Systematic Botany (2008), 33(2): pp. 462–468

The Predominantly South American Clade of Lobeliaceae

Eric B. Knox,[1,4] A. Muthama Muasya,[2] and Nathan Muchhala[3]

[1]Department of Biology, Indiana University, Bloomington, Indiana 47405 U.S.A.
[2]Botany Department, University of Cape Town, 7701 Rondebosch, South Africa
[3]Department of Biology, University of Miami, Coral Gables, Florida 33143 U.S.A.; present address: Department of Ecology and Evolution, University of Toronto, 25 Harbord Street, Toronto, Ontario, M5S 3G5 Canada
[4]Author for correspondence (eknox@indiana.edu)

Communicating Editor: James F. Smith

***Abstract*—**A 3.7 kilobase region of chloroplast DNA that includes *atpB*, *rbcL*, and their intergenic spacer was sequenced in 61 samples from 45 species of South American Lobeliaceae plus two outgroup samples from Australia. A clade of four hexaploid *Lobelia* species from Chile is sister to a clade comprising *Lysipomia*, *Siphocampylus*, *Centropogon*, and *Burmeistera*. *Lysipomia* is a monophyletic group of small cushion-forming plants endemic to the high Andes, and is sister to the clade comprising the remaining three shrubby genera, which are most diverse in the Andes, but also extend to Central America, Mexico, and the West Indies. *Siphocampylus* has capsular fruit and is inferred to be paraphyletic relative to fleshy-fruited *Centropogon* and *Burmeistera*, but fleshy fruits have evidently evolved repeatedly, making *Centropogon* polyphyletic. *Burmeistera* is primarily bat-pollinated and monophyletic, having evolved from one group of species in *Centropogon*. The phylogenetic relationships within *Burmeistera* indicate that this genus underwent repeated episodes of rapid diversification when organismal diversification outpaced the accumulation of mutations in this region of chloroplast DNA.

Keywords*—atpB–rbcL*, *Burmeistera*, *Centropogon*, *Lobelia*, *Lysipomia*, *Siphocampylus*.

The Lobeliaceae comprise about 1,200 species in 31 genera. *Lobelia* L. is the 'core genus' of the family, being paraphyletic to the remaining segregate genera (Knox et al. 2006). The Lobeliaceae originated in southern Africa (Knox et al. 2006) and have subsequently colonized all continents except Antarctica. South America has a diverse assemblage of autochthonous lobelioids that includes: four hexaploid species of *Lobelia* that grow in Chile [Lammers and Hensold 1992; Lammers 2002; placed by Wimmer (1953) in section *Tupa* (G.Don) Benth. subsection *Primanae* E.Wimm. (invalid; see Lammers 2000)]; the genus *Lysipomia* Kunth, which comprises 30 species of small, cushion-forming plants endemic to the high Andes (Lammers 2007); and three shrubby neotropical genera (*Centropogon* C.Presl [212 spp.], *Burmeistera* Triana [102 spp.], and *Siphocampylus* Pohl [231 spp.]; Lammers 2007; referred to as the "CBS clade" by Batterman and Lammers 2004), which collectively comprise almost half of the species of Lobeliaceae, and are most diverse in the Andes. South America is also home to: 11 *Lobelia* species derived from African ancestry that are part of the pan-tropical radiation of the giant lobelias (Knox et al. 1993; Knox and Palmer 1998); one species of *Diastatea* Scheidw. [*D. micrantha* (Kunth) McVaugh] and two species of *Lobelia* (*L. nana* Kunth and *L. laxiflora* Kunth) that have North American origins; and three species previously placed in the problematic genera *Pratia* and *Hypsela*, now treated as synonyms of *L. oligophylla* Lammers (see Lammers 1999), that had an Australian origin (E. Knox and A. Muasya, unpubl. data). This leaves only a dozen herbaceous species of *Lobelia* growing in South America that have uncertain phylogenetic affinities.

Wimmer's (1943, 1953, 1968) monograph of these plants followed Presl's (1836) tribal delimitation based on whether the fruit is fleshy (baccate) or dry (capsular). This single-character taxonomy separates genera that otherwise share coherent sets of morphological features and biogeographical provenance, including the shrubby neotropical genera with fleshy fruits (*Burmeistera* and *Centropogon*) and their obvious capsular relative, *Siphocampylus*. Wimmer's (1943) introductory discussion of the morphology and geographical distribution of these plants indicates his awareness of this situation, and his infrageneric taxonomy occasionally belies the natural affinities. For example, the species of *Centropogon* are divided into two sections, *Centropogon* and *Siphocampyloides* Benth. Wimmer's keys frequently use a standardized progression of morphological features, which make them easy to use, but a compelling argument cannot be made that Wimmer's taxonomy was intended as a phylogenetic hypothesis suitable for testing.

