PHYLOGENY, BIOGEOGRAPHY, FLORAL MORPHOLOGY OF CYPHOCARPOIDEAE (CAMPANULACEAE)

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ABSTRACT

PHYLOGENY, BIOGEOGRAPHY, FLORAL MORPHOLOGY

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KIMBERLY M. HANSEN

Campanulaceae is a family of flowering plants in the Asterales that is composed of five morphologically distinct subfamilies. Historically, systematic studies have focused within the two large subfamilies and have largely ignored the relationships among the subfamilies. Furthermore, studies of the anomalous Cyphocarpoideae, consisting of three species distributed in the Atacama Desert of Chile, are all but absent from the literature. Historical hypotheses concerning the evolution of the subfamilies and the placement of Cyphocarpoideae are tested with molecular phylogenies constructed from 57 plastid coding sequences for 78 taxa and 3 nuclear ribosomal genes for 47 taxa. All five subfamilies are sampled including a phylogenetically diverse representation of the larger subfamilies and all three extant species of Cyphocarpoideae. A rapid radiation early in the evolution of Campanulaceae is evident including an initial divergence into two lineages. In the lineage comprised of Lobelioideae, Nemacladoideae and Cyphocarpoideae, Cyphocarpoideae is sister to Nemacladoideae. Divergence dates, geologic events and floristic affinities are used to reconstruct a biogeographic history that includes a single long distance dispersal event from South Africa (origin of Lobelioideae) to the Neotropics via GAARlandia followed by a second dispersal to the Nearctic (origin of Nemacladoideae). The distribution of Cyphocarpoideae can be explained by a dispersal from either the Nearctic or Neotropics.

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INTRODUCTION

The Campanulaceae, comprised of five subfamilies, 84 genera and more than 2300 species (Lammers, 2007a), is the second largest family in the Asterales and third largest family in the Campanulidae. Jussieu formally described it in 1789. Historically, some authors expanded it to include Goodeniaceae, *Pentraphragma* and/or *Sphenoclea* (Tahktajan, 1980, 1983; Dahlgren, 1983). Molecular phylogenies suggest it is the sister taxon to Rousseaceae and their divergence is estimated at 86-71 million years ago (Ma) (Wikstrom et al., 2001; Magallon et al., 2015; Tank et al., 2015). The radiation of Campanulaceae began significantly later as evidenced by the estimated dates for the crown group at 67-41 Ma (Wikstrom et al., 2001; Bell et al., 2010; Knox, 2014). The distribution of the family is cosmopolitan, although significant overlap among the subfamilies is limited to Africa, where the family is proposed to have originated (Knox, 2006; Antonelli, 2009; Crowl et al., 2016). Classification and evolutionary studies within the family have been generally limited to the species rich Campanuloideae and Lobelioideae (Givnish, 1995; Stace and James, 1996; Schultheis, 2001; Sales, 2004; Koopman and Ayers, 2005; Park, 2006; Knox, 2008; Roquet, 2008, 2009; Antonelli, 2009; Borsch, 2009; Cellinese, 2009; Frajman and Schneeweiss, 2009; Haberle, 2009; Stefanović and Lakušić, 2009; Wendling et al., 2011; Mansion et al., 2012; Prebble et al., 2012; Wang et al., 2013; Lagarmosino, 2014; Crowl et al.,2016)

The Cyphocarpoideae, composed of a single genus and three species, is the smallest of the five subfamilies. It is restricted to the southern Atacama Desert of Chile (Figure 1) and is poorly represented in herbaria. Aside from the original species descriptions and illustrations, the Kew World Checklist and Bibliography of Campanulaceae (Lammers, 2007a) cites a mere four additional publications focused on this subfamily: two taxonomic descriptions for the type

species, *Cyphocarpus rigescens* Miers (Gay, 1849; Wimmer, 1968), and two pollen morphology studies that were also limited to the type species (Dunbar 1976, 1984). *Cyphocarpus innocuus* Sandwith and *C. psammophilus* Ricardi have not been included in any studies following their original descriptions in 1914 and 1959 respectively.

Morphology—Although the five subfamilies are morphologically distinct from each other (Table 1), the floral morphology of Cyphocarpoideae is unique among Asterales (Lammers, 1992). Like all Campanulaceae, Cyphocarpoideae have articulated lacticifers associated with the phloem throughout the vegetative and floral organs all of which produce milky sap. All three species of Cyphocarpoideae are annuals, a condition shared with all but one species of Nemacladoideae and some Campanuloideae and Lobelioideae. Unlike Lobelioideae and some Nemacladoideae, Cyphocarpoideae are non-resupinate. Similar to Lobelioideae, Cyphioideae, and some Nemacladoideae, Cyphocarpoideae have zygomorphic, bilabiate corollas. However, only in Cyphocarpoideae is the upper lip comprised of a single corolla lobe that forms a hood. The margins of the hood are connivent so that it remains tightly closed. The margin is winged and at the distal end of the hood, the wings fuse (and become notably elongate in *Cyphocarpus rigescens* and *C. innocuus*) to form an appendage that projects off the hood. The appendage resembles, but is smaller than, the four lobes of the lower lip. The point of attachment of the appendage, is keeled and this results in the appendage being erect. (Plates 1-3). The palate has three prominent yellow ridges (Plate 4), which are the result of induplicate aestivation of the corolla lobes, which is also unique to this subfamily. The stamens are epipetalous, as in many Lobelioideae, and the free portion of the filaments are pubescent (Plate 5). The anthers are included in the corolla tube, a true synapomorphy with Campanuloideae and Cyphioideae. In Lobelioideae the anthers are connate but in Cyphocarpoideae and the remaining subfamilies they

