Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae)[1]

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- *Premise of the study:* The species-rich Neotropical genera *Centropogon*, *Burmeistera*, and *Siphocampylus* represent more than half of the ~1200 species in the subfamily Lobelioideae (Campanulaceae). They exhibit remarkable morphological variation in floral morphology and habit. Limited taxon sampling and phylogenetic resolution, however, obscures our understanding of relationships between and within these genera and underscores our uncertainty of the systematic value of fruit type as a major diagnostic character.

- *Methods:* We inferred a phylogeny from five plastid DNA regions (*rpl32-trnL*, *ndhF-rpl32*, *rps16-trnK*, *trnG-trnG-trns*, *rbcL*) using maximum-likelihood and Bayesian inference. Ancestral character reconstructions were applied to infer patterns of fruit evolution.

- *Key results:* Our results demonstrate that the majority of species in the genera *Centropogon*, *Burmeistera*, and *Siphocampylus* together form a primarily mainland Neotropical clade, collectively termed the "centropogonids." Caribbean *Siphocampylus*, however, group with other Caribbean lobelioid species. We find high support for the monophyly of *Burmeistera* and the polyphyly of *Centropogon* and mainland *Siphocampylus*. The ancestral fruit type of the centropogonids is a capsule; berries have evolved independently multiple times.

- *Conclusions:* Our plastid phylogeny greatly improves the phylogenetic resolution within Neotropical Lobelioideae and highlights the need for taxonomic revisions in the subfamily. Inference of ancestral character states identifies a dynamic pattern of fruit evolution within the centropogonids, emphasizing the difficulty of diagnosing broad taxonomic groups on the basis of fruit type. Finally, we identify that the centropogonids, *Lysipomia*, and *Lobelia* section *Tupa* form a Pan-Andean radiation with broad habitat diversity. This clade is a prime candidate for investigations of Neotropical biogeography and morphological evolution.

Key words: Andes; *Burmeistera*; Campanulaceae; *Centropogon*; fruit evolution; Lobelioideae; Neotropics; phylogenetic systematics; plastid; *Siphocampylus.*

The bellflower family, Campanulaceae Juss., is cosmopolitan in its distribution and includes ~2300 species in 84 genera (Lammers, 2007b). Members of the family share at least three putative synapomorphies: laticifers, stamens attached to the disc of the ovary, and epigynous flowers (Lammers, 2004, 2007a). Additionally, plants are usually herbaceous, with alternate, estipulate leaves and five-merous, protandrous flowers with various forms of secondary pollen presentation. Five subfamilies are recognized: Campanuloideae Burnett, Lobelioideae Burnett, Cyphioideae Walp., Nemacladoideae Lammers, and Cyphocarpoideae Miers. The largest of these is Lobelioideae, which includes ~1200 species and is characterized by species with resupinate, zygomorphic flowers, connate stamens, and styles with brush hairs that facilitate secondary pollen presentation by a pump-and-piston mechanism (Erbar and Leins, 1995;

Leins and Erbar, 2006). Lobelioideae are ecologically diverse, ranging from northern temperate herbs and small aquatics to the giant tropical pachycaul rosettes, cliff succulents, and epiphytes that comprise the canonical Hawaiian island radiation (Givnish et al., 2009). Lobelioideae are widely distributed, but absent from the Arctic, Middle East, and large regions of central Asia (Stevens, 2003). Some Lobelioideae are well known for their horticultural importance in temperate latitudes, including *Lobelia cardinalis* L., *L. siphilitica* L., and *L. erinus* L. The majority of species, however, are native to tropical regions, with the highest diversity in the New World tropics (Lammers, 2007b).

Approximately half of all species in Lobelioideae fall within three exclusively Neotropical genera: *Centropogon* C. Presl (~210 species), *Burmeistera* Triana (~100 species), and *Siphocampylus* Pohl (~230 species) (Lammers, 2007b). Previous studies have suggested that these genera form a clade (Antonelli, 2008, 2009; Knox et al., 2008), which has been informally named the "CBS clade" (Batterman and Lammers, 2004). After the cosmopolitan and polyphyletic *Lobelia* L. (Antonelli, 2008, 2009; Lammers, 2011), these are the three largest genera within the Lobelioideae. Species in *Burmeistera*, *Centropogon*, and *Siphocampylus* are distinguished by their shrubby habit and entire corolla tubes, which are neither fenestrate nor dorsally cleft, as in many *Lobelia* species. The exception is *Siphocampylus* section *Macranthi* E. Wimm. subsection *Hemisiphocampylus* (A. DC.) E. Wimm., whose species possess fenestrate corollas. The corolla lobes in *Centropogon*, *Siphocampylus*, and *Burmeistera* are either monomorphic or dimorphic; if dimorphic, the dorsal pair are larger than the ventral three (Lammers, 2002). The staminal tube almost always extends beyond the corolla, with the anther tube frequently bearing a tuft of hair on the ventral apex (Fig. 1). Most species are scandent or erect suffruticose shrubs and subshrubs. However, plants can be hemiepiphytes (e.g., many *Burmeistera* spp.), herbs (e.g., *C. coccineus* [Hook.] Regel ex B. D. Jacks.), xerophytes with substantial woody bases (e.g., *S. smilax* Lammers), or, very rarely, trees (e.g., *S. tunarensis* Zahlbr.). Like many other woody species in Lobelioideae, all species in the genera *Centropogon* and *Siphocampylus* whose cytology has been studied are tetraploid ($n = 14$), though chromosome numbers have never been reported for *Burmeistera* species (Lammers, 1993). Genera have been distinguished primarily by fruit type: *Burmeistera* and *Centropogon* produce berries (Fig. 2B–F), whereas *Siphocampylus* produces capsules (Fig. 2A). *Burmeistera* is further distinguished on the basis of several characters, including ebracteolate pedicels, an inflated corolla opening (Fig. 1A), a dilated anther orifice, and isodiametric seeds (Lammers, 1998; Muchhala and Lammers, 2005). Aside from fruit type, there are no characters that readily distinguish *Centropogon* and *Siphocampylus*; their morphology overlaps in nearly every vegetative and reproductive character. As a result, specimens are often misfiled between these two genera in herbarium collections. Additionally, character overlap has caused many species to be described as ambiguously placed between the two genera, particularly when fruit information is missing (e.g., *C. dubius* [Zahlbr.] E. Wimm.). A major goal of the present study is to determine whether fruit type is a useful taxonomic character for delineating these large genera within Neotropical Lobelioideae.

The >540 species in the genera *Burmeistera*, *Centropogon*, and *Siphocampylus* exhibit a high degree of morphological