McVaugh (1949) and Lammers (1998) recognized many of the problems with Wimmer's taxonomy for the shrubby neotropical genera. The capsular *Siphocampylus* is reasonably hypothesized to be paraphyletic relative to the fleshy-fruited genera (Lammers 1998). *Burmeistera* has well-circumscribed generic features (McVaugh 1949) with an inflated corolla limb, filaments free from the corolla, and a dilated orifice of the anther tube (Wimmer 1943), and is reasonably hypothesized to be monophyletic (Lammers 1998). *Centropogon*, however, is problematic. Section *Centropogon* is reasonably hypothesized to be monophyletic because the two lower anthers are tipped with a single scale-like structure of concrescent hairs (Wimmer 1943; McVaugh 1949; Jeppesen 1981; Lammers 1998), but Wimmer's section *Siphocampyloides* is regarded by McVaugh (1949) as "an agglomeration of several species-groups which have little in common" and is reasonably hypothesized to be polyphyletic (Lammers 1998). Working within the traditional circumscription of *Centropogon*, McVaugh (1949) and Lammers (1998) revised section *Siphocampyloides* s.l. into perceived natural groups, recognizing four sections, two of which have two subsections. Plants in section *Wimmeriopsis* McVaugh are glabrous or possess simple trichomes, with long corolla tubes that are red or orange, and the subsections are distinguished by whether the corolla lobes are falcate and strongly deflexed (*Falcati* McVaugh) or triangular and erect or spreading (*Columbiani* McVaugh). Plants in sections *Niveopsis* Lammers and *Siphocampyloides* s.s. have stalked, branched trichomes, and the subsections of the latter are distinguished by whether the corolla tube is significantly longer than the lobes (*Brevilimbati* E.Wimm.) or about the same length (*Peruviani* McVaugh). Plants in section *Burmeisteroides* Gleason also lack the char-

acteristic trichomes of section *Siphocampyloides* s.s. and have corollas that are greenish or cream-colored; as the name implies, they may be closely related to *Burmeistera* (McVaugh 1949).

As is typical of the Lobeliaceae, plants in the "CBS clade" have zygomorphic flowers with precise mechanisms of pollen deposition (Erbar and Leins 1995; Muchhala 2007), and are primarily pollinated by either bats or hummingbirds (Feinsinger and Colwell 1978; Stein 1992; Buzato et al. 2000; Muchhala 2003, 2006a). The extensive diversification of this group may have been driven by the combination of specialized pollinator-mediated selection in microallopatric Andean distributions and dramatic historical changes in geology and climate, as has been suggested for other diverse Andean-centered taxa (Gentry 1982; Kay et al. 2005; Smith and Baum 2006). *Siphocampylus* and *Centropogon* are predominantly hummingbird-pollinated, with some bat-pollinated species known in *Centropogon*, whereas, *Burmeistera* is predominantly bat-pollinated, with one apparent reversion to hummingbird pollination (Muchhala 2006a, b).

In this study we include all four hexaploid Chilean species of *Lobelia*, two species of *Lysipomia* that represent the most basal branching within this clade (T. Ayers, pers. comm.; relationships within *Lysipomia* are the subject of an independent study by T. Ayers), and a sampling of the CBS clade that includes both sections of *Siphocampylus* (Wimmer 1953), four of the five sections of *Centropogon* (Lammers 1998; the monotypic section *Niveopsis* Lammers is not sampled), and both sections of *Burmeistera* (Wimmer 1943). A complete understanding of the evolutionary history of this large CBS clade is beyond the scope of this study. Our modest aims are to: 1) establish the relationships among the main autochthonous groups of South American Lobeliaceae; 2) provide a preliminary assessment of phylogenetic relationships among *Siphocampylus*, *Centropogon*, and *Burmeistera*; and 3) document the relationships among 21 species of *Burmeistera*.

Material and Methods

Plant Material—A modified CTAB method (Doyle and Doyle 1987) was used to extract total DNA from fresh or silica-dried samples of leaf tissue. Eight samples were taken from plants grown in botanical gardens, one sample was taken from an herbarium specimen, and the remaining samples were field-collected and vouchered. One cultivated *Centropogon* (UCBG902279) has not been identified to species, and one field-collected *Burmeistera* (Mu142) is potentially an undescribed species related to *B. brachyandra* E.Wimm., but additional material is needed to establish its identity. Source and voucher information for the 63 plants used in this study is presented in the Appendix.

DNA Amplification and Sequencing—The adjacent chloroplast genes *atpB* and *rbcL* and their intergenic region were amplified as three overlapping fragments (Table 1) that were purified using Elu-Quik (Whatman, Brentford, U.K.), QIAquick columns (Qiagen, Valencia, California), or ExoSAP-IT (USB, Cleveland, Ohio). The sequencing reactions used BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) with various internal primers and were run on an Applied Biosystems 3730. Primer 55202F is located in *atpE*, which overlaps *atpB*, and the sequences were trimmed at the end of *atpB*. The sequences were assembled using Sequencher (GeneCodes, Ann Arbor, Michigan). Three sequences are missing 2–6 nucleotides just before the primer at the end of *rbcL* (59002R; < 0.005%), but there is no variation at these positions among the remaining sequences, and the phylogenetic results are unaffected by these missing data. The sequences were aligned manually, and 30 alignment gaps were characterized and coded for inclusion in the phylogenetic analyses. One alignment gap in the intergenic region, involving a variable number (6–11) of bases in a homonucleotide run, was not included in the gap coding because the nucleotide homology cannot be determined, but all gaps are treated as missing data, and the variation in number has no effect on the phylogenetic analysis.