are connivent around the style. Pollen is 3-colporate, prolate-spheroidal, and the surface sculpture is reticulate with protrusions in the lumina (Plate 5) (Dunbar, 1984). Nearly all Campanulaceae flowers are epigynous and the capsules are hemispheric or turbinate but in Cyphocarpoideae the capsules are notably elongate and sickle-shaped or falcate (Plates 1-3). The arrangement and function of stylar hairs vary among the subfamilies. In Cyphocarpoideae they are found only on the distal most portion, on the abaxial sides of the immature stigmatic lobes (Plate 5). Similar to Nemacladoideae the style curves abruptly just below the stigma (Plate 5). In *Nemacladus* Nutt. it curves away from the odd sepal whereas in *Pseudonemacladus* (B.L.Rob.) McVaugh and Cyphocarpoideae it curves toward the odd sepal. In Lobelioideae, Nemacladoideae and Cyphocarpoideae the stigma is two-lobed. Like most Lobelioideae and all Cyphioideae and Nemacladoideae, the fruit is a two-locular capsule and placentation is axile (apical in Cyphioideae). However, in Cyphocarpoideae the septum is extremely thin and, early in development, it tears and largely disintegrates and thus the capsule appears to have a single locule with free central placentation. Additionally, in Cyphocarpoideae dehiscence is along a single longitudinal slit rather than apical valves, the predominant condition in the other subfamilies. Chromosome numbers and phytochemistry are completely unknown.

Classification— Historically, relationships among the subfamilies have been largely ignored. Although several classification schemes have been proposed (Table 2), detailed discussions are wholly absent (Bremer,1876; Schonland, 1889;Wimmer, 1968; Takhtajan, 1980, 1983, 1987, 1997; Cronquist 1980,1987; Thorne, 1983; Lammers, 1992, 1998, 2007). This is due, in part, to the fact that little has been published about Cyphocarpoideae and Nemacladoideae and Cyphioideae have only recently received attention (Knox, 2014). Subfamily level classification is further complicated by the prevalence of floral autapomorphies (e.g.

actinomorphic corollas in Campanuloideae, staminal column in Lobelioideae, stigma containing a fluid-filled stigmatic chamber in Cyphioideae, stellately spreading anthers in Nemacladoideae, galeate upper lip in Cyphocarpoideae), symplesiomorphies shared with other Asterales (i.e. estipulate leaves, protandrous flowers, antesepalous stamens, tetrasporangiate, dithecal anthers that dehisce introrsely along longitudinal slits, a syncarpous gynoecium that bears a solitary style, and small, numerous seeds) and traits that vary widely (e.g. carpel number in Campanuloideae, pollen surface sculpture in Lobelioideae, filament fusion in Cyphia, floral resupination in Nemacladoideae). It is evident that the few traits that are shared among subfamilies are largely a result of homoplasy given that nearly all combinations can be made (Table 1).

Bentham's 1876 treatment was the first to include species from all five currently recognized subfamilies. In his classification, Campanulaceae was composed of three tribes, Campanuleae, Cyphieae and Lobelieae. Nemacladoideae and Cyphocarpoideae, as currently recognized, were placed in the Cyphieae with *Cyphia* P.J. Bergius. Bentham's Campanuleae and Lobelieae are in agreement with current circumscription of the Campanuloideae and Lobelioideae, respectively. In his 1875 prelude, he noted that his circumscription of Cyphieae should not be considered a natural group and no further discussion was provided to clarify why he did not recognize Cyphioideae, Nemacladoideae and Cyphocarpoideae as distinct tribes. Subsequent treatments (Schonland 1889, Wimmer 1968, Takhtajan 1980, 1983, Lammers 1992) recognized Bentham's broadly circumscribed Cyphioideae and included similar, undetailed disclaimers regarding doubts of its monophyly. Wimmer (1968), echoed the opinion of Miers (1848) in his original description, but added that *Cyphocarpus* was so morphologically distinct that family level recognition was more appropriate yet he did not formally propose this

classification due to the lack of available material for study. Thorne (1983) included both Nemacladoideae and Cyphocarpoideae within Lobelioideae and thus narrowed Cyphioideae to include only *Cyphia*. Cronquist (1980) was the first to formally propose the five distinct subfamilies that are currently recognized. He later speculated that Cyphioideae, Nemacladoideae and Cyphocarpoideae (or some combination) were transitional between the ancestral Campanuloideae and the derived Lobelioideae (Cronquist, 1987). When Takhtajan (1987, 1997) and Lammers (1998, 2007b) revisited their classification systems, they also recognized five distinct subfamilies but failed to propose relationships among them.