Fig. 1.   Floral diversity of *Burmeistera*, *Centropogon*, and *Siphocampylus*, the majority of whose species we informally designate as the centropogonids. (A) *Burmeistera tenuiflora* Donn. Sm. (burmeisterid), (B) *C. dombeyanus* E. Wimm. (peruvianid), (C) *C. macbridei* Gleason (brevilimbatid), (D) *C. leucocarpus* McVaugh (colombianid), (E) *C. yungasensis* Britton (eucentropgonid), and (F) *Siphocampylus rusbyanus* Britton. (Photos: L. Lagomarsino.)
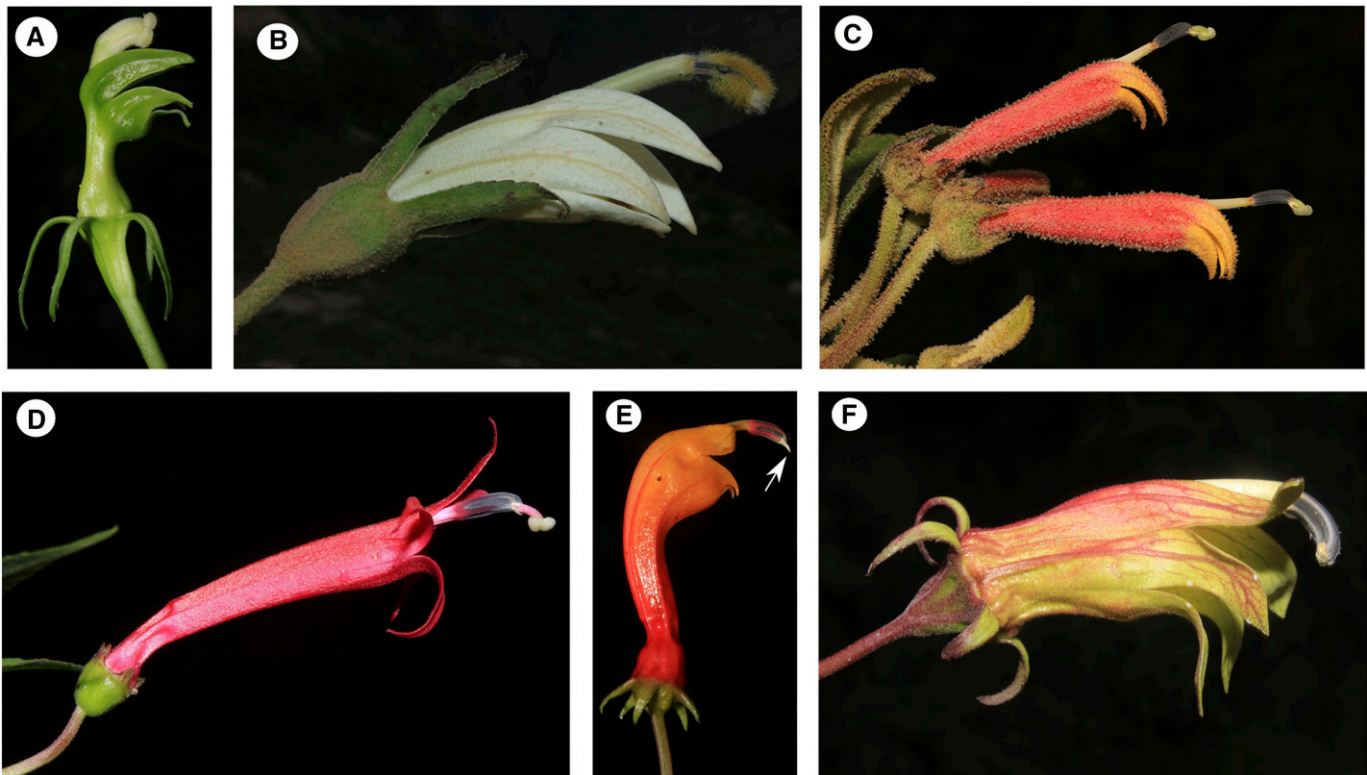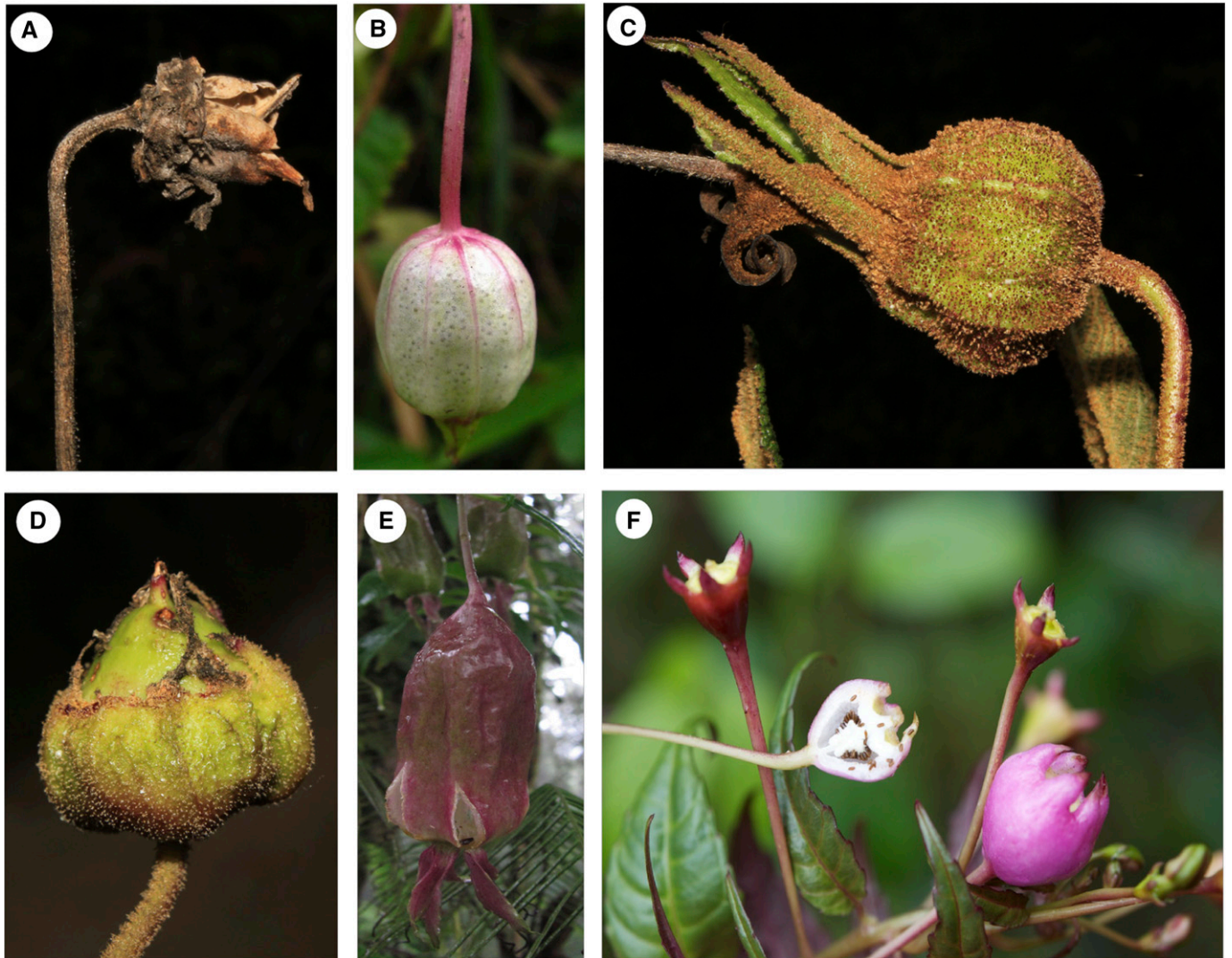
Fig. 2. Fruit diversity of *Burmeistera*, *Centropogon*, and *Siphocampylus*, the majority of whose species we informally designate as the centropogonid clade. (A) Capsule of *Siphocampylus corynoides* E. Wimm., (B) berry of *Centropogon coccineus* (Hook.) Regel ex B. D. Jacks. (colombianid), (C) berry of *C. viriduliforus* E. Wimm. (peruvianid), (D) berry of *C. weberbaueri* Zahlbr. (brevilimbatid), (E) berry of *B. vulgaris* E. Wimm. (burmeisterid), and (F) berry of *B.* aff. *minutiflora* Garzón & Gonzalez (burmeisterid). (Photos: A–E, L. Lagomarsino; F, A. Antonelli.)

variation, particularly in floral form, habit, and fruit type. Together, their high species diversity, striking morphological variation, and relatively recent origin (estimated to be approximately 5–12 mya; Antonelli, 2009; Givnish et al., 2009) make the group particularly interesting for studies of species diversification. Factors that have likely contributed to their diversification include pollinator interactions and climatic and geological events (particularly the orogeny of the Andes; see below). Variation in floral morphology is associated with diverse pollinator interactions: *Centropogon* and *Siphocampylus* species are primarily adapted to a variety of hummingbird pollinators (Knox et al., 2008) (Fig. 1C–E), though bat-pollination is relatively common (Fig. 1B, F). With the exception of one documented hummingbird-pollinated species, however, *Burmeistera* species are almost exclusively bat-pollinated (Muchhala, 2006b) (Fig. 1A). Floral morphology in these species and the pollinators that they attract (Stein, 1992; Muchhala, 2006b) are consistent with classical descriptions of pollination syndromes (Baker, 1961;

Faegri and van der Pijl, 1979; Fenster et al., 2004). For example, the hummingbird-pollinated *B. rubrosepala* (E. Wimm.) E. Wimm. (Muchhala, 2006b) and *C. valerioi* Standl. (Colwell et al., 1974) have flowers that are typical of species pollinated by hummingbirds—they lack odor, are brightly colored (red, pink, orange), and have narrow corolla openings. *Centropogon granulosus* Presl. and many other members of *Centropogon* section *Centropogon* Benth. & Hook. (Stein, 1992) (Fig. 1E), while still hummingbird-pollinated, have abruptly curved corollas and inflorescences that allow for perching, as is typical of species pollinated by sicklebill hummingbirds. Many *Burmeistera* species (Muchhala, 2006b) and *C. nigricans* Zahlbr. (Muchhala and Thomson, 2009), on the other hand, are generally pale in color, produce a strong skunk-like odor, and open at night, like most bat-pollinated species. At least two species, *S. sulfureus* E. Wimm. (Sazima et al., 1994) and *B. tenuiflora* Donn. Sm. (Muchhala, 2003), are effectively pollinated by both bats and hummingbirds, and produce flowers that are intermediate

between hummingbird and bat pollination syndromes. Flower length is highly variable in these three genera and varies from <1 cm in *B. minutiflora* Garzón and F. Gonzalez (Garzón Venegas et al., 2012) to >8 cm in *C. nigricans*. In the latter, long flowers are an adaptation to specialized pollination by the bat species *Anoura fistulata* Muchhala, Mena-Valenzuela & Albuja (Muchhala and Thomson, 2009), which has the longest tongue protrusion known among mammals (Muchhala, 2006a).

Centropogon, *Burmeistera*, and *Siphocampylus* are a mostly continental radiation, ranging from southern Mexico through northern Argentina, with a handful of species occurring in the Caribbean region. These genera have a broad ecological amplitude, occurring from midmontane cloud forests (the majority of species) to lowland tropical rainforests (e.g., *C. cornutus* [L.] Druce, *C. tessmannii* E. Wimm.), seasonally dry subtropical forest (e.g., *S. orbignianus* A. DC., *S. smilax* Lammers), and high elevation grasslands (e.g., *S. jelskii* Zahlbr., *C. ferrugineus* [L.f.] Gleason). *Burmeistera* is distributed from Guatemala through northern Peru; *Centropogon* is distributed from southern Mexico to Bolivia and Brazil, with two species in the Lesser Antilles; and *Siphocampylus* is distributed from Costa Rica to Argentina, and in the Greater Antilles. These genera form an especially conspicuous element in the cloud forest understories of montane regions of South America, particularly in the Andes. As a result of extensive plant diversification, the Andean cordilleras, where Neotropical lobelioids are most abundant, are the richest of the global biodiversity hotspots (Myers et al., 2000). In a survey of various Andean angiosperm lineages, *Burmeistera* was found to have the highest speciation rate of all groups examined (Pennington et al., 2010: table S1). The Andean orogeny is thought to have stimulated diversification in numerous groups (von Hagen and Kadereit, 2003; Bell and Donoghue, 2005; Winkworth and Donoghue, 2005; Hughes and Eastwood, 2006; Smith and Baum, 2006; Antonelli et al., 2009; Madriñán et al., 2013), the ultimate causes of which are attributed to a variety of factors (Luebert and Weigend, 2014). The Miocene diversification (approximately 5–12 mya) of *Centropogon*, *Burmeistera*, and *Siphocampylus* (Antonelli, 2009; Givnish et al., 2009) is similarly consistent with the hypothesis that Andean orogeny was a major factor promoting diversification in this group.

Many scholars have expressed frustration with the existing classifications of *Burmeistera*, *Centropogon*, and *Siphocampylus*, particularly in the latter two genera (e.g., McVaugh, 1949; Lammers, 1998, 2007a). Wimmer's treatments for Lobelioideae in Das Pflanzenreich (Wimmer, 1943, 1953, 1968) constitute the most recent monograph of these three genera. Wimmer divided each genus into a series of hierarchical divisions, including sections, subsections, greges (singular: grex), subgreges, and series. This work often relies on single, seemingly arbitrary characters and narrow species concepts to define taxa. An outline of the classification by Wimmer (1943), including his refinements (Wimmer, 1953, 1968), is presented in Table 1. McVaugh (1949) substantially improved on the taxonomy of *Centropogon* by erecting new sections and recircumscribing existing taxa using a combination of characters. McVaugh's concept of *Centropogon*, plus an additional section described in Lammers (1998), is preferred by current taxonomists of Lobelioideae (Lammers, 1998, 2002; Batterman and Lammers, 2004) (Table 1). There has been no substantial taxonomic revision within *Siphocampylus* or *Burmeistera* since Wimmer (1943, 1953, 1968), though a systematic treatment of Colombian *Burmeistera* species is in progress (Garzón Venegas and González, 2012).