Maximum Parsimony Analysis—The aligned data matrix (comprising all 1,497 nucleotides of *atpB*, 934 positions in the intergenic region, the first 1,407 nucleotides of *rbcL*, and 30 coded alignment gaps) was analyzed using PAUP* (Swofford 2002). A heuristic search was conducted with 1,000 replicates of random sequence additions, TBR swapping, MulTrees, and Fitch parsimony (Fitch 1971). Taxonomic subsets of the data matrix were used to isolate all variation that contributes to the number of equally most-parsimonious (EMP) trees. Trees with zero-length branches that create 'false' topologies (Knox and Palmer 1998) were eliminated by condensing trees (collapsing branches with minimum length of zero, but without eliminating duplicate trees) and then filtering with the option to "not retain a nonbinary tree if a more highly resolved compatible tree exists." The minimum number of point mutations and indels supporting each node was determined for the resulting EMP trees and decay values (Donoghue et al. 1992) were determined. The DNA alignment is deposited with TreeBASE (study number S1930).

Maximum Likelihood Analysis—The analysis was conducted using the HKY85 + G+I model with a molecular clock not enforced. Likelihood parameters were estimated for each of the EMP trees, and the mean values were used to recalculate the likelihood score for each tree using a common set of parameters.

Results

Phylogenetic Analyses—Of the 3,838 nucleotide positions in the aligned data matrix, 419 (10.9%) are variable and of these 192 (45.8%) are potentially phylogenetically informative. None of these 63 sequences has indels in *atpB* or *rbcL*. The intergenic region in 36 sequences comprises 817 collinear nucleotides, and this region in the remaining 25 sequences ranges from 754–877 nucleotides due to 15 small deletions, 14

Table 1. DNA amplification primers named according to the position of the 3′ nucleotide and the orientation of the corresponding sequence in the chloroplast genome of *Nicotiana tobacum* (Z00044).

Primer	Sequence	Target
55202F	5′-GGYAAKACGCCRATTTGTCCAC-3′	*atpB* amplification
55799R	5′-GGTCKATAACRTCKATTCAAGCAG-3′	*atpB* sequencing
56069F	5′-GGYGGTTCRTTCATCTGACC-3′	*atpB* sequencing
56158R	5′-GGAGTHGGBGAACGKACTCG-3′	*atpB* sequencing
56876R	5′-GAAMTCRCSTGCTAACTCCC-3′	*atpB* amplification
56069F	see above	intergenic amplification
56627F	5′-GGTTKACCAAGAGTATCTCGACC-3′	intergenic sequencing
57707R	5′-GGTTGAGGRGTTACTCGRAAKGCTG-3′	intergenic sequencing
58169R	5′-ATCMAGRCCACCVCGAAGAC-3′	intergenic amplification
57620F	5′-ATGTCACCACAAACAGARACTAAAGC-3′	*rbcL* amplification
58169R	see above	*rbcL* sequencing
58966F	5′-TCAYATCCAYCGHGCHATGC-3′	*rbcL* sequencing
58558R	5′-GMGTGAATATGATCKCCACC-3′	*rbcL* sequencing
59002R	5′-TTATAAAGTATCCATTGCGGC-3′	*rbcL* amplification

tandem duplications, and one insertion of a single nucleotide in one of the outgroup samples. Twenty-four of the 30 coded gaps are autapomorphic, and the remaining six are potentially phylogenetically informative.

Maximum parsimony analysis of the aligned DNA sequences for *atpB*, *rbcL*, and the intergenic region from 63 samples yields a single set of 560 EMP trees with 578 steps, a consistency index of 0.69, and a retention index of 0.88. The 560 EMP trees result from 2×5×4×(2×7) alternative topologies. Three of the alternative topological components are 'false' topologies (Knox and Palmer 1998) with alternative zero-length branches that depend on the character optimization and collapse to one or another alternative topology. Purging these 'false' topologies leaves 2×4×4×(2×5) = 320 EMP trees. The empirical base frequencies are: A = 0.286; C = 0.204; G = 0.211; T = 0.299. The mean values for the estimated likelihood parameters are: ti/tv = 0.917789; invariable = 0.620390; and gamma = 1.05360. The individually calculated −ln likelihood scores range from 9137.231–9152.985. Using a common set of parameters, the likelihood scores range from 9137.238–9152.992 and the ranked order does not change.