Although only a single species (*C. rigescens*) was sampled, Cosner et al. (1994) constructed the first molecular phylogeny that included Cyphocarpoideae. The monophyly of the family was well supported, however, the monophyly of the subfamilies could not be confirmed from a single gene (rbcL) and with their limited taxon sampling. Although additional studies expanded the number of taxa representing Campanuloideae and Lobelioideae, several important taxa considered to be basal were not included and rbcL continued to be the only locus sampled for Cyphocarpoideae (Gustafsson and Bremer, 1995; Gustafsson et al., 1996; Backlund and Bremer, 1997; Lundberg and Bremer, 2003). Gustafson and Bremer (1995) inferred a phylogeny of the Asterales based on chemical and morphological traits however, significant amounts of missing data for Cyphocarpoideae and Nemacladoideae resulted in an unresolved topology. Only recently has a multigene phylogeny been constructed for the Campanulaceae that included more than one locus for Cyphocarpoideae (Crowl et al., 2016). This phylogeny was the first to include a sufficient number of taxa from the Campanuloideae and Lobelioideae that allowed for the unambiguous confirmation of the monophyly of the subfamilies. It was also able to clarify, to some degree, the relationships among them. An initial divergence into two major clades is highly

supported and suggests a novel topology. One clade contains Campanuloideae and Cyphioideae and the second, an unresolved clade, is composed of Lobelioideae, Nemacladoideae and Cyphocarpoideae (Crowl et al., 2016). The topology confirms Bentham's (1875) suspicion that Cyphiodeae, Nemacladoideae and Cyphocarpoideae are not a monophyletic clade and lends some support to the treatment of Thorne (1983) who suggested that Lobelioideae, Nemacladoideae and Cyphocarpoideae are closely related. Unfortunately, the sister taxon of Cyphocarpoideae remains unknown.

Biogeography— Cyphocarpoideae is restricted to the southern Atacama Desert of Chile, the oldest, extant, non-polar desert in the world. Semi-arid conditions characterized the Atacama Desert in the late Jurassic 150 Ma (Hartley, 2005). Following the opening of the Drake Passage 32-28 Ma and the development of the Humboldt Current, arid conditions were prevalent by the middle Oligocene (Graham, 2010). The alluvial soils characteristic of the Peruvian and Atacama Deserts were deposited in the early Miocene (23 Ma) (Graham, 2010). The onset of hyper-aridity occurred in pulses. The first two pulses were the most significant and were related to the uplift of the Andes. In the middle Miocene 19-13 Ma, the Andes reached 2 km, which was sufficient to block the moisture-rich winds from the east and a second pulse occurred following a surge of rapid uplift in the central Andes 10-6 Ma to reach heights of 4 km (Garzione, et al., 2008; Schlunegger et al., 2010). Aridity increased again as modern water surface circulation patterns in the eastern tropical pacific (responsible for El Nino weather events) developed following the closing of the Panama Seaway 4.5-4.2 Ma and yet again 1.5 Ma in response to global climate change associated with Northern Hemisphere glaciation (Cannariato and Ravelo, 1997).

Dated phylogenies that have included endemic taxa suggest the flora of the Atacama Desert is relatively young. All lineages diverged from their closest relatives 14-1 Ma (Lavin et

al., 2003; Huertas et al., 2007; Catalano et al., 2008; Luebert and Wen, 2008; Scherson et al., 2008; Dillon et al.,2009; Guerrero et al., 2013) with a single exception (*Tiquilia* Pers., 59-48 Ma; Moore and Jansen, 2006). A review of well resolved phylogenies with relatively complete taxon sampling identified four distinct elements of the flora of the Atacama Desert based on the distributions of the sister taxa of native species: i) neotropical; ii) central Chilean; iii) trans-Andean; and iv) amphitropical (Luebert, 2011).

Nemacladoideae is composed of two geographically disjunct genera. Monotypic *Pseudonemacladus* occurs on exposed limestone at the edges of pine-oak forests on the dry eastern slopes of the Sierra Madre Oriental in eastern Mexico. Although the geologic history of the Sierra Madre Oriental is complex and not well understood, it is well accepted that the orogeny was complete by the beginning of the Tertiary ca. 65 Ma (Graham, 2010). Due to high levels of endemism and disparate community assemblages that include both Nearctic and Neotropical elements, Mexico has long been recognized as an important biogeographic transition zone composed of several distinct subunits, including the Sierra Madre Oriental (Marshall and Liebherr, 2000). *Pseudonemacladus* occurs in the transition zone between two floristic regions that each have a significant endemic element. Additionally, one has a strong influence from the floras of western North American and Central America while the other has affinities with southern montane regions, primarily the Andes in South America (Rzedowski, 1966). *Nemacladus* typically occurs in dry, sandy habitats from Oregon and Idaho in the US south to Baja California and Sonora, Mexico. All of the basal lineages of *Nemacladus* occur in, but are not necessarily endemic to, Baja California. The peninsula, and its flora, became isolated after rifting began 12.5 Ma and the spreading of the Gulf of California commenced 6-10 Ma (Londsdale, 1989). The flora of northern Baja California has a significant endemic element as

well as affinities to the mainland Sonoran Desert to the east and the flora of California to the north (Wiggins, 1980).

The cosmopolitan Lobelioideae is most diverse in South America where nearly half of the species are distributed. Another 17% occur in North America, 14% in Africa and 12% are Polynesian. It is poorly represented in Australasia (5%), Asia (3%) and Europe (<1%; Lammers, 2007b). Noteworthy radiations include the monophyletic Hawaiian clade composed of ca. 126 species (Givnish, 2009) and the neotropical Centropogon-Siphocampylos-Burmeistera clade with ca. 541 species (Lagarmosino, 2014). The detailed biogeographical analysis presented by Crowl, et al., (2016) inferred several long distance dispersals from the Afrotropics to the Neotropics and suggested that bidirectional exchanges between the Nearctic and Neotropics were common. South Africa is proposed as the origin of Lobelioideae (Knox, 2014; Crowl et al., 2016).

The purpose of this study is to resolve the relationships within the Lobelioideae-Nemacladoideae-Cyphocarpoideae clade in order to understand character evolution, divergence times and biogeographic patterns in Campanulaceae with a particular focus on Cyphocarpoideae. This is the first study to include more than a single species of Cyphocarpoideae and therefore date, for the first time, the crown node of this subfamily.