Lammers (1998, 2004) suggested that the monophyly of genera and subgenera would remain dubious until a broad phylogenetic analysis is conducted.

Phylogenetic analyses of the Neotropical Lobelioideae, however, remain limited despite their remarkable floral diversity and broad ecological amplitude. Three previous studies have attempted to reconstruct relationships within and among *Burmeistera*, *Centropogon*, and *Siphocampylus* using molecular data (Antonelli, 2008, 2009; Knox et al., 2008). These studies suggested that together these genera form a clade, but that the two largest genera, *Centropogon* and *Siphocampylus*, are not monophyletic. *Burmeistera* was found to be monophyletic, consistent with the presence of numerous putative morphological synapomorphies (see above). These were important findings, but of the ~530 species in these genera, only 41 (Knox et al., 2008) and 14 (Antonelli, 2008) species were included, respectively. Nonetheless, their results suggested that many of the subgeneric taxa of Wimmer and McVaugh are nonmonophyletic. *Centropogon* section *Centropogon*, however, was found to be monophyletic in both studies; it is characterized by scale-like concrescent hairs on the tips of the ventral anthers (Fig. 1E, arrow). Both studies also resolved *Lysipomia* Kunth, a genus of minute, occasionally suffrutescent herbs restricted to the high Andes, as sister to *Burmeistera*, *Centropogon*, and *Siphocampylus*. Despite these discoveries, however, much of the phylogeny remains uncertain because of low taxon sampling and the lack of phylogenetic resolution, especially along the spine of the tree. This presents a substantial obstacle for understanding how (1) fruit characters inform classification, (2) floral morphology has evolved, and (3) biogeographic history has influenced evolution in the group.

Here, we present a greatly revised phylogeny of *Centropogon*, *Burmeistera*, and *Siphocampylus* and their close relatives, based on five plastid DNA regions (*rpl32-trnL*, *ndhF-rpl32*, *rps16-trnK*, *trnG-trnG-trns*, *rbcL*). Our phylogeny includes nearly six times more species than the most comprehensive published phylogeny (Knox et al., 2008). This sampling covers most of the taxonomic, morphological, and geographic variation in these genera. It provides a strong starting point for recircumscription of taxa and sheds insight on the evolution of fruit type, a trait that is taxonomically, ecologically, and evolutionarily important. Finally, our study lays the foundation for future investigations into the pattern of floral diversification and biogeography in this charismatic and conspicuous group.

MATERIALS AND METHODS

Taxon selection—Members of all subdivisions of Wimmer's taxonomy within *Centropogon*, *Burmeistera*, and *Siphocampylus* were sampled, including more than one species from 11 of his 14 subsections and all 13 of his greges. Multiple individuals representing distinct populations were sampled in widespread and/or morphologically variable species. Additionally, multiple close relatives (*sensu* Antonelli, 2008) were sampled, including five *Lysipomia* species and three of the four species in the hexploid Chilean *Lobelia* section *Tupa* (G. Don.) Benth (Lammers, 2000). The majority of species we sampled are Neotropical. Two species in Campanuloideae, *Campanula medium* L. and *Triodanis perfoliata* (L.) Nieuwl., were used as outgroups to root the phylogeny (Antonelli, 2008). The majority of specimens were collected in the field by A.A., A.T., L.L., and N.M. during trips to Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Panama, Peru, and the United States. Leaf tissue was preserved using silica gel, and vouchers were deposited in various herbaria. Leaf fragments were extracted from dried herbarium specimens when Wimmer's subsections/greges were not represented by field collections. In total, 268

TABLE 1. Comparison of the phylogenetic results from this study with the subgeneric classification of *Burmeistera*, *Centropogon*, and *Siphocampylus*, largely following Wimmer. Subgeneric classifications of Wimmer (1943, 1953, 1968), McVaugh (1949), and Lammers (1998) are shown for *Centropogon*. "N/A" denotes insufficient taxon sampling to determine phylogenetic status; asterisk denotes a monotypic taxon.

Genus	Section	Subsection	Grex	Phylogenetic status
Burmeistera Triana				
	Imberbes nom. invalid. (= *Burmeistera*)			Nonmonophyletic
		Genuinae E. Wimm.		Nonmonophyletic
		Aequilatae E. Wimm.		N/A
	Barbatae E. Wimm.			Nonmonophyletic
Siphocampylus Pohl				
	Macrosiphon E. Wimm.			
		Hemisiphocampylus E. Wimm.		Nonmonophyletic
		Siphocampylus (A. DC.) E. Wimm.		Nonmonophyletic
			Ectropici E. Wimm.	Nonmonophyletic
			Verticillati E. Wimm.	Nonmonophyletic
			Umbellati E. Wimm.	Nonmonophyletic
			Botryoides E. Wimm.	Nonmonophyletic
			Dissitiflori E. Wimm.	Nonmonophyletic
		Byrsanthes (Presl) E. Wimm.		N/A
	Brachysiphon E. Wimm.			Nonmonophyletic
		Secundiflori E. Wimm.		Nonmonophyletic
		Altofissi E. Wimm.		Nonmonophyletic
			Barbiceps E. Wimm.	Nonmonophyletic
			Cremochilus E. Wimm.	Monophyletic*
		Megastomi E. Wimm.		Nonmonophyletic
		Megalandri E. Wimm.		N/A
Centropogon Presl (sensu Wimmer)				
	Centropogon Benth & Hook.			Monophyletic
		Corymboides E. Wimm.		Nonmonophyletic
			Campylobotrys E. Wimm.	Nonmonophyletic
			Amplifolii Zahlb.	Monophyletic
		Brevilimbati E. Wimm.		Nonmonophyletic
		Axillares Gleason		Monophyletic
	Siphocampyloides Benth & Hook			Nonmonophyletic
				Nonmonophyletic
			Formosi E. Wimm.	Nonmonophyletic
			Stellato-tomentosi Gleason	Nonmonophyletic
		Macranthi E. Wimm.		Nonmonophyletic
			Lehmannioides E. Wimm.	Nonmonophyletic
			Mandonioides E. Wimm.	Nonmonophyletic
Centropogon (sensu McVaugh and Lammers)				
	Centropogon Benth & Hook			Monophyletic
	Siphocampyloides Benth & Hook			Nonmonophyletic
		Brevilimibatii E. Wimm.		Nonmonophyletic
		Peruviani McVaugh		Nonmonophyletic
	Wimmeriopsis McVaugh			Nonmonophyletic
		Falcati McVaugh		Nonmonophyletic
		Colombiani McVaugh		Nonmonophyletic
	Burmeisteroides McVaugh			Nonmonophyletic
	Niveopsis Lammers			Monophyletic*

individuals were included; 93, 105, and 41 individuals represent *Siphocampylus*, *Centropogon*, and *Burmeistera*, respectively. Appendix S1 (see Supplemental Data with the online version of this article) contains a list of species with associated collection information and GenBank accession numbers.

***Molecular methods*—**Genomic DNA was extracted from leaf tissue using the Qiagen DNeasy kit (Qiagen, Valencia, California, USA). Rapidly evolving plastid markers were initially tested by L.L. in 10 phylogenetically diverse Lobelioideae using primers discussed in Shaw et al. (2007). Species for this preliminary analysis were selected according to the phylogenetic trees in Antonelli (2008) and Knox et al. (2008) and included *B. cyclostigmata* Donn. Sm., *B. vulgaris* E. Wimm., *S. umbellatus* (Kunth.) G. Don., *S. westinianus* Pohl, *C. cornutus* (L.) Druce, *C. granulosus*, *C. ferrugineus* (L.f.) Gleason, *C. costaricae* (Vatke) McVaugh, *Lysipomia muscoides* Hook f., and *Lobelia laxiflora* Kunth. We selected the four markers that showed the most variation: *rpl32-trnL*, *ndhF-rpl32*, *rps16-trnK*, and *trnG-trnG-trns*. The use of low-copy nuclear markers, which provides an important test of plastid phylogenies (Sang, 2002; Small et al., 2004), was attempted but was not successful. *Waxy* (Mason-Gamer et al., 1998) showed little sequence

variation, whereas *LFY* (Howarth and Baum, 2005), *cam* (Johansen, 2005), *NIA* (Howarth and Baum, 2002, 2005), and *rpb2* (Denton et al., 1998) exhibited complex duplication histories, which posed challenges for correct orthology assessment (data not shown). Additionally, the internal transcribed spacer from the nuclear ribosomal DNA (nrITS), a marker commonly used in plant phylogenetics, produced sequences with many multiply overlapping peaks, indicating multiple copies in our focal taxa (data not included). Moreover, aligned nrITS sequences did not yield reliable topologies in a preliminary phylogenetic analysis; they differed greatly from the plastid markers and contradicted previously published phylogenies (Antonelli, 2008, 2009; Knox et al., 2008). Explanations for these problems include duplication of tandem arrays, incomplete homogenization, misleading signal from concerted evolution following hybridization or introgression, and the presence of pseudogenes (Álvarez and Wendel, 2003). Chloroplast capture is an additional mechanism by which our plastid phylogeny would contradict a nuclear phylogeny (Rieseberg and Soltis, 1991; Soltis et al., 1991).

Polymerase chain reaction (PCR) amplification of the plastid markers used the following reaction mixture with the Qiagen *Taq* DNA polymerase kit: 1× CoralLoad Buffer, 0.4 mM MgCl2, 0.2 mM dNTPs, 0.4 µM forward primer,

0.4 μM reverse primer, 0.4 U Taq polymerase, for a final volume of 10 μL. The following thermocycler protocol was used: 7 min at 94°C, followed by 35 cycles of 1 min at 60°C, 1 min at 49°C (*rpl32-trnL*, *ndhF-rpl32*, *rps16-trnK*) or 55°C (*trnG-trnG-trnS*), 2 min at 72°C, and 10 min at 72°C. Amplification products were subsequently visualized on 1.5% agarose gels.