Samples of the hexaploid Chilean *Lobelia* species have silent variation at *atpB*174, the third-base position in AA58 (Pro). *Lobelia polyphylla* and *L. bridgesii* have the ancestral G, whereas *L. excelsa* has an A and *L. tupa* has a T. Three other mutations unambiguously unite the latter three samples, a result consistent with previous analysis (Knox et al. 1993). The two alternative topologies result from interpretation as independent mutations (G→A and G→T; leaving the node unresolved) versus successive mutations (G→A, A→T or G→T, T→A; uniting the samples of *L. excelsa* and *L. tupa*). Both topologies are equally supported in the maximum likelihood analysis because the alternatives minimally require one transition and one transversion. However, the previous restriction-site analysis of these same samples (Knox et al. 1993) identified *L. tupa* and *L. bridgesii* as sister-species, a result consistent with the morphology. With no additional variation in this data-set to resolve the matter, it seems imprudent to regard the possibility of successive mutations as positive evidence of relationship, and the relationships according to these data are best left unresolved, which is also the strict consensus (Fig. 1).

Six *Centropogon* samples have conflicting variation at two sites that generate four alternative topologies. A silent transition at *atpB*414, the third-base position in AA138 (Ala) unites five samples (UC810952, Mu139, Mu152, Mu174, and Mu173) with a synapomorphic T, whereas *C. comosus* has the ancestral C. This conflicts with a T→G transversion in the intergenic region just upstream of *rbcL* shared by *C. comosus* and *C. salviiformis*. Maximum likelihood analysis equally favors two of these alternatives (that differ based on character-state optimization), one of which is also supported by the morphology (Fig. 1). With the exception of *C. grandidentatus*, the remaining species have a pubescence of stalked, branched trichomes that may be a general feature of this clade (Lammers 1998).

Four topological alternatives affecting seven species of *Burmeistera* (*B. rubrosepala* to *B. glabrata*; Fig. 1) result from conflicting variation at two sites in the intergenic region (one transition and one transversion). Maximum likelihood analysis favors the two topologies with a single transversion, and one of the character-state optimization alternatives is clearly favored because all seven species have a distinctive inflated berry (Muchhala and Lammers 2005) that is consistent with these seven species forming a clade (Fig. 1).

The remaining 10 alternative topologies within *Burmeistera* result from a complex pattern of conflicting variation at seven sites. Half of these alternatives result from a conflict among six mutations (two transitions and an 18-base-pair direct duplication in intergenic region versus a transversion in the intergenic region and two first-base-position transversions in *rbcL* that cause amino acid substitutions). Although maximum likelihood favors one subset of topologies that minimizes the number of transversions, the alternative subset is favored by morphology because it unites all three samples of *B. succulenta* (Fig. 1). Within each subset of five topologies, four result from conflict between one of the first-base-position transversions in *rbcL* (above) and a silent transversion in *atpB*, the fifth is a variant that is only supported using ACCTRAN character-state optimization. Maximum likelihood analysis equally favors the two alternatives that differ based on the character-state optimization. The ACCTRAN-based topology is presented because a subsequent reversal unites all five samples of *B. sodiroana* (Fig. 1); in the alternative topology these samples form a polytomy at the node below.

Discussion

Inferred Phylogenetic Relationships—Phylogenetic analysis of this predominantly South American clade of Lobeliaceae indicates that the hexaploid Chilean species of *Lobelia* are sister to the remaining species, and the high Andean genus *Lysipomia* is sister to the shrubby neotropical genera *Siphocampylus*, *Centropogon*, and *Burmeistera* (Fig. 1). *Lysipomia* and the "CBS clade" are inferred to have evolved independently from a *Lobelia*-like ancestral species; it is hard to imagine how the shrubby genera could have been derived from a *Lysipomia*-like ancestral species or vice versa.

Within the shrubby neotropical clade, the sampled species of *Burmeistera* are clearly monophyletic and derived from a group of *Centropogon* species, with *C. nigricans* (section *Burmeisteroides*) only weakly supported as the species most closely related to *Burmeistera* among those sampled (Fig. 1). Our sampling of *Siphocampylus* and *Centropogon* is not sufficient to reconstruct the evolutionary history of these large genera, but our results are consistent with the hypothesis that the capsular-fruited *Siphocampylus* evolved from a *Lobelia*-like ancestral species, and provisionally confirm the suggestion by Lammers (1998) that fleshy fruits evolved repeatedly, making *Centropogon* polyphyletic. *Centropogon granulosus*, *C. baezanus*, and *C. solanifolius* have scale-like, concrescent hairs on the two lower anthers (Jeppesen 1981) and form a clade, which suggests that section *Centropogon* is monophyletic. The remaining *Centropogon* species have the lower anthers tipped with non-concrescent hairs characteristic of Wimmer's section *Siphocampyloides* s.l., which is evidently polyphyletic, as suggested by McVaugh (1949). Much additional work is required to fully delimit the various clades of *Centropogon*. For example, *C. tessmannii* (section *Wimmeriopsis* subsection *Columbiani*) does not group with two representatives of subsection *Falcati* (*C. aequitorialis* and *C. sodiroanus*; Fig. 1). *Centropogon grandidentatus* is also placed in subsection *Falcati*, but it groups with plants in section *Siphocampyloides* s.s. subsection *Brevilimbati*. Additional sampling of species in section *Bur-*