METHODS

***Taxon Sampling* —** Eighty accessions representing 79 species were included in this study; 48 were newly sequenced for this study and 32 were available in GenBank or TreeBase (Table 3). Seventy-eight accessions were included in the analyses of plastid DNA (cpDNA); 46 were newly sequenced and 32 were available in GenBank or TreeBase. Two taxa, *Downingia cuspidata* and *Cuscuta exaltata*, were removed from the data set due to high proportions of missing data before performing the divergence dating analysis in BEAST. The ribosomal DNA (nrDNA) analyses included only the 47 newly sequenced taxa. Outgroups were composed of 15 euasterid taxa. Campanulidae was represented by one taxon from the sister family of Campanulaceae (*Carpodetus serratus* J.R. Forst. & G. Forst., Rousseaceae), five additional taxa from the Asterales (*Acourtia wrightii* (A. Gray) Reveal & R.M. King, *Helianthus annuus* L., *Praxelis clematidea* (Griseb.) R.M. King & H.Rob., *Silybum marianum* (L.) Gaertn., Asteraceae; *Scaevola aemula* R.Br., Goodeniaceae) and one taxon from Dipsacales (*Lonicera japonica* Thunb., Caprifoliaceae). More distantly related outgroups were composed of six taxa from the Lamiidae (*Asclepias syriaca* L., Apocynaceae, Gentianales; *Boea hygrometrica* (Bunge) R. Brown, Gesneriaceae, Lamiales; *Coffea Arabica* L., Rubiaceae, Gentianales; *Cuscuta exaltata* Engelm., *Ipomoea batatas* (L.) Lam., Solanaceae, Solanales; *Olea europaea* L., Oleaceae, Lamiales) and two taxa from the basal euasterid lineage Ericales (*Camellia sinensis* (L.) Kuntze, Theaceae; *Primula poissonii* Franch., Primulaceae). Only two outgroup taxa (*Acourtia wrightii* and *Scaevola aemula*) were newly sequenced for this study and included in the nrDNA analyses.

Where possible, each of the major lineages within each subfamily of Campanulaceae is represented. Previously published molecular phylogenies were referenced to select representative taxa of Campanuloideae (Eddie, et al., 2003; Roquet, et al., 2008, 2009; Haberle, et al. 2009;

Mansion, et al., 2012; Crowl, et al., 2014, 2016), Lobelioideae (Antonelli, 2008; Knox, et al., 2008; Knox, 2014) and Cyphioideae (Knox, 2014). The cpDNA data set included 17 (14 newly sequenced and included in nrDNA dataset) Campanuloideae and 25 (20 newly sequenced and included in nrDNA dataset) Lobelioideae taxa. All eleven Cyphioideae taxa with previously published plastome data (Knox, 2014) were included in the cpDNA dataset. Two *Cyphia* species were newly sequenced for this study and included in the rDNA analyses. The five Nemacladoideae taxa included in the cpDNA dataset (six in nrDNA dataset), represent both major lineages and all major clades within Nemacladus based on unpublished atpB and nrITS trees (Ayers, unpublished data). All three species of Cyphocarpoideae are included. The nine taxa representing these two subfamilies are newly sequenced for this study; eight are included in the cpDNA dataset and all nine are included in the nrDNA dataset.

DNA Extraction— A modified sorbitol protocol (Storchova, et al., 2000) was used to extract genomic DNA from herbarium collections or tissue preserved in silica gel. All samples were eluted in 10mM Tris-Cl pH 8.0. Only samples with high molecular weight (assessed visually on a 1% agarose gel) were selected for Illumina library preparation. A NanoDrop spectrophotometer (Thermo Scientific, Carlsbad, California, USA) was used to quantify DNA concentration and assess purity. A solid phase reversible immobilization (SPRI) bead solution containing 18% PEG and Sera-Mag carboxylate modified beads (ThermoFisher Scientific, Waltham, MA) was used to purify samples if 260/280 absorbance values were below 1.7 and when necessary, concentrate samples before proceeding to library preparation.

Illumina Library Prep and Sequencing—Sequence data was generated from three Illumina 500 cycle MiSeq runs (Illumina, San Diego, CA USA) to produce paired-end 250 bp reads. The first run consisted of a multiplex of 24 libraries (representing 21 taxa) prepped with a

Nextera XT DNA Sample Preparation Kit (Cat no.: FC-131-1001, Illumina, San Diego, CA, USA). These libraries were prepared according to the manufacturer's protocol with ca. 100 ng of starting DNA and no size selection procedure was performed. Quantitative PCR was performed using universal Illumina P7 and P5 primers to quantify libraries and multiplex them in equimolar concentrations. The samples sequenced on the second and third MiSeq runs were prepped as follows. One to two µg of genomic DNA was fragmented with NEBNext fragmentase (Cat no.: M0348, New England BioLabs, Ipswich, Massachusetts, USA) for an initial duration of 5 minutes. Fragment size distribution was assessed on a 1% agarose gel and when necessary, samples were fragmented for an additional two-six minutes until the highest concentrations of fragments were ca. 600-1000 bp in length. Samples were cleaned and concentrated with AMPure SPRI bead solution (Cat no.: A63880, Agencourt, Beverly, Massachusetts, USA) before proceeding with library preparation using NEBNext DNA Library Prep Master Mix Set for Illumina kit (Cat no.: E6040L, New England BioLabs, Ipswich, Massachusetts, USA). The manufacturer's instructions were followed except in all instances where 1:1.8 (library: SPRI bead solution) bead clean ups were recommended, instead, a 1:1 or 1:0.8 ratio was substituted. NEBNext Multiplex Oligos for Illumina (Cat no.: E7335 and E7500, New England BioLabs, Ipswich, Massachusetts, USA) were used to barcode libraries for multiplexing. No size selection procedure was performed. Quantitative PCR was performed using universal Illumina P7 and P5 primers to quantify libraries and multiplex them in equimolar concentrations. The second run consisted of 24 multiplexed libraries (representing 24 taxa) and the third run consisted of 22 multiplexed libraries (representing 22 taxa).