The PCR products were Sanger sequenced at Functional BioSciences (http://functionalbio.com) and Genewiz (http://www.genewiz.com/). Chromatograms were checked for quality, assembled into contigs, and edited using Geneious version 6.1.8 (http://www.geneious.com). Sequences of *rbcL* from previous molecular phylogenetic analyses (Knox et al., 2008, Antonelli, 2008, 2009) were obtained from GenBank and included for species that overlapped with our sampling. With the exception of these *rbcL* sequences, all molecular data were newly generated for this study. Alignments were inferred using MUSCLE (Edgar, 2004) as implemented in Geneious and then edited manually. Gaps were coded using gapcode.py version 2.1 (R. Ree, personal communication; http://www.bioinformatics.org/~rick/software.html) based on the method of Simmons and Ochoterena (2000). Individual markers did not show discordant relationships (>70 maximum-likelihood bootstrap percentage [BP]) and were combined and analyzed simultaneously.

Phylogenetic analysis—PartitionFinder version 1.1.1 (Lanfear et al., 2012) was used to determine an appropriate data-partitioning scheme from potential partitions that were defined a priori (in this case, each plastid locus), as well as the best-fitting model of molecular evolution for each partition, using the Bayesian Information Criterion. Maximum-likelihood analyses of the optimally partitioned data were performed using RAxML version 7.7.5 (Stamatakis, 2006; Stamatakis et al., 2008). The GTRGAMMA model of sequence evolution was determined to be the best-fitting model for each nucleotide partition. The MK model of character evolution for a binary state (Lewis, 2001) was determined to be the best model for the gap partition. The search for an optimal maximum likelihood tree was combined with a rapid bootstrap analysis of 1000 replicates. After an initial RAxML run, RogueNaRok (Aberer et al., 2013) was used to prune unstable taxa from the analysis. Each of the 18 excluded individuals lacked sequences for at least two of the four noncoding plastid markers. The resulting taxon sampling was used in all analyses presented. The RAxML analysis was repeated with this reduced taxon-sampling scheme. All RAxML analyses were performed on the Harvard Odyssey 2.0 cluster (http://rc.fas.harvard.edu/).

Two phylogenetic analyses with the reduced taxon sampling were conducted using Bayesian inference. The first was a partitioned analysis in MrBayes version 3.2.2 (Altekar et al., 2004; Ronquist et al., 2012) performed on the CIPRES cluster (http://www.phylo.org/). The parameters of each of the partitions were the same as in the maximum-likelihood analysis. Rate heterogeneity, base frequencies, and substitution rates across partitions were unlinked. The analysis was allowed to run for 100 million generations across eight independent runs each with four chains, sampling every 10 000 generations. Convergence of the chains across runs was explored in Tracer version 1.5 (Rambaut and Drummond, 2003), and convergence of topologies and clade stability were confirmed using AWTY (Nylander et al., 2008). The first 25% of the trees from all runs were excluded as burn-in before making a majority-rule consensus of the 7500 posterior distribution trees using the "sumt" function. This analysis was repeated with identical parameters but excluding gap characters to confirm that these characters did not bias the results (see Supplemental Data with the online version of this article, Appendix S2).

We performed a second Bayesian inference analysis on CIPRES using PhyloBayes, implementing a mixture model using CAT (Lartillot and Philippe, 2004). This analysis was performed with two independent chains on the partitioned molecular data using a different CAT-GTR model for each partition, with gap data excluded. The analysis was allowed to run until the maxdiff value was 0.1, and the minimum effective size was 50. Convergence parameters were calculated after excluding the first 500 generations, and assessed using Tracer.

Alternative topology testing—We used the approximately unbiased (AU) test (Shimodaira, 2002) to examine alternative suboptimal topologies. Three phylogenetic hypotheses based on Wimmer's (1953) taxonomy that were not supported in our analyses were tested: (1) the monophyly of *Centropogon*, (2) the monophyly of *Siphocampylus*, and (3) the monophyly of mainland *Siphocampylus* (i.e., excluding the Caribbean subsection *Hemisiphocampylus*). Additionally, two hypotheses based on fruit evolution were tested: (1) monophyly of species with capsular fruits and (2) monophyly of species with berry fruits. Topologies conforming to these hypotheses were created in Mesquite version 2.5 (Maddison and Maddison, 2011) and used to perform constrained phylogenetic analyses in RAxML using the settings described above. The per site likelihood scores were calculated in RAxML (-f g option) from the constrained and unconstrained maximum-likelihood topologies. The AU test was performed using the site-likelihoods and the relltest function in scaleboot (Shimodaira, 2008) in R version 3.0.2 (R Core Development Team, 2013).

Fruit evolution—Fruit evolution was explored via Bayesian stochastic character mapping (Bollback, 2006). All taxa were coded as berry (0) or capsule (1), from specimens or the literature (see Supplemental Data with the online version of this article, Appendix S3). Before performing the stochastic character mapping, the optimal maximum-likelihood tree, trimmed to include only the clade containing *Centropogon*, *Burmeistera*, mainland *Siphocampylus*, *Lysipomia*, and *Lobelia* section *Tupa*, was made ultrametric using the chronoPL function in the ape package (Paradis et al., 2004) in R. This implements the penalized likelihood method of Sanderson (2002). Ancestral state reconstructions were initially performed using standard maximum likelihood methods using the equal rates ("ER") and all-rates-different ("ARD") models using the ace function. A likelihood-ratio test was subsequently performed to determine the optimal model. Ancestral character states were then estimated from 1000 iterations of Bayesian stochastic character mapping under the optimal model ("ER") using the make.simmap function in the phytools package (Revell, 2012). The Q transition matrix was fixed at the most likely values (i.e., the empirical Bayes method; Q = "empirical"). This analysis calculated ancestral states at each node as the marginal posterior probabilities for both character states, which relies on the ultrametric RAxML topology.

RESULTS

Characteristics of the plastid data set—The final concatenated matrix included 7282 bp of aligned sequence data and 207 gap characters for 268 individuals from 199 species; 239 individuals from 172 species in the genera *Centropogon*, *Burmeistera*, and *Siphocampylus* were represented—approximately one-third of the described species in these genera. Table 2 contains the summary statistics for the individual markers and for the coverage of each marker in our data set.

Phylogenetic reconstructions, topology congruence, and taxonomic implications—The results of each of the three phylogenetic analyses were largely congruent (Fig. 3). None of the relationships that differed between trees were well supported (i.e., >80 BP or PP), and any differences below this threshold were generally restricted to closely related taxa. All data matrices are deposited in the Dryad repository (doi:10.5061/dryad.8t4gp).

Centropogon, *Siphocampylus*, and *Burmeistera* do not form a clade. Five *Siphocampylus* species form a paraphyletic grade that fall outside of the "CBS clade" with high support (BP = 100,

TABLE 2.   Summary statistics of plastid data sets.

Locus	Total length (bp)	Variant characters (*n*)	Parsimony-informative characters (*n*)	Gap characters (*n*)	Coverage (%)
ndhF-rpl32	1623	589	361	67	87.7
rpl32-trnL	1083	878	522	51	97.0
rps16-trnK	1188	656	439	63	98.9
trnG-trnG-trnS	1694	759	434	24	80.2
rbcL	3821	404	165	3	12.7

Bayesian posterior probability percentage [PP] = 100 [PP from MrBayes]/100 [PP from PhyloBayes]) (Fig. 3). All species in this grade are included in Wimmer's *Siphocampylus* subsection *Hemisiphocampylus*, which comprises all and only Caribbean *Siphocampylus* species. This grade is most closely related to the pantropical weed *Hippobroma longiflora* (L.) G. Don., which is native to Jamaica, and to the Caribbean endemic *Lobelia martagon* Hitchc.

We refer to the newly identified clade that includes all *Centropogon*, *Burmeistera*, and mainland *Siphocampylus* species as the "centropogonids" (Fig. 3; BP = 96, PP = 100/100). *Siphocampylus fulgens* Hort. is well supported (BP = 80, PP = 98/100) as sister to the rest of the centropogonids. As has been the case in previous phylogenetic analyses of the centropogonids (Antonelli, 2008; Knox et al., 2008), branch lengths separating species were short (Fig. 3, inset phylogram; see Supplemental Data with the online version of this article, Appendix S4). However, unlike in previous analyses, moderate to high support values were estimated along the majority of the backbone, and often toward the tips of the phylogeny (Fig. 3).

Among the focal genera, *Burmeistera* is the only monophyletic genus (BP = 100, PP = 100/100) (Fig. 3). *Centropogon* and *Siphocampylus* are polyphyletic with respect to one another (Fig. 3). Moreover, subgeneric taxa in all three genera are largely not monophyletic. The exceptions are *Centropogon* section *Centropogon* and the monotypic taxa highlighted in Table 1. Members of *Centropogon* form at least seven clades that are broadly distributed within the centropognid clade. Most *Centropogon* species (89%) belong to five subclades that correspond to morphologically cohesive units, which reflect currently accepted taxonomy (*sensu* McVaugh, 1949). These five well-supported subclades, which we characterize in detail in the Discussion, are (1) the brevilimbatiids (BP = 64, PP = 100/100), (2) the peruvianids (BP = 85, PP = 100/100), (3) the colombianids (BP = 93, PP = 96/99), (4) the burmeisterids (including the entire genus *Burmeistera*) (BP = 87, PP = 100/100), and (5) the eucentropogonids (BP = 95, PP = 100/100). The eucentropogonids can be further divided into three subclades, which closely reflect the classification of Stein (1987a). By contrast, *Siphocampylus* species are scattered throughout the phylogeny, and most species do not fall into well-supported, morphologically coherent clades.

AU tests rejected the monophyly of (1) *Centropogon* and (2) *Siphocampylus* ($P < 0.01$ and $P < 0.01$ respectively). They additionally rejected the monophyly of (3) mainland *Siphocampylus* (i.e., the monophyly of *Siphocampylus* when excluding the more distantly related Caribbean *Siphocampylus* in subsection *Hemisiphocampylus*; $P < 0.01$).

We find the large cosmopolitan genus *Lobelia* to be polyphyletic. Members of the genus form a series of lineages including species in the genera *Diastatea* Scheidw., *Hippobroma* G. Don., *Isotoma* (R. Br.) Lindl., and *Siphocampylus*, which are successively sister to the *Lysipomia* plus centropogonid clade (Fig. 3). These findings corroborate previous studies (Antonelli, 2008, 2009; Knox, 2014). This highlights that additional phylogenetic efforts within *Lobelia* will be important, because this genus represents more than a third of the species within the subfamily Lobelioideae, yet remains sparsely sampled. Lammers' (2011) revision of *Lobelia* will provide an important starting point for further targeted phylogenetic sampling to facilitate future reclassification.