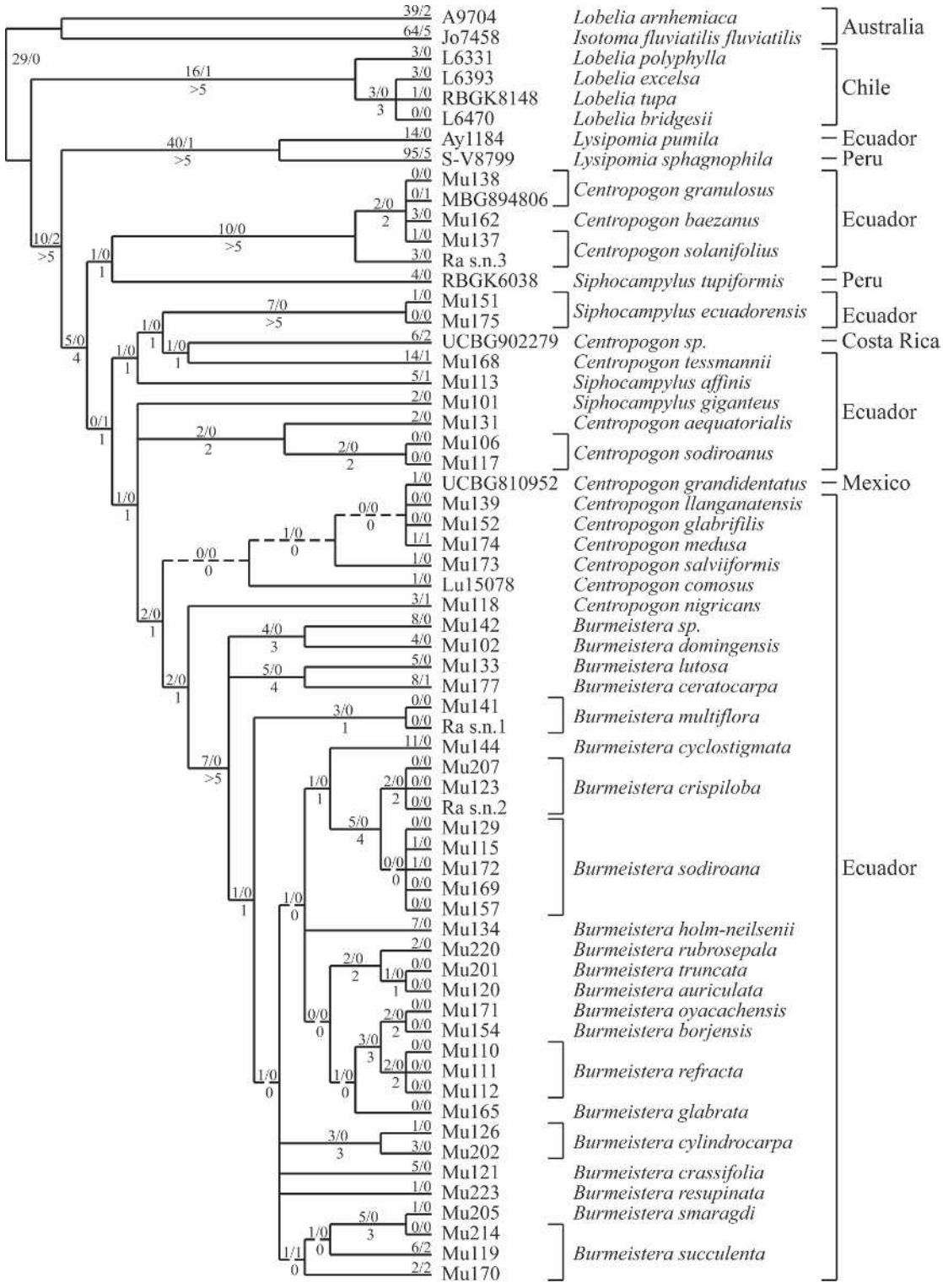

FIG. 1. One of 320 equally most-parsimonious trees found in a maximum parsimony analysis of the *atpB–rbcL* data-set for 61 samples of Lobeliaceae in the predominantly South American clade (plus two outgroup samples), and the single tree favored by maximum likelihood analysis of this data-set and consideration of the morphology of these plants. Dashed lines indicate the nodes that collapse in the strict consensus tree. The numbers above the line at each node indicate the minimum support for that node from point mutations (before the slash) and indels (after the slash), and the amount of variation in each terminal segment is similarly indicated. The numbers below the lines indicate decay values.

meisteroides is necessary to determine whether it is a monophyletic group that is sister to *Burmeistera* or a paraphyletic group from which *Burmeistera* was derived.

Evolution of Burmeistera—The phylogenetic structure within *Burmeistera* suggests that there have been repeated episodes during which speciation has out-paced the accumulation of mutations within the chloroplast region used for this study. Several nodes are well supported by the available data, but the remaining nodes are only weakly supported, often by single mutations at highly labile positions. Future

evidence may necessitate some revision in this phylogenetic estimate.