Short Read Processing, Assembly and Alignment— Unique barcode sequences were entered into the MiSeq prior to sequencing and samples were subsequently de-mulitplexed by the

instrument. The fastq-mcf program (within the ea-utils package; Aronesty, 2011) was used to trim adaptors and low quality bases and remove reads with a PHRED score less than 30 for read pools sequenced on the first and second run. Read pools from the third run were trimmed and quality filtered using default settings for the BBDuk plug-in available in Geneious Pro v9.0.5 (http://www.geneious.com, Kearse et al., 2012). Because read pools were not size selected during library prep, fastq-mcf was also used to de-couple paired reads for all read pools. Geneious Pro (versions 6.1.6, 7.1.7, 8.1.3, 9.0.5) was used to remove duplicate reads and retain only reads greater than 70 bp.

The *Trachelium caeruleum* (Campanuloideae, Campanulaceae) reference plastome (NC010442; Haberle, 2008) and the *Asclepias syriaca* nrDNA cistron (JF312046; Straub, et al., 2011) were retrieved from GenBank and used for reference guided assembly. Read pools were aligned to the references using the medium sensitivity algorithm for 25 iterations in Geneious Pro (versions 6.1.6, 7.1.7, 8.1.3, 9.0.5). Assemblies were visually inspected for misassembly and masked where coverage was less than five reads. Visual inspection also revealed areas of high sequence divergence, which were also masked. Missing data was recovered by using the newly assembled contigs of a more closely related taxon as a reference in a second round of reference-guided assembly in Geneious Pro using the same settings. Coding portions of 57 single copy genes were extracted from each plastome assembly according to the annotations available for the *Trachelium caeruleum* reference. Genes encoding the 18s, 5.8s and 28s ribosomal subunits were extracted from each nrDNA cistron assembly according to the annotations available for the *Asclepias syriaca* reference. For both the cpDNA and nrDNA datasets, all genes were aligned individually and then concatenated into a single alignment. All alignments were performed in MAFFT v7 (Katoh, 2013) using default settings and edited

manually in BioEdit v7.2.3 (Hall, 1999). Geneious Pro (versions 6.1.6, 7.1.7, 8.1.3, 9.0.5) was used to concatenate alignments.

Phylogenetic Analyses— Maximum parsimony (MP), maximum likelihood (ML), and Bayesian (BI) analyses were performed on the unpartitioned cpDNA (78 taxa) and nrDNA (47 taxa) alignments. Maximum parsimony analyses for each dataset were performed in PAUP* 4.0a147 (http://people.sc.fsu.edu/~dswofford/paup_test/; Swofford, 2002) using a heuristic search algorithm and 1000 bootstrap replicates. Gaps were treated as missing data, starting trees were obtained via stepwise random addition with 1000 replicates, and branch-swapping was performed using the TBR method. MEGA v6.0.5 (Tamura, et al., 2013) was used to determine the optimal model of molecular evolution by running the Find Best-Fit Substitution Model (ML) analysis with default settings. Maximum likelihood analyses were performed in Geneious Pro v9.0.5 using the RAxML v7.2.8 (Stamatakis, 2014) plug-in. A Rapid Bootstrapping and Search for the Best-Scoring Maximum Likelihood Tree analysis was performed with 1000 replicates. Default settings were used for all remaining parts of the analyses including generation of the starting tree via randomized stepwise addition parsimony. The same analysis was performed on the 76 taxa cpDNA dataset to generate a starting tree for the divergence data analysis. The Bayesian analyses were performed in Mr. Bayes v.3.2.2 (Huelsenbeck and Ronquist, 2001) using the optimal model of molecular evolution and *Primula poissonii* was designated as the outgroup. Each Markov chain Monte Carlo (MCMC) analysis was performed using four heated chains with a chain temperature of 0.2. The MCMC was run for 1,000,000 generations sampling the posterior every 100 generations. An initial burn-in of 1,000 trees (10%) was designated. Parameter outputs were visualized in Geneious Pro v9.0.5 to determine chain stabilization and verify an appropriate

burn-in was designated. Default settings were used for all remaining aspects of the analyses including priors and starting trees.