Lysipomia is monophyletic (BP = 100, PP = 100/100) and placed sister to the centropogonids with high support (BP= 100,

PP = 100/100) (Fig. 3), agreeing with the results of Antonelli (2008, 2009) and Knox et al. (2008). Within *Lysipomia*, the monophyly of subgenus *Lysipomia* (A. DC.) E. Wimm. is supported (BP = 100, PP = 100/100), but subgenus *Rhizocephalum* (Wedd.) E. Wimm. is paraphyletic.
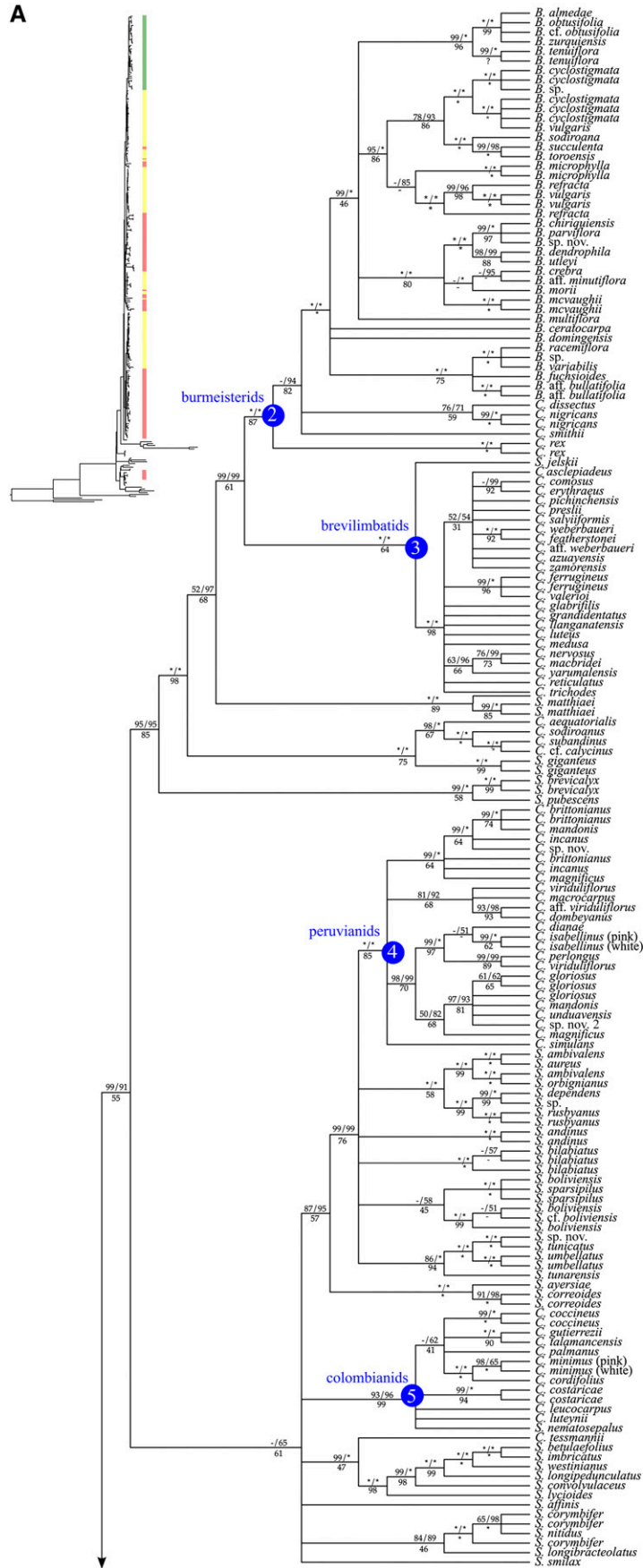
The hexaploid ($n = 21$) species that constitute *Lobelia* section *Tupa* (Lammers and Hensold, 1992; Lammers, 1993, 2000), the Chilean lobelias, form a well-supported clade (BP = 100, PP = 100/100) that is moderately to weakly placed as sister to the centropogonids plus *Lysipomia* (BP = 58, PP = 65/85; Fig. 3). This clade collectively represents a broad pan-Andean distribution spanning temperate and tropical latitudes. Knox et al. (2008) similarly placed the Chilean lobelias sister to the centropogonids plus *Lysipomia*. Antonelli (2008) placed them as more distantly related to the centropogonids, but with low support.

Fruit evolution—Bayesian stochastic character mapping demonstrated that the ancestral fruit type of centropogonids is unequivocally capsular (PP = 100; Fig. 4). These results were corroborated by our maximum-likelihood character state reconstruction (see Supplemental Data with the online version of this article, Appendix S5). On average, 12.2 transitions between character states were estimated on the phylogeny of the ((centropogonid, *Lysipomia*), *Lobelia* section *Tupa*) clade. Of these, 7.5 were from capsule to berry and 4.7 were transitions from berry to capsule. On average, 26.1% of the branch space (i.e., including branch lengths) is reconstructed as in the berry state and 73.9% as in the capsule state. The monophyly of capsular and berry-fruited species was rejected by the AU test (both $P < 0.01$).

DISCUSSION

We present the first well-resolved, densely sampled phylogeny of *Burmeistera*, *Centropogon*, and *Siphocampylus*. Our plastid phylogeny greatly improves on prior taxon-sampling efforts by including ~33% of the species in the centropogonid genera; previous studies have included at most ~8% (Knox et al., 2008). Our results indicate that the taxonomy of the centropogonids does not, for the most part, reflect evolutionary relationships. In lieu of proposing a new formal classification for the group, we discuss the existing classification of the centropogonids in light of our new phylogeny. We then discuss the dynamic pattern of fruit evolution in centropogonids and suggest future directions for investigating Andean biogeography.

Systematics—*Intergeneric phylogeny and taxonomy*—The well-supported placement (BP = 100, PP = 100/100) of the Caribbean-endemic *Siphocampylus* subsection *Hemisiphocampylus* in a clade with other Caribbean lobeloids, including *Lobelia martagon* and *Hippobroma longiflora*, renders the CBS clade (*sensu* Batterman and Lammers, 2004) nonmonophyletic (Fig. 3). Despite their shared Caribbean distribution, members of this clade exhibit diverse morphologies: species in *Siphocampylus* subsection *Hemisiphocampylus* are suffrutescent subshrubs with reddish flowers, whereas *Hippobroma longiflora* (L.) G. Don. is a low, weedy rosette plant that produces white flowers with long, narrow, tubular corollas. These phylogenetic results provide strong support for taxonomic separation of the Caribbean *Siphocampylus* from mainland centropogonids. Species in subsection *Hemisiphocampylus* have traditionally been circum-

A

B. almedae
B. obtusifolia
B. cf. obtusifolia
B. zurquiensis
B. tenuiflora
B. tenuiflora
B. cyclostigmata
B. cyclostigmata
B. sp.
B. cyclostigmata
B. cyclostigmata
B. cyclostigmata
B. cyclostigmata
B. vulgaris
B. sodiroana
B. succulenta
B. toroensis
B. microphylla
B. microphylla
B. refracta
B. vulgaris
B. vulgaris
B. refracta
B. chiriquiensis
B. parviflora
B. sp. nov.
B. dendrophila
B. utleyi
B. crebra
B. aff. minutiflora
B. morii
B. mcvaughii
B. mcvaughii
B. multiflora
B. ceratocarpa
B. domingensis
B. racemiflora
B. sp.
B. variabilis
B. fuchsioides
B. aff. bullatifolia
B. aff. bullatifolia

burmeisterids ②

C. dissectus
C. nigricans
C. nigricans
C. smithii
C. rex
C. rex
S. jelskii
C. asclepiadeus
C. comosus
C. erythraeus
C. pichinchensis
C. preslii
C. salviiformis
C. weberbaueri
C. featherstonei
C. aff. weberbaueri
C. azuayensis
C. zamorensis
C. ferrugineus
C. ferrugineus
C. valerioi
C. glabrifilis
C. grandidentatus
C. tunganatensis
C. luteus
C. medusa
C. nervosus
C. macbridei
C. yarumalensis
C. reticulatus
C. trichodes
S. matthiaei
S. matthiaei
C. aequatorialis
C. sodiroanus
C. subandinus
C. cf. calycinus
S. giganteus
S. giganteus
S. brevicalyx
S. brevicalyx
S. pubescens

brevilimbatids ③

C. brittonianus
C. brittonianus
C. mandonis
C. incanus
C. sp. nov.
C. brittonianus
C. incanus
C. magnificus
C. viriduliflorus
C. macrocarpus
C. aff. viriduliflorus
C. dombeyanus
C. dianae
C. isabellinus (pink)
C. isabellinus (white)
C. perlongus
C. viriduliflorus
C. gloriosus
C. gloriosus
C. gloriosus
C. mandonis
C. unduavensis
C. sp. nov. 2
C. magnificus
C. simulans