The infrageneric classification of *Burmeistera* presented by Wimmer (1943) was based on whether the two lower anthers are barbate (Section *Barbatae*) or sparsely pilose or naked (Section *Burmeistera*; presented as '*Imberbes*' nom. invalid.; see Lammers 1998, 2002). The presence of hairs on the lower anthers is widespread in the Lobeliaceae and is a critical part of the pollination mechanism. For most species, the orifice of the anther tube is blocked by the tips of the anthers, and the hairs function as a trigger to open the anther tube and release pollen during pollinator visits (Stein 1992). In *Burmeistera*, the orifice of the anther tube is dilated (a synapomorphy of the genus), so this 'trigger' function is lost (Stein 1987a), and the lower anther hairs likely represent a vestigial feature. Our data suggest that these hairs have been lost repeatedly within *Burmeistera*: only *B. lutosa*, *B. ceratocarpa*, *B. cylindrocarpa*, and *B. crassifolia* (Fig. 1) retain the barbate condition. Jeppesen (1981) notes that *B. domingensis* has only sparse hairs on the lower two anthers and that this species is related to *B. brachyandra*, in which all anthers are villous. The unnamed species (Mu142) is also closely related to *B. brachyandra*, but all anthers are glabrous. Although often a useful phylogenetic character in other lobelioids, the hairs on the tips of the anthers are not a good character in *Burmeistera*.

The modification of anther morphology in *Burmeistera* is likely related to the mode of pollination. While the majority of *Centropogon* and *Siphocampylus* species are hummingbird-pollinated, the clade including *C. nigricans* and *Burmeistera* are predominantly bat-pollinated (Muchhala 2006a, b). Bat-pollinated flowers typically produce more pollen than those of other pollination syndromes (Baker 1961; Helversen 1993); accordingly, the anther tube of *C. nigricans* is larger than other *Centropogon* species, and that of *B. rubrosepala* (the only known reversion to hummingbird pollination in the genus; Muchhala 2006a) is smaller than other *Burmeistera* species (N. Muchhala, pers. obs.). The open anther tube of *Burmeistera* may represent an additional means to maximize pollen deposition per visit. Bats also visit flowers more forcefully than hummingbirds, readily dislodging pollen (Muchhala 2007), which probably reduces the need of a trigger mechanism for pollen deposition. More evidence for a link between anther morphology and pollination can be seen in *Centropogon* section *Burmeisteroides* (which includes *C. nigricans*); flowers of this group fit the chiropterophilous syndrome in terms of floral morphology and color, and the tuft of hairs on the lower anthers is soft rather than stiff or fused into a scale (Gleason 1924). Additionally, the chiropterophilous *Siphocampylus oscitans* B.A.Stein has a dilated anther-tube orifice (like that of *Burmeistera*) and very sparse, soft hairs around this opening (Stein 1987b).

Within *Burmeistera*, relatively few morphological traits display clear nested hierarchical patterns when mapped onto our phylogenetic estimate (not shown). These include aspects of fruit morphology, leaf shape and orientation, and plant habit. Seven of the sampled species (*B. rubrosepala* to *B. glabrata*; Fig. 1) have distinctive inflated berries. Other species have fleshy berries that are brightly colored when ripe (red, pink, purple, yellow, or blue) and are likely bird-dispersed. The inflated berries are dull-colored (green with occasional splotches of magenta) and are not removed from the plant by frugivorous animals, but instead simply fall to the ground when mature (N. Muchhala, pers. obs.). Further study is needed to determine whether biotic or abiotic factors secondarily disperse the seeds. The shared possession of this inflated berry was the basis for favoring the DNA-based topology that resolved these seven species as a clade.

The *Burmeistera* species with inflated berries can be subdivided into two groups based on biogeography and leaf shape and orientation. *Burmeistera rubrosepala*, *B. truncata*, and *B. auriculata* grow on the western slopes of the Andes, and the alternately arranged leaves are ovate to lanceolate with an obtuse base, and are oriented in a distichous pattern, with two rows along the stem in a plane parallel to the ground. *Burmeistera oyacachensis*, *B. borjensis*, *B. refracta*, and *B. glabrata* grow on the eastern slopes, the leaves are elliptical with cuneate bases, and have a spiral orientation.

The inferred ancestral habit of *Burmeistera* is as free-standing herbs or subshrubs, with a scandent habit having evolved in the ancestral lineage of the clade comprising *B. cyclostigmata* to *B. succulenta* (Fig. 1). Exceptions include *B. ceratocarpa*, *B. cyclostigmata*, and *B. rubrosepala*, which are free-standing or scandent. A distichous leaf orientation roughly corresponds with the scandent habit. The species with a free-standing habit have a spiral leaf orientation, and except for *B. cyclostigmata* and the four east Andean species with inflated berries (discussed above), the scandent species have a distichous leaf orientation.