***Divergence Dating*—**Divergence times were calculated in BEAST v1.8.2 (Drummond, 2012) from the cpDNA dataset consisting of 57 coding genes and 76 taxa. MEGA v6.0.5 (Tamura, et al., 2013) was used to determine the optimal model of molecular evolution for individual genes by running the Find Best-Fit Substitution Model (ML) analysis with default settings. The dataset was partitioned by gene and the optimal model of molecular evolution was specified for each gene (Table 4). The only reported Campanulaceae fossils consisting of seeds dated to 17-16 Ma, are thought to be most closely related to *Campanula pyramidalis* (Lancucka-Srodoniowa, 1977, 1979). A lognormal prior distribution with mean= 5.0, standard deviation= 1.0 and offset= 16 was applied to the node representing the MRCA of *C. parryi* and *C. garganica*, the most closely related taxa included in this study. The Asterales and Campanulaceae were each constrained as monophyletic and the crown nodes were used as calibration points by applying a normal distribution using means and 95% highest posterior densities from previously published date ranges (Bell et al., 2010). The uncorrelated lognormal clock model and Yule process speciation model were assigned. A maximum likelihood tree was generated in RAxML (see details above) and provided as a starting tree. The MCMC was run for 40 million generations sampling every 1000 generations. Tracer v1.6 (Heled and Drummond 2010) was used to assess effective sample size and chain convergence and estimate an appropriate burn in (20%). TreeAnnotator v1.8.2 (Drummond et al., 2012) was used to produce a maximum clade credibility summary tree.

***Character reconstruction*—**Mesquite v3.04 was used to map unambiguous synapomorphies among subfamilies in the Lobelioideae-Nemacladoideae-Cyphocarpoideae

clade onto the maximum likelihood tree constructed from cpDNA using maximum parsimony criteria.

RESULTS

***Read Pools, Assemblies and Alignments*—**Three MiSeq runs produced a total of 81,522,150 reads that were successfully de-multiplexed and assigned to a specific index. This initial pool was reduced to 66,825,699 reads (72%) after trimming and filtering (Table 5). Read pools were composed of 128,729-2,825,500 clean reads per read pool (mean 954,653; median 899,528). The number of clean reads per read pool that mapped to the plastid reference ranged from 7,885-333,062 (mean 82,176; median 51383). Mapped reads spanned 60.3-100% of the length (162,321 bp) of the reference (mean 91.5%; median 93.4%). Mean read depth ranged from 9.3-425.5 (median 55.9). The number of clean reads per read pool that mapped to the rDNA reference ranged from 780-296,919 (mean 40899.54; median 24240). Mapped reads spanned 84.2-100% of the length (8016 bp) of the reference (mean 98.9%; median 100%). Mean read depth ranged from 12.9-4236.4 (median 363.6).

The plastid alignment, consisting of 57 genes and 78 taxa, was 41,621 bp in length and as a whole contained 1.5% missing data (Table 6). *Cuscuta exalta* had the most missing data among taxa (14%) and nhdI had the most missing data (8.1%) among genes. There were 18,868 variable characters and 13,433 of these were parsimony informative. The rDNA alignment, consisting of three genes and 47 taxa, was 5361 bp in length and contained 4% missing data (Table 7). *Cyphocarpus rigescens* had the most missing data among taxa (3%). The 18s and 5.8s coding sequences contained no missing data and the 28s coding sequence had 5.8% missing data. There were 415 variable characters and 233 of these were parsimony informative.

***Phylogenetic Analyses*—**Congruent with previously published studies, all cpDNA analyses (Figures 2-4) and nrDNA analyses (Figures 5-7) show high support for the monophyly of Campanulaceae. The maximum parsimony bootstrap values (MPBV) and maximum

likelihood bootstrap values (MLBV) for both the cpDNA and nrDNA datasets are all 100% and the Bayesian inference posterior probabilities (BIPP) for both data sets are one. The sister relationship to Rousseaceae is also highly supported (MPBV=91.2, MLBV=100, BIPP=1). Each subfamily is highly supported as monophyletic in all analyses with the exception of Nemacladoideae and Campanuloideae in some analyses (Table 8). Within Cyphocarpoideae, the relationships among *Cyphocarpus* species are consistent in all analyses.

Analyses of cpDNA— The ML and BI analyses of the cpDNA dataset produced identical and highly supported backbone topologies (Figures 2-3). The backbone topology of Campanulaceae is congruent with previously published results (Crowl et al., 2016) and shows an initial divergence into two clades. The first clade contains Campanuloideae sister to Cyphioideae and it is weakly supported in the ML analyses but highly supported in the BI analysis (MLBV=72, BIPP=1). The second clade contains Lobelioideae, Nemacladoideae and Cyphocarpoideae and is highly supported (MLBV=100, BIPP=1). Within this clade, the sister relationship between Nemacladoideae and Cyphocarpoideae is highly supported (MLBV=100, BIPP=1).

The backbone topology of the strict consensus tree constructed from the MP analysis is a polytomy among the subfamilies with one exception (Figure 4). Although weakly supported, the only relationship among subfamilies that is inferred is a monophyletic Cyphocarpoideae and Nemacladoideae (MPBV=64.7) where Cyphocarpoideae is nested within a paraphyletic Nemacladoideae. *Nemacladus* and *Cyphocarpus* are each highly supported as monophyletic (MPBV=100 for each), although their sister relationship is weakly supported (MPBV=71.8) Among the eight equally most parsimonious trees (Figure 8), two are identical and congruent with the backbone topologies of the ML and BI analyses except that Cyphocarpoideae is nested

within Nemacladoideae. Furthermore, none of the eight equally most parsimonious trees support a monophyletic Nemacladoideae but six topologies support Cyphocarpoideae nested within Nemacladoideae. Three of these six trees infer a close relationship among Lobelioideae, Cyphocarpoideae and Nemacladoideae while two suggest Cyphocarpoideae and Nemacladoideae are more closely related to Campanuloideae than Lobelioideae. The topology of the two trees that do not support Nemacladoideae and Cyphocarpoideae as a monophyletic group have identical backbone topologies which initially diverge into two clades; the first clade contains *Pseudonemacladus* sister to Cyphocarpoideae with Lobelioideae basal and the second clade contains *Nemacladus* sister to Campanuloideae with Cyphioideae basal.