peruvianids ④

S. ambivalens
S. aureus
S. ambivalens
S. orbignianus
S. dependens
S. sp.
S. rusbyanus
S. rusbyanus
S. andinus
S. andinus
S. bilabiatus
S. bilabiatus
S. bilabiatus
S. boliviensis
S. sparsipilus
S. sparsipilus
S. boliviensis
S. cf. boliviensis
S. boliviensis
S. sp. nov.
S. tunicatus
S. umbellatus
S. umbellatus
S. tunarensis
S. ayersiae
S. correoides
S. correoides
C. coccineus
C. coccineus
C. gutierrezii
C. talamancensis
C. palmanus
C. minimus (pink)
C. minimus (white)
C. cordifolius
C. costaricae
C. costaricae
C. leucocarpus
C. luteynii
S. nematosepalus
S. tessmannii
S. betulaefolius
S. imbricatus
S. westinianus
S. longipedunculatus
S. convolvulaceus
S. lycioides
S. affinis
S. corymbifer
S. corymbifer
S. nitidus
S. corymbifer
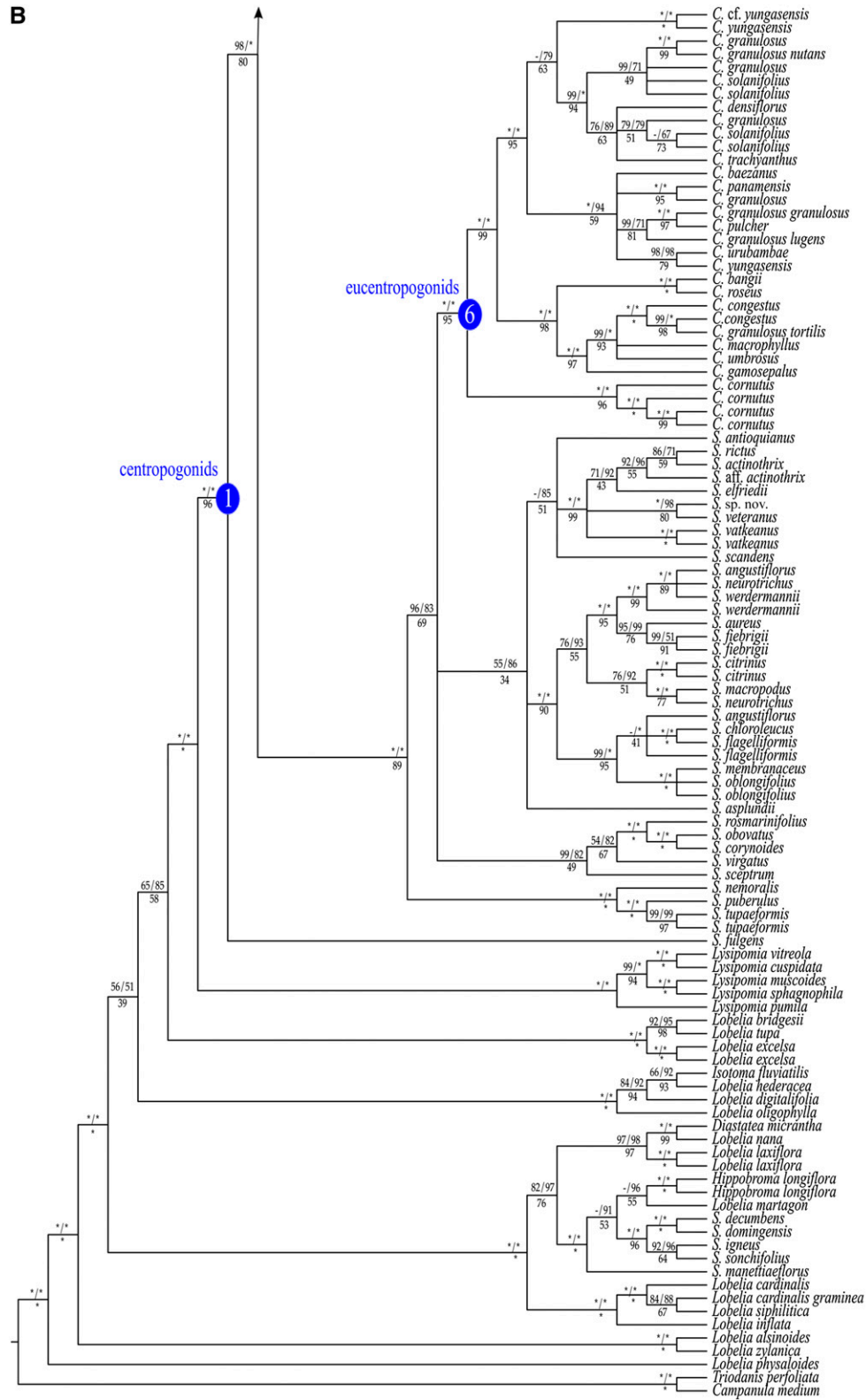S. longibracteolatus
S. smilax

colombianids ⑤

Fig. 3.   Five-locus plastid phylogeny of Neotropical Lobelioideae. Bayesian posterior probabilities (MrBayes/PhyloBayes) are shown above branches. Maximum-likelihood bootstrap support values are shown below branches. Asterisk indicates highest possible support value; dash indicates that the branch was not supported in the relevant analysis. The phylogram, depicting branch lengths, is inset (also see Appendix S4 for full phylogram with tip labels and scale bar). Species in the genera *Burmeistera*, *Centropogon*, and *Siphocampylus* are color-coded green, yellow, and red, respectively, in the rectangles at right in both the cladogram and phylogram. Blue circles at nodes in the cladogram indicate the six well-supported subclades described in detail in the text: (1) centropogonids, (2) burmeisterids, (3) brevilimbatids, (4) peruvianids, (5) colombianids, and (6) eucentropogonids.
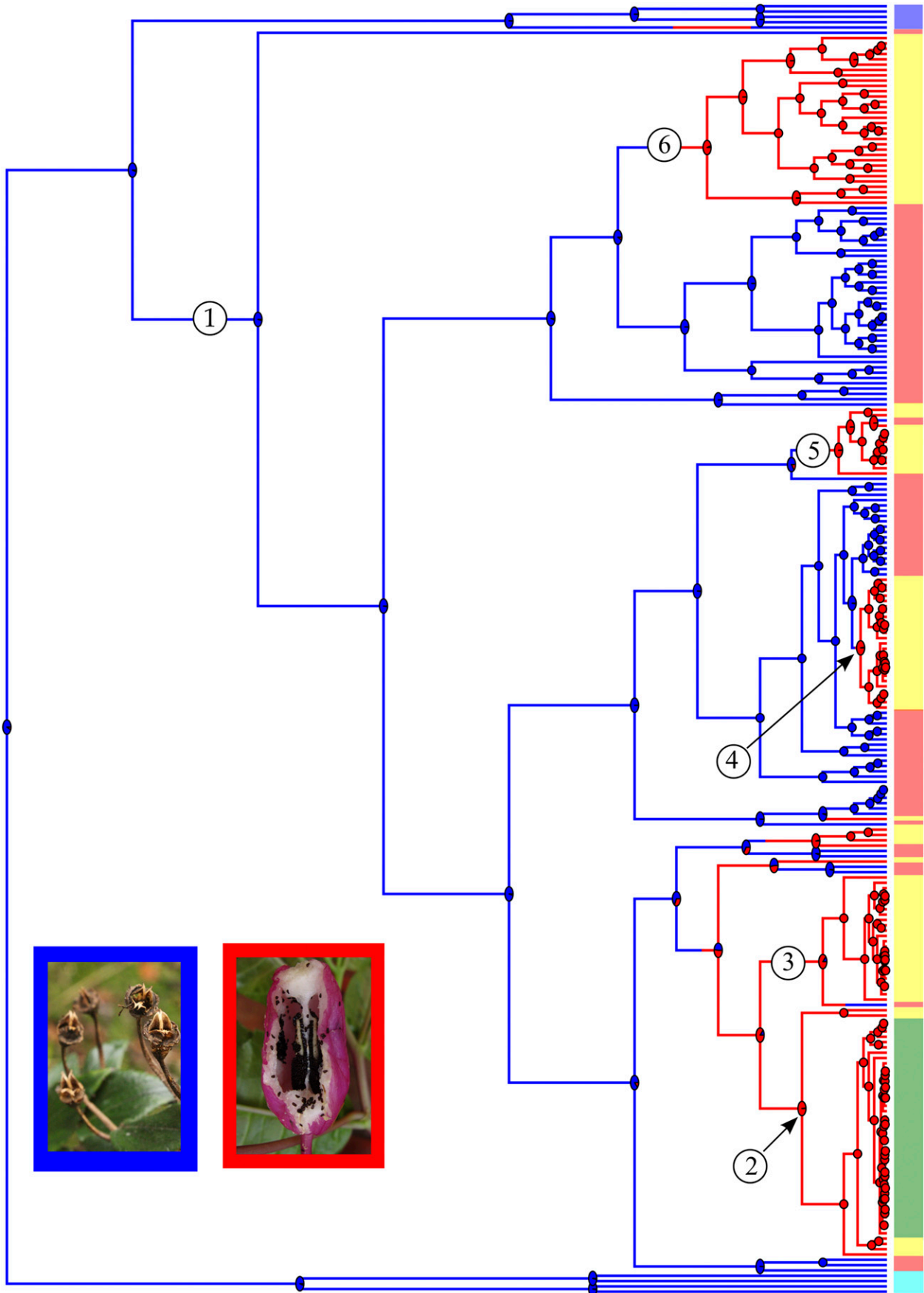
scribed within *Siphocampylus* largely on the basis of their shared Caribbean distribution. However, they also differ notably from other *Siphocampylus* in possessing a fenestrate corolla, and they have a generally lower vegetative stature. Further phylogenetic and morphological sampling are required before names can be applied to the *Siphocampylus*, *Hippobroma*, and *Lobelia* species that constitute this clade.

Our newly discovered mainland centropogonid clade is not reflected in any previous classification. Our phylogeny renders the two tribes within subfamily Lobelioideae (Wimmer, 1943, 1953, 1968), the berry-fruited Delisseeae Rchb. (= Bacciferae E. Wimm.), and the capsular-fruited Lobelieae Presl., nonmonophyletic, a result that corroborates the reconstruction of fruit type within the subfamily by Givnish et al. (2009). This suggests that tribal circumscriptions of Lobelioideae have been misled by an overreliance on the single character of fruit type. This is not unique to the Lobelioideae; the classification schemes of several large tropical angiosperm clades have been guided by fruit type, only to have elements of these schemes shown to be nonmonophyletic by subsequent molecular phylogenetic systematics (e.g., Melastomataceae Juss. [Clausing et al., 2000], Malpighiaceae Juss. [Davis et al., 2001; Davis and Anderson, 2010], and Verbenaceae J. St.-Hil. [Marx et al., 2010]). In the case of Lobelioideae, a major overhaul of the tribal classification within the subfamily is necessary, as recognized previously by Lammers (2004, 2007a, 2011).

We determined that *Burmeistera* form a well-supported clade, which confirms previous findings (Antonelli, 2008, 2009; Knox et al., 2008). *Burmeistera* was originally described by Triana (1854), and his concept for the genus has been retained and subsequently refined (Wimmer, 1943, 1953, 1968; Wilbur, 1975; Nash, 1976; Jeppesen, 1981; Stein, 1987b). Species in the genus are characterized by several putative synapomorphies, including an inflated corolla opening, a dilated anther orifice, and isodiametric seeds. In the past, many *Centropogon* and *Siphocampylus* species have been placed in *Burmeistera* because of their superficial similarity due to convergent evolution in floral morphology (e.g., many species in *Centropogon* section *Burmeisteroides* McVaugh, and the former *Burmeistera* section *Aequilatae* E. Wimm., now placed in *Siphocampylus*; Stein, 1987b).