Like the barbate condition of the anthers, other morphological characters commonly used to identify species of *Burmeistera* do not show clear nested hierarchical patterns when mapped onto our DNA-based phylogenetic estimate (not shown). For example, two types of anther tube morphologies are interspersed throughout this phylogenetic estimate. Ten species have an obliquely cup-shaped anther tube and the other eleven have a curved-cylindrical anther tube. If cup-shaped is the ancestral condition, a minimum of four transformations to cylindrical and one reversion back to cup-shaped is required to account for the distribution of this character. Similarly, calyx lobe morphology is highly variable, with multiple transformations between linear, triangular, ovate, or lanceolate shapes and erect, spreading, patent, or reflexed positions. The shape of the leaves and the shape of the hypanthium also vary with no clear phylogenetic patterns.

Lack of clear infrageneric structure in the morphology of the members of this large genus may be indicative of extremely rapid rates of speciation, as suggested by our molecular evidence. Alternatively or additionally, it may be due to frequent hybridization events. A comparison of nuclear DNA with our chloroplast DNA results would be useful to better understand the importance of reticulate evolution in the diversification of the Lobeliaceae.

In conclusion, the predominantly South American clade of Lobeliaceae originated with colonization of the continent by a *Lobelia*-like ancestral species that diversified to form 1) the Chilean clade of four hexaploid species; 2) possibly other, as yet unsampled, diploid species of *Lobelia*; 3) the derived, segregate genus *Lysipomia*; and 4) a large clade with almost 550 species in the derived, segregate genera *Siphocampylus*, *Centropogon*, and *Burmeistera*. The capsular-fruited *Siphocampylus* is paraphyletic relative to the fleshy-fruited species of *Centropogon* (which is evidently polyphyletic) and *Burmeistera*, which is monophyletic, primarily bat-pollinated, and evidently derived from *Centropogon* as currently delimited. Many of the other native species of Lobeliaceae in South

America are derived from more recent colonization from Africa, North America, and Australia.

ACKNOWLEDGMENTS. We thank Tina Ayers for providing DNA samples of *Lysipomia*, Tom Lammers for seed of Chilean *Lobelia* species, David Albrecht, Peter Jobson, and Rob Raguso for silica-dried tissue samples, the Missouri Botanical Garden, University of California Botanical Garden, and Royal Botanic Gardens, Kew, for fresh tissue samples, and two anonymous reviewers for their helpful comments and suggestions. This work was supported in part by a grant from the National Science Foundation (DEB-0074354).

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APPENDIX 1. Voucher information and GenBank accession numbers for taxa used in this study. Voucher specimens are deposited in the following herbaria: ASC = Northern Arizona University, CPUN = Universidad Nacional de Cajamarca, F = Field Museum of Natural History, IND = Indiana University, K = Royal Botanic Gardens, Kew, MO = Missouri Botanical Garden, NT = Northern Territory Herbarium, OSH = University of Wisconsin Oshkosh, QCA = Pontificia Universidad Católica del Ecuador, UC = University of California. Information is presented in the following order: *Taxon*; GenBank accession: *atpB–rbcL*; Tissue source; *Voucher* (herbarium); Country.

Burmeistera. *B. auriculata* Muchhala & Lammers; EF174650; Field; *Muchhala 120* (QCA); Ecuador. *B. borjensis* Jeppesen; EF174652; Field; *Muchhala 154* (QCA); Ecuador. *B. ceratocarpa* Zahlbr.; EF174635; Field; *Muchhala 177* (QCA); Ecuador. *B. crassifolia* (E.Wimm.) E.Wimm.; EF174659; Field; *Muchhala 121* (QCA); Ecuador. *B. crispiloba* Zahlbr.; EF174640; Field; *Muchhala 123* (QCA); Ecuador; EF174639; Field; *Muchhala 207* (QCA); Ecuador; EF174641; Field; *Raguso s.n. 2* (IND); Ecuador. *B. cyclostigmata* Donn.Sm.; EF174638; Field; *Muchhala 144* (QCA); Ecuador. *B. cylindrocarpa* Zahlbr.; EF174657; Field; *Muchhala 126* (QCA); Ecuador; EF174658; Field; *Muchhala 202* (QCA); Ecuador. *B. domingensis* Jeppesen; EF174633; Field; *Muchhala 102* (QCA); Ecuador. *B. glabrata* (Kunth) Benth. & Hook.f. ex B.D.Jacks.; EF174656; Field; *Muchhala 165* (QCA); Ecuador. *B. holm-nielsenii* Jeppesen; EF174647; Field; *Muchhala 134* (QCA); Ecuador. *B. lutosa* E.Wimm.; EF174634; Field; *Muchhala 133* (QCA); Ecuador. *B. multiflora* Zahlbr.; EF174636; Field; *Muchhala 141* (QCA); Ecuador; EF174637; Field; *Raguso s.n. 1* (IND); Ecuador. *B. oyacachensis* Jeppesen; EF174651; Field; *Muchhala 171* (QCA); Ecuador. *B. refracta* E.Wimm.; EF174653; Field; *Muchhala 110* (QCA); Ecuador; EF174654; Field; *Muchhala 111* (QCA); Ecuador; EF174655; Field; *Muchhala 112* (QCA); Ecuador. *B. resu-*