Analyses of nrDNA— The topologies of the nrDNA analyses are incongruent, however, most of the conflicting relationships were weakly supported (Figures 5-7). All nodes forming the backbone topologies of the ML tree (Figure 5) and bootstrapped MP strict consensus tree (Figure 7) are weakly supported and collapse into a polytomy if a threshold of 70% bootstrap support is imposed. In the BI analysis (Figure 6) there is high support for the sister relationships of Nemacladoideae and Cyphocarpoideae (BIPP=0.996) and Campanuloideae and Lobelioideae (BIPP=0.995). When a threshold of 0.7 posterior probability is imposed, a three-way polytomy forms among these two clades and Cyphioideae.

Divergence Time Estimation—Stem lineages of all five subfamilies diverged within 5.5 million years. The ages of crown lineages varied from 12.8 Ma (Cyphocarpoideae) to 55.7 Ma (Lobelioideae) (Figure 9; Table 9) and are in agreement with previously published estimates (Knox, 2014; Crowl et al., (2016).

DISCUSSION

The topology of the ML and BI cpDNA trees suggests that the early evolution of Campanulaceae included an initial divergence into two clades followed by a rapid radiation into five monophyletic groups consistent with current circumscription of the five subfamilies. This is in agreement with the tree presented by Crowl et al., (2016). The rapid radiation did not allow sufficient time for shared morphological characters to accumulate among the subfamilies before they diverged. Likewise, the relatively long branches leading to the crown group of each subfamily are indicative of a longer shared history. The resolution of the Lobelioideae-Nemacladoideae-Cyphocarpoideae clade permits ancestral character reconstruction and thus a better understanding of morphological evolution within the family. The resolved phylogeny, in combination with the divergence dates, also provides some insight into the biogeographical history of Cyphocarpoideae.

Morphology— A close relationship between Lobelioideae, Nemacladoideae and Cyphocarpoideae was proposed by Thorne (1983) although he did not provide any morphological context to support his hypothesis. Two-lobed stigmas are the only morphological synapomorphy that unites the Lobelioideae-Nemacladoideae-Cyphocarpoideae clade. Although this character is found in other Asterales, ancestral character reconstruction suggests that it has evolved independently in Campanulaceae (Figure 10). The only definitive synapomorphy that supports the sister relationship of Nemacladoideae and Cyphocarpoideae is the curved style just below the stigma. In Cyphocarpoideae and *Pseudonemacladus* it curves abruptly toward the lower lip (i.e. toward the odd sepal). In *Nemacladus,* it curves to varying degrees toward the upper lip (lower lip in resupinate taxa) but always away from the odd sepal. Although this trait is

also shared with *Scaevola aemula* and other Goodeniaceae, the ancestral character reconstruction supports two independent evolutionary events (Figure 11).

***Biogeography*—** Given that an African origin for Lobelioideae has been proposed (Knox, 2006; Antonelli, 2009), the resolution of this subfamily as basal in the Lobelioideae-Nemacladoideae-Cyphocarpoideae clade further supports the African origin for Campanulaceae proposed by Crowl, et al. (2016). However, the sister relationship of Nemacladoideae and Cyphocarpoideae is inconsistent with the claim that the distributions of these two subfamilies are the result of two independent dispersal events from the Afrotropics to the Nearctic and Neotropics, respectively. Instead, the sister relationship proposed here infers a single dispersal event would better explain their New World distribution.

Cyphocarpoideae and Nemacladoideae diverged ca. 57 Ma during the initial rapid radiation of Campanulaceae. The edaphic and climatic conditions that restrict the distribution of extant Cyphocarpoideae had not yet developed. However, the deposition of modern soils was complete when the crown group diverged ca. 12.8 Ma and *Cyphocarpus rigescens* and *C. innocuus* diverged ca. 8.3 Ma following hyper-aridification resulting from uplift pulses of the Andes. The recent diversification of Cyphocarpoideae is consistent with divergence times of <14 Ma for other endemic taxa in the Atacama Desert (Lavin et al., 2003; Huertas et al., 2007; Catalano et al., 2008; Luebert and Wen, 2008; Scherson et al., 2008; Dillon et al.,2009; Guerrero et al., 2013). The divergence from Nemacladoideae before the formation of suitable habitat and the significant lag time until the diversification of crown Cyphocarpoideae suggests that the dispersal event from South Africa was not directly to the Atacama Desert. This is further supported by the affinities of the flora of the Atacama Desert (i.e. central Chilean, neotropical,

transandean and amphitropical elements but lacking an African element) and ages of other endemic taxa.

In Nemacladoideae, *Nemacladus* diverged early from *Pseudonemacladus*, ca. 52.5 Ma. The relatively short branch leading to *Pseudonemacladus* and the coincidental timing of habitat formation could be interpreted as evidence that this taxon represents a relictual lineage. The diversification of all major lineages of *Nemacladus* occurred 27.1-14.1 Ma, before the isolation of the Baja peninsula 10-6 Ma. This suggests that the distribution of *Pseudonemacladus* may better represent the ancestral distribution of Nemacladoideae. A dispersal event from South Africa directly to the Sierra Madre Orientale is not supported by the floristic affinities of the Sierra Madre Oriental (i.e. the presence of a significant Central and South American element and lack of an African element) (Rzedowski, 1966).