Our findings indicate that generic concepts of *Centropogon* and *Siphocampylus* are problematic. The discrepancy between taxonomy and phylogeny we have identified likely results from the fact that fruit type was the basis for circumscription of each genus: *Centropogon* have berries, and *Siphocampylus* have capsules. Many students of Lobelioideae have questioned the utility of fruit type in distinguishing these two genera, given the wide overlap of nonfruit characters (Gleason, 1921; Stein, 1987b; Lammers, 1998). Although previous studies have documented the nonmonophyly of *Centropogon* and *Siphocampylus* (Antonelli, 2008, 2009; Knox et al., 2008), our results provide much greater detail on the extent to which these genera are polyphyletic. *Siphocampylus* are the least resolved: its species

fall into at least 11 distantly related clades. *Centropogon* fall into six well-supported clades and one grade that constitutes at least two separate clades (Fig. 3).

Infrageneric phylogeny and taxonomy—Most previously circumscribed subgenera in *Burmeistera*, *Centropogon*, and *Siphocampylus* are polyphyletic. We discuss this finding in the context of two important contributions to the taxonomy of lobelioids: Wimmer's (1943, 1953, 1968) comprehensive monograph of the Lobelioideae, and McVaugh's refinements to Wimmer's classification of *Centropogon* (McVaugh, 1949). Together, these works form the basis of the currently accepted classification in these genera (Lammers, 1998, 2002, 2007b). In the following section, we consider the classification within each genus, and denote nonmonophyletic genera, when necessary, with quotation marks.

"*Siphocampylus*"—The 11 subclades of *Siphocampylus* that we identified are not well resolved with respect to each other. Moreover, the species that constitute each of these clades are often not easily characterized by morphology, distribution, or ecology, even in cases where clade support is high. Refining the placement of the constituent subclades of *Siphocampylus* remains fertile ground for future investigation. When this information is combined with detailed morphological investigations, we anticipate a clearer direction for dividing this group into monophyletic subunits.

Burmeistera—The monophyly of *Burmeistera* is well supported here (BP = 100, PP = 100/100) (Fig. 3) and in previous studies (Antonelli, 2008; Knox et al., 2008). Within *Burmeistera*, however, Wimmer's two sections are not monophyletic (Table 1). Those sections, "*Imberbes*" nom. invalid (section *Burmeistera*, *sensu* Lammers, 1998) and *Barbatae* E. Wimm., are based on anther hair type (glabrous or sparsely pubescent, vs. barbate). This character appears to have a complex evolutionary history in this clade and should not be used alone to delineate taxa in *Burmeistera*.

"*Centropogon*"—Among the minimum of eight lineages of *Centropogon*, only one of Wimmer's taxa, section *Centropogon*, is monophyletic (Table 1). Five of these *Centropogon* subclades form the basis of our subsequent discussion because they can be delineated by morphology and exhibit reasonable overlap with currently accepted taxonomies (McVaugh, 1949; Lammers, 1998). We refer to these well-supported subclades informally as the brevilimbatids (BP = 64, PP = 100/100), eucentropogonids (BP = 95, PP = 100/100), peruvianids (BP = 85, PP = 100/100), colombianids (BP = 99, PP = 93/96), and burmeisterids (BP = 87, PP = 100/100) (Fig. 3). All clade names are derived from previous taxonomies (Wimmer, 1943; McVaugh, 1949).

The burmeisterids. The burmeisterid clade includes *Burmeistera* plus a grade of robust, green-flowered *Centropogon* species that fall into at least two separate lineages, which are successively sister to *Burmeistera*. These *Centropogon* species

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Fig. 4.   Bayesian stochastic character mapping of fruit evolution. Pie charts at nodes represent ancestral states that were calculated as the marginal posterior probability of capsule (blue) and berry (red). White circles at internodes or indicated by arrows correspond to the six subclades we define in the text, numbered according to Fig. 3: (1) centropogonids, (2) burmeisterids, (3) brevilimbatids, (4) peruvianids, (5) colombianids, and (6) eucentropogonids. Bars to the right of the phylogeny are color-coded according to taxon: *Lysipomia* (purple), *Centropogon* (yellow), *Siphocampylus* (red), *Burmeistera* (green), and *Lobelia* section *Tupa* (light blue). Photos of representative fruits are of *Siphocampylus ayersiae* Lammers (capsule) and *Burmeistera toroensis* Wilbur (berry). (Photos: L. Lagomarsino.)

are placed in two greges of *Centropogon* section *Siphocampyloides* Benth. & Hook. subsection *Macranthi* E. Wimm. by Wimmer. In McVaugh (1949), they are placed in *Centropogon* section *Burmeisteroides* Gleason.

The burmeisterids occur from Guatemala to northern Peru. They share an inflated corolla opening, which has previously been recognized as a defining trait of *Burmeistera*. This represents a potential synapomorphy for the entire burmeisterid clade. Additionally, burmeisterid species tend to have robust, green flowers, many of which are known to be pollinated by long-tongued bats (Muchhala, 2006b; Muchhala and Potts, 2007; Muchhala and Thomson, 2009). The grade of *Centropogon* species differs from *Burmeistera* in their erect, soft-woody shrub habit, larger flowers, constricted anther orifice, and seeds that are slightly longer than wide. The morphology of berries within burmeisterids is quite variable (e.g., Fig. 2E–F).

Species within McVaugh's section *Burmeisteroides* fall into at least two distinct and distantly related clades, the burmeisterids and the peruvianids. This is an example where the classification groups distantly related species using features that appear to have evolved convergently. Both clades include shrubby species that have large, cream-colored or greenish flowers with wide openings and large anthers that produce abundant pollen. This combination of characters is widely accepted as part of the bat-pollination syndrome (e.g., Fleming et al., 2009), which likely arose more than once within the centropogonids (L. Lagomarsino, unpublished data).

The brevilimbatids. The brevilimbatids are sister to the burmeisterids and include two subclades: the capsular-fruited *S. jelksii*, and a second, well-supported (BP = 98, PP = 100/100) subclade including numerous berry-fruited *Centropogon* species. The berry-fruited brevilimbatids largely comprise species from Wimmer's *Centropogon* section *Siphocampyloides* subsection *Brevilimbatii* grex *Stellato-tomentosi*. This grex is not monophyletic, as it also includes species that we place separately in the peruvianid clade (see below). Additionally, the glabrous species we place in the brevilimbatid clade (i.e., *C. valerioi*, *C. grandidentatus* [Schltdl.] Zahlbr., and *C. trichodes* E. Wimm.) include some, but not all, of Wimmer's subsection *Brevilimbatii* E. Wimm. grex *Formosi* E. Wimm. This grex is also nonmonophyletic, with numerous members belonging in our colombianid clade (see below). Within McVaugh's taxonomy, the berry-producing brevilimbatid subclade corresponds closely to *Centropogon* section *Siphocampyloides* subsection *Brevilimbatii*. However, glabrous species in the brevilimbatid clade were placed elsewhere by McVaugh in his *Centropogon* section *Wimmeriopsis* McVaugh subsection *Falcati* McVaugh.

The berry-fruited brevilimbatids are scandent plants of mostly exposed areas with a uniform floral form. They possess long, narrow, orange corolla tubes with falcate, narrowly triangular yellow lobes and steel gray anthers that are glabrous or sparsely pubescent (Fig. 1C). Berries in these species are leathery (Fig. 2D). Most species have leaves, stems, and corollas that are covered in tawny, branched trichomes. *Siphocampylus jelskii*, the sister to the rest of the brevilimbatids, differs in its capsular fruit and growth from, which is characterized by tall stems with large terminal leaf rosettes, much like species in the genus *Espeletia* Mutis ex Bonpl. (Asteraceae), which also occur in high-altitude Andean grasslands (e.g., páramo and puna). In addition, its large greenish flower with wide opening is more similar to the burmeisterids than the rest of the brevilimbatids. While *S. jelskii* shares these aspects of its floral morphology with the burmeiste-

rids, it overlaps in its high-elevation habitat with the rest of the brevilimbatids, many of which prefer habitats above tree line.

The peruvianids. The peruvianids mainly comprise taxa from Wimmer's *Centropogon* section *Siphocampyloides* subsection *Macranthi* grex *Mandonioides* E. Wimm., and to a lesser extent from his subsection *Brevilimbatii* grex *Stellato-tomentosi* E. Wimm. Most peruvianids were placed in *Centropogon* section *Siphocampyloides* subsection *Peruvianii* by McVaugh. However, one species from outside McVaugh's subsection *Peruvianii* falls into this clade: *C. incanus* (Britton) Zahlbr. (section *Burmeisteriodes*; see discussion above). *Centropogon dianae* Lammers, the only species in the monotypic section *Niveopsis* Lammers (Lammers, 1998), is deeply nested within the peruvianids.

The peruvianids are tall (>3 m), robust shrubs of the central Andes of Peru and Bolivia. They frequently have branched trichomes, and both vegetative surfaces and flowers emit a musky odor. They have large, sturdy flowers with corollas of various colors. The corolla lobes are usually narrowly triangular and falcate (Fig. 1B), but these can sometimes be deltate and decurved (e.g., *C. dianae*). Anther connectives bear dense, long, simple hairs. They produce the largest berries among the centropogonids (Fig. 2C).

The colombianids. The colombianids encompass part of Wimmer's *Centropogon* section *Siphocampyloides* subsection *Brevilimbatii* grex *Formosii*, with the exception of *Siphocampylus nematosepalus* (Donn. Sm.) E. Wimm., which he placed in *Siphocampylus* section *Macrosiphon* E. Wimm. subsection *Siphocampylus* (A. DC.) E. Wimm. grex *Dissitiflori* E. Wimm. Remaining members of grex *Formosii* occur throughout the centropogonid phylogeny, rendering it nonmonophyletic. In McVaugh's treatment, the colombianids correspond closely to *Centropogon* section *Wimmeriopsis* subsection *Colombiani* McVaugh, with two exceptions. His treatment did not extend to *Siphocampylus* and so could not have included *S. nematosepalus* (which we place among the colombianids). Also, we place *C. tessmannii* E. Wimm., which McVaugh included in subsection *Colombiani*, outside of the colombianids.

