the genus is paraphyletic. But once again, its monophyly cannot be fully rejected by the SH test (Table 6). *Siphocampylus macropodus* forms together with *S. scandens* a strongly to moderately supported clade, sister to *Centropogon* section *Centropogon*. Neither section *Siphocampylus* ("Macrosiphon", Wimmer, 1953), represented here by *S. macropodus*, *S. scandens*, *S. affinis*, *S. brevicalyx*, and *S. fulgens*, nor grex *Dissitiflori*, represented by *S. fulgens*, *S. affinis*, and *S. macropodus*, is monophyletic as presently circumscribed. All other relationships in this clade remain obscure.

Possible synapomorphies for Clade 8 include adnation of the filament tube to the corolla (at least basally), and a long bristle (in *Lysipomia*) or tufts of hair (in the other genera) at the apex of the ventral anthers, which are presumably homologous structures. The frutescent habit seems to be a synapomorphy uniting the genera *Centropogon*, *Siphocampylus*, and *Burmeistera*. In the latter clade, two of the analyzed species have been reported to have 14 chromosomes: *Siphocampylus giganteus* and *Centropogon cornutus* (Lammers, 1993). Since all 13 species in *Siphocampylus* and *Centropogon* summarized by Lammers (1993) also have this basic number, tetraploidy may be an additional synapomorphy for the group. However, as no chromosome numbers have been reported for *Burmeistera*, this generalization is uncertain.

4.2. Evolutionary trends in morphology (Fig. 3)

The most parsimonious optimizations of the shifts in fruit type, corolla tube, and habit on the tree generated by molecular data provide some novel insights into the morphological evolution of subfamily Lobelioideae. It is clear from the analyses that corolla tubes that are totally cleft dorsally represents a symplesiomorphic condition in the group, from which other types of corolla tubes have arisen at least 4 times. Lineages producing berries seem to have arisen at least 5 times from capsule-producing ancestors, shifting back to capsules at 5 other occasions. This result provides additional evidence to the ever-growing picture (e.g., Janson, 1992; Bolmgren and Eriksson, 2005) that shifts between capsules and berries occur much more frequently than once thought. Although the independent origin of fleshy fruits may generally be attributed to shifts in habitat (Bolmgren and Eriksson, 2005), it is not evident whether this correlation may also explain these shifts in the Lobelioideae, given the variety of habitats occupied by the berry-producing species. The analyses also show that the herbaceous habit is plesiomorphic in the family, suggesting that giant herbs (Clade 4) are derived from herbaceous ancestors, a result consistent with that by Knox et al. (1993). The frutescent habit has evolved

independently in at least three other clades (Clades 5, 6, and 8), only returning to herbaceous in *Hippobroma*. In five occasions, shifts in the form of the corolla tube occurred along the same lineage as shifts in fruit type; as was the case between shifts in habit and corolla tube, correlated twice.

The plotting of chromosome numbers and types of seed coat provides a much less clear pattern on the evolution of these features in the Campanulaceae. Due to the incompleteness of reports and the variation found in the species situated at the base of the phylogenetic tree, it is difficult to identify a basic chromosome number in the Lobelioideae. However, these limited accounts seem to contradict the hypothesis that a high chromosome number ($x = 21$) is plesiomorphic in the Lobelioideae (Stace and James, 1996), favoring instead the hypothesis of a lower basic number ($x = 7$; Lammers, 1993). The seed type D (Table 7) seems plesiomorphic in the subfamily, corroborating the interpretations by Lammers (1993).

4.3. Effects of missing data on phylogenetic accuracy

Whereas the failure in sequencing the *rbcL* gene for six taxa may be attributed to poor plant material, the failure in sequencing the *ndhF* region for over forty collections is more probably due to primer-associated problems. For instance, the amplification of this gene in a single fragment using the terminal primers *ndhF1f/2110r* has almost always failed, while its amplification in two fragments (using these two primers with the internal primers *ndhF1600r* and *ndhF1320f*, respectively) resulted in a much higher degree of success (Blomby, unpublished data). Reasonably, the terminal primers in this case appear to be interacting and becoming inactive. As a number of alternative primers have been tested, substantial laboratory work may be needed in order to design more suitable primers for the subfamily.

Several studies have discussed how missing data may affect the inference of phylogenetic trees (e.g., Wiens, 2003). Contrary to what is commonly believed, analyses of real as well as simulated data sets indicate that phylogenetic inaccuracy is caused by too few complete characters, rather than too many missing data cells (Wiens, 2003). In order to estimate the effect of the incomplete *ndhF* partition on the results obtained, additional phylogenetic analyses were conducted using the same methods as described above, but excluding the *ndhF* partition from the combined matrix. The resulting trees (not shown) were totally consistent with the trees inferred from the complete data set, except that: (i) the reduced data set resulted in lower support values, especially within distal clades, causing a higher number of unresolved polytomies; (ii) in contrast, the reduced data set yielded higher jackknife supports for two nodes, namely the node uniting Clades 5–8 (92 instead of 71 in the analysis of the complete data set), and for the node uniting Clades 6–8 (83 instead of 63). However, as shown by the results from the Partition Homogeneity Test, this apparently conflicting signal from *ndhF*—or interfer-

ence due to missing data—was not statistically significant. Interestingly, the Bayesian analysis seemed to be less sensitive to the large amount of missing data or conflicting signal, since it yielded the same (± 0.01) posterior probabilities for those two nodes with and without the *ndhF* partition.

5. Conclusions

Generally, Wimmer's (1953) classification of the Lobelioideae has been demonstrated to be suboptimal. This is especially evident in his circumscription of the genus *Lobelia*, where morphologically very distinct species were treated together on the sole basis of having the corolla dorsally cleft in its entire length. As already noted by Maberley (1974), Wimmer was obviously influenced by Candolle, who emphasized this character in his *Prodromus* (Candolle, 1830). Since this and the two other characters largely used by Wimmer (fruit type, for delimiting groups at and above the generic level; and habit, mostly for intra-generic groups) have very low consistency and retention indices, this clearly shows that these characters, albeit useful for practical identification purposes, are in fact of little taxonomic value. Unfortunately, the same applies for the cytological and micromorphological features studied more recently. Although the species in some clades seem to have a more or less constant number of chromosomes (Clades 4, 5, 6, and 8 in Fig. 3), and others share the same type of seed coat (Clades 2, 6, 7, and 8), both these features have a high level of homoplasy in the family.

However, although most of the larger groupings recognized by Wimmer are shown to be unnatural, this study has equally shown that several of his smaller groups are supported as monophyletic. Although classifications based on morphology alone may—as in the case with the Lobelioideae—fail in revealing evolutionary affinities, they may nevertheless provide a useful framework for testing phylogenetic hypotheses in the light of molecular methods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2007.06.015](https://doi.org/10.1016/j.ympev.2007.06.015).

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