pinata Zahlbr.; EF174660; Field; *Muchhala 223* (QCA); Ecuador. *B. rubrosepala* (E.Wimm.) E.Wimm.; EF174648; Field; *Muchhala 220* (QCA); Ecuador. *B. smaragdi* Lammers; EF174661; Field; *Muchhala 205* (QCA); Ecuador. *B. sodiroana* Zahlbr.; EF174643; Field; *Muchhala 115* (QCA); Ecuador; EF174642; Field; *Muchhala 129* (QCA); Ecuador; EF174646; Field; *Muchhala 157* (QCA); Ecuador; EF174645; Field; *Muchhala 169* (QCA); Ecuador; EF174644; Field; *Muchhala 172* (QCA); Ecuador. *B. sp. nov.*; EF174632; Field; *Muchhala 142* (QCA); Ecuador. *B. succulenta* H.Karst; EF174663; Field; *Muchhala 119* (QCA); Ecuador; EF174664; Field; *Muchhala 170* (QCA); Ecuador; EF174662; Field; *Muchhala 214* (QCA); Ecuador. *B. truncata* Zahlbr.; EF174649; Field; *Muchhala 201* (QCA); Ecuador. ***Centropogon***. *C. aequatorialis* E.Wimm.; EF174622; Field; *Muchhala 131* (QCA); Ecuador. *C. baezanus* Jeppesen; EF174612; Field; *Muchhala 162* (QCA); Ecuador. *C. comosus* Gleason; EF174630; Herbarium specimen; *Luteyn et al. 15078* (OSH); Ecuador. *C. glabrifilis* (E.Wimm.) Jeppesen; EF174627; Field; *Muchhala 152* (QCA); Ecuador. *C. grandidentatus* (Schltdl.) Zahlbr.; EF174625; Cult.; *University of California Botanical Garden 810952* (UC); Mexico. *C. granulosus* C.Presl; EF174611; Cult.; *Missouri Botanical Garden 894806* (MO); Ecuador; EF174610; Field; *Muchhala 138* (QCA); Ecuador. *C. llanganatensis* Jeppesen; EF174626; Field; *Muchhala 139* (QCA); Ecuador. *C. medusa* E.Wimm.; EF174628; Field; *Muchhala 174* (QCA); Ecuador. *C. nigricans* Zahlbr.; EF174631; Field; *Muchhala 118* (QCA); Ecuador. *C. salviiformis* Zahlbr.; EF174629; Field; *Muchhala 173* (QCA); Ecuador. *C. sodiroanus* Zahlbr.; EF174623; Field; *Muchhala 106* (QCA); Ecuador; EF174624; Field; *Muchhala 117* (QCA); Ecuador. *C. solanifolius* Benth.; EF174613; Field; *Muchhala 137* (QCA); Ecuador; EF174614; Field; *Raguso s.n. 3* (IND); Ecuador. *C. sp.*; EF174618; Cult.; *University of California Botanical Garden 902279* (UC); Costa Rica. *C. tessmannii* E.Wimm.; EF174619; Field; *Muchhala 168* (QCA); Ecuador. ***Isotoma***. *I. fluviatilis* (R. Br.) F.Muell. ex Benth. subsp. *fluviatilis*; EF999977; Field; *Jobson 7458* (IND); Australia. ***Lobelia***. *L. arnhemiaca* E. Wimm.; EF694737; Field; *Albrecht 9704* (NT); Australia. *L. bridgesii* Hook. & Arn.; EF174607; Field-collected seed; *Lammers & Baeza 6470* (F); Chile. *L. excelsa* Bonpl.; EF174605; Field-collected seed; *Lammers et al. 6393* (F); Chile. *L. polyphylla* Hook. & Arn.; EF174604; Field-collected seed; *Lammers et al. 6331* (F); Chile. *L. tupa* L.; EF174606; Cult.; *Royal Botanic Gardens, Kew 1985-8148* (K); Chile. ***Lysipomia***. *L. pumila* (Wedd.) E.Wimm.; EF174608; Field; *Ayers 1184* (ASC); Ecuador. *L. sphagnophila* Griseb. ex Wedd.; EF174609; Field; *Sánchez-Vega 8799* (CPUN); Peru. ***Siphocampylus***. *S. affinis* (Mirb.) McVaugh; EF174620; Field; *Muchhala 113* (QCA); Ecuador. *S. ecuadorensis* E.Wimm.; EF174616; Field; *Muchhala 151* (QCA); Ecuador; EF174617; Field; *Muchhala 175* (QCA); Ecuador. *S. giganteus* (Cav.) G.Don; EF174621; Field; *Muchhala 101* (QCA); Ecuador. *S. tupiformis* Zahlbr.; EF174615; Cult.; *Royal Botanic Gardens, Kew 1986-6038* (K); Ecuador.