The colombianids are restricted to premontane and montane regions of Central America and the adjacent Chocó region of Colombia. Members of this clade are scandent or climbing vines, either woody or herbaceous, with a small stem diameter and leaves with pronounced dentation. The corolla is always long–tubular and bright pink or red, with narrowly triangular lobes that are usually erect or spreading (Fig. 1D). Fruit form is variable: berries and capsules are both present, and berries are either dry–leathery or fleshy with a thin exocarp (Fig. 2B). Given its fruit type, *S. nematosepalus* is not currently placed in the same genus as the rest of the colombianids. However, despite its being the only member of its clade that produces capsules, the rest of its morphology is otherwise very similar to that of other colombianid species.

The eucentropogonids. The eucentropogonid clade corresponds completely to Wimmer's and McVaugh's section *Centropogon*, which was elevated to subgeneric rank and renamed *Centropogon* (Wilbur, 1976). The most recent monograph and taxonomic revision of this clade was by Stein (1987a). The name "eucentropogonids" comes from their taxonomic history: *Centropogon* section *Centropogon* was originally described as section *Eucentropogon*, but the International Code of Botanical Nomenclature no longer allows this naming convention, as detailed in Article 21.3.

The eucentropogonids' primary distinguishing characteristic is a cornute scale-like appendage of concrescent hairs at the base of the two lower anthers (Fig. 1E, arrow), a trait that functions in their pollination biology. Flowers, which are oriented vertically, are often abruptly curved (Fig. 1E) and are frequently arranged in erect terminal inflorescences. This is likely an adaptation for pollination by sicklebill hummingbirds (*Eutoxeres*: Phaethornidae; Stein, 1992). Plants are vegetatively glabrous or sparsely pubescent and occur at lower elevations than the rest of the centropogonids, which occur more broadly from sea level to ~1500 m.

Based on our sampling, the eucentropogonids can be subdivided into three subclades. One consists solely of the widespread, though morphologically homogenous, *Centropogon cornutus*, whose monophyly is strongly supported (BP = 96, PP = 100/100). It is a scandent subshrub with light pink, moderately curved corollas that grows at low elevations, including in the Amazon basin. Although it is a single species, branch lengths between individuals are among the longest within the ingroup (Appendix S4), which suggests that there may be cryptic species within this group. The second subclade (BP = 95, PP = 100/100) corresponds largely to Wimmer's grex *Campylobotrys* E. Wimm. (and completely to Stein's subsection of the same name). These are vining subshrubs of low to mid-elevations with corollas with tubes that are are extremely curved and tend to be red in color (Fig. 1E). The corollas have bright yellow deltate lobes; the dorsal pair are strongly recurved, and the ventral three are reflexed. The most common and widespread species in this clade is *Centropogon granulosus*, which we find is not monophyletic (Fig. 3). The third subclade (BP = 98, PP = 100/100) consists of species that are placed in various greges of Wimmer's section *Centropogon*, but whose membership corresponds exactly to Stein's subsection *Amplifolii*. These species are erect clonal herbs with bright pink flowers that have extremely curved corollas. They grow in wet soils at low to mid-elevations. When they occur at lower elevations, they frequently develop extrafloral nectaries between calyx lobes, thought to attract ants as defense against herbivory (Stein, 1992).

Fruit evolution—The lability of fruit evolution in the centropogonids is striking. Stochastic character mapping shows that the ancestor of the centropogonids was capsular, with an average of 7.5 more recent origins of berries (Fig. 4). While the majority of transitions are from capsule to berry, on average 4.7 reversions from berry to capsule are inferred, suggesting that fruit evolution is not unidirectional in the centropogonids. Two of these inferred reversals, representing the independent evolution of capsules in *S. jelskii* and *S. nematosepalus*, are supported very strongly in our reconstructions. Given the homoplasy in fruit type, it is unlikely that fruit types are homologous across the centropogonids. In this regard, it is not surprising that the gross morphology of berries is quite variable among centropogonids. They can be dry and leathery with a rounded distal end (e.g., the brevilimbatids; Fig. 2D); round with opaque, juicy mesocarp and a leathery, ribbed exterior (e.g., in the eucentropogonids and peruvianids; Fig. 2C); somewhat inflated with spongy mesocarp (e.g., *B. toroensis* Wilbur; Fig. 2F); much inflated and thin-walled, lacking a fleshy mesocarp (e.g., *B. vulgaris*; Fig. 2E); or oblong with thin exocarp and viscous pericarp (Fig. 2B). By contrast, capsule morphology within the centropogonids is not very diverse: they are bilocular and open apically via two valves (Fig. 2A). This suggests that the ancestral, centropogonid-type capsule was modified in various ways to form a fleshy, animal-dispersed berry.

More broadly within Campanulaceae, Givnish et al. (2009) demonstrated that fleshy fruits have evolved in at least six distantly related clades outside of the centropogonids. Our analysis is consistent with this broader view that fruit evolution is particularly labile in Campanulaceae, but our results suggest that patterns of fruit evolution may be far more dynamic. An in-depth morphological and developmental survey of fruits in Campanulaceae would aid in characterizing fruit evolution in the family. This could be coupled with molecular developmental genetics to determine the genes underlying the independent, potentially distinct developmental transitions between fruit type, within both the closely related centropogonids and the more distantly related Campanulaceae. The FRUITFULL MADS-box gene lineage may be of particular interest in this endeavor (Pabón-Mora and Litt, 2011; Knapp and Litt, 2013; A. Litt et al., unpublished data).

More generally, transitions between fleshy and dry fruits have been important in angiosperm evolution. It is thought that the evolution of fleshy fruit may be an adaptation to facilitate animal dispersal in forest understories (Gentry, 1982; Givnish et al., 2005), including within Campanulaceae (Givnish et al., 2009). Some of our data are consistent with this hypothesis. For example, capsular taxa in the centropogonids (i.e., *Siphocampylus*) tend to occur in exposed environments, either above tree line, in shrubland, or on cliffs. Additionally, *Burmeistera*, all of which produce berries, occur exclusively in forest interiors. There are also specific cases of contrasting fruit type in sister lineages that support this hypothesis: *S. jelskii* (brevilimbatid) and *S. nematosepalus* (colombianid), both of which represent reversals from berries to capsules (Fig. 4), occur above tree line, whereas their closest berry-fruited relatives tend to occur in forest interiors. Also, berry-producing *C. tessmannii* grows in lowland tropical rainforest interior, whereas its capsular-fruited sister group consists of species that all grow on exposed montane cliffs in Brazil. This pattern is not universal, however. Many berry-fruited *Centropogon* species occur in exposed or disturbed habitat (e.g., a small number of baccate brevilimbatids can grow in páramo, and many species occur only in exposed, open areas, including *C. coccineus*, *C. granulosus*, and *C. smithii* E. Wimm.). These observations suggest that a combination of elevation, precipitation, and habitat type may have greatly influenced fruit evolution within the centropogonids, such that the group may provide an additional test of the correlation between fleshy fruits and forest understories first proposed by Gentry (1982) and tested across monocots by Givnish et al. (2005).

Biogeography: A Neotropical, primarily Andean radiation—The centropogonids are part of a larger Neotropical radiation that includes *Lysipomia* and *Lobelia* section *Tupa*. *Lysipomia* and the centropogonids together represent a tropical, primarily Andean component of this radiation that is estimated to be approximately 15–18 Ma old (Antonelli, 2009; Givnish et al., 2009), although the two groups have markedly different natural histories. The centropogonids, whose crown group age is estimated to be approximately 5–12 Ma (Antonelli, 2009; Givnish et al., 2009), are shrubby, robust, cloud-forest plants. *Lysipomia*, whose crown group age is estimated to be ~11 Ma (Antonelli, 2009), by contrast, are diminutive herbs with extreme adaptations to the highest elevations of the Andes. If we expand our phylogenetic view to also include the closely related, species-poor *Lobelia* section *Tupa*, this clade forms a mainly pan-Andean radiation that encompasses both tropical and temperate ecosystems. Within this larger clade, *Lysipomia* represents an extremely

derived morphology, specialized to its high-elevation habitat. *Lobelia* section *Tupa* share many similarities with the centropogonids, despite their occurrence in temperate latitudes of the southern Andes of central Chile. These similarities include their suffrutescent habit and tendency toward red, long–tubular corollas, typical of hummingbird-pollinated plants. These traits are likely pleisiomorphic within the centropogonids and are shared with the species that is sister to the rest of the centropogonid clade, *Siphocampylus fulgens*. An in-depth analysis to determine the origin of the progenitor of this Andean-centered radiation will be possible when additional taxa have been sampled.

Given their myriad growth forms and extreme floral diversity, the centropogonids are one of the most dramatic primarily Andean radiations in angiosperms. Many other Andean plant groups (e.g., *Lupinus* L. [Hughes and Eastwood, 2006], *Valeriana* L. [Bell and Donoghue, 2005], *Vasconella* Walp. [Antunes Carvalho and Renner, 2012], and *Puya* Molina [Jabaily and Sytsma, 2013]) are thought to have radiated rapidly in a similar timeframe as the centropogonids, within the last 10 Ma, likely in response to a combination of the last phases of Andean orogeny, climate fluctuations, and biotic interactions (e.g., with pollinators) (Luebert and Weigend, 2014). To our knowledge, fine-scale phylogenetic relationships of these clades have remained largely unresolved, which hinders focused biogeographic investigation. Our robust centropogonid phylogeny is the foundation for a unique investigation of fine-scale patterns of diversification and niche evolution in this hotspot of biodiversity. The importance of centropogonids in this regard results from their exceptional breadth of habitat diversity compared to many other Andean plant groups.

The future inclusion of phylogenetic data from the nuclear genome will undoubtedly improve our understanding of evolution within the centropogonids, especially in light of polyploidization within this young (<12 Ma), rapidly diversifying clade. Fortunately, the acquisition of genomic-scale nuclear data is quickly becoming feasible in nonmodel organisms (Grover et al., 2012, Weitemier et al., 2014), including within Asterales, which includes Campanulaceae (Mandel et al., 2014). Along these lines, owing to their rapid radiation, it seems possible that incomplete lineage-sorting has led to discordance between gene trees and species trees within the centropogonids. Future efforts will explore the effect of this phenomenon with additional data through phylogenetic reconstruction using coalescent methods (e.g., Liu et al., 2009).

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