The Rio-Grande Rise-Walvis Ridge Complex, a series of submerged islands located off the coasts of Brazil and western Africa (Figure 12), were exposed until the Oligocene (Hekinian, 1979; Parrish, 1993; Bermingham and Martin, 1998) and provided a stepping stone migration route between Africa and South America when these continents were significantly closer together (Morely, 2003). At this time, the Greater Antilles and Aves Ridge (GAARlandia) provided another migration route from northern South America to Central and North America (Iturralde-Vicent and MacPhee, 1999) (Figure 13). Both routes were available during the rapid radiation of the family and could have facilitated a dispersal from South Africa to the Neotropics, which was then followed by a dispersal to the Nearctic (Nemacladoideae) via GAARlandia. This pattern is supported by the floristic affinities where *Pseudonemacladus* is distributed. The floristic affinities of the Atacama Desert would support a subsequent dispersal from the Sierra

Madre Orientale in Mexico (Figure 14A) or a dispersal following migration across the Rio-Grande Rise Wavis-Ridge Complex (Figure 14B).

***Conclusions*—** The resolution of the relationships in the Lobelioideae-Nemacladoideae-Cyphocarpoideae clade allows for the identification of morphological traits that have been conserved in the evolutionary history of this clade of Campanulaceae. In particular, it has allowed for the interpretation of those associated with the historically enigmatic Cyphocarpoideae. Definitive synapomorphies are rare, but all three subfamilies have a two-lobed stigma and Nemacladoideae and Cyphocarpoideae have styles that curve just below the stigma. Resolution of the relationships has allowed for the clarification of some aspects pertaining to the early biogeographic history of Campanulaceae while others remain ambiguous. An African origin for the family is now unequivocal given that Lobelioideae is basal. The sister relationship of Nemacladoideae and Cyphocarpoideae suggests that a single dispersal from the Afrotropics occurred. The timing coincides with available migration routes between South Africa and the Neotropics as well as between the Neotropics and eastern Mexico. The divergence time of Nemacladoideae and Cyphocarpoideae coincides with the habitat formation of *Pseudonemacladus* but precedes that of *Nemacladus* and Cyphocarpoideae. The recent diversification of Cyphocarpoideae and the floristic affinities of the Atacama Desert do not support a dispersal event from South Africa, but instead equally support two scenarios of dispersal from either the Nearctic or the Neotropics.

LITERATURE CITED

ANTONELLI, A. 2008. Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: Molecular signal overshadows morphology. *Molecular phylogenetics and evolution*, *46*(1), 1-18.

ANTONELLI, A. 2009. Have giant lobelias evolved several times independently? Life form shifts and historical biogeography of the cosmopolitan and highly diverse subfamily Lobelioideae (Campanulaceae). *BMC Biology* 7: 82-103.

ARONESTY, E. 2011. ea-utils : Command-line tools for processing biological sequencing data. Expression Analysis, Durham, NC http://code.google.com/p/ea-utils.

BACKLUND, A., & BREMER, B. 1997. Phylogeny of the Asteridae s. str. based on rbcL sequences, with particular reference to the Dipsacales. *Plant Systematics and Evolution*, *207*(3-4), 225-254.

BELL, C. D., SOLTIS, D. E., & SOLTIS, P. S. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, *97*(8), 1296-1303.

BENTHAM, G. 1875. Notes on the gamopetalous orders belonging to the campanulaceous and oleacous groups. *Botanical Journal of the Linnean Society*, 15: 1-16.

BENTHAM, G. 1876. Campanulaceae. *In* G. Bentham and J.D. Hooker, Genera plantarum, 2: 541-564. L. Reeve, London. England.

BERMINGHAM, E., & MARTIN, A. P. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, *7*(4), 499-517.

BORSCH, T., KOROTKOVA, N., RAUS, T., LOBIN, W., & LÖHNE, C. 2009. The petD group II intron as a species level marker: utility for tree inference and species identification in the diverse genus *Campanula* (Campanulaceae). *Willdenowia*, 7-33.

CANNARIATO, K. G., & RAVELO, A. C. 1997. Pliocene-Pleistocene evolution of eastern tropical Pacific surface water circulation and thermocline depth. *Paleoceanography*, 12(6), 805-820.

CATALANO, S. A., VILARDI, J. C., TOSTO, D., & SAIDMAN, B. O. 2008. Molecular phylogeny and diversification history of *Prosopis* (Fabaceae: Mimosoideae). *Biological Journal of the Linnean Society*, *93*(3), 621-640.

CELLINESE, N., SMITH, S. A., EDWARDS, E. J., KIM, S. T., HABERLE, R. C., AVRAMAKIS, M., & DONOGHUE, M. J. 2009. Historical biogeography of the endemic Campanulaceae of Crete. *Journal of Biogeography*, 36(7), 1253-1269.

CROWL, ANDREW A., EVGENY MAVRODIEV, GUILHEM MANSION, ROSEMARIE HABERLE, ANNALAURA PISTARINO, GEORGIA KAMARI, DIMITRIOS PHITOS, THOMAS BORSCH, AND NICO CELLINESE. 2014. Phylogeny of Campanuloideae (Campanulaceae) with emphasis on the utility of nuclear pentatricopeptide repeat (PPR) genes. *PloS one* 9, no. 4: e94199.

CROWL, A. A., MILES, N. W., VISGER, C. J., HANSEN, K., AYERS, T., HABERLE, R., & CELLINESE, N. 2016. A global perspective on Campanulaceae: Biogeographic, genomic, and floral evolution. *American journal of botany*, *103*(2), 233-245.

CRONQUIST, A. 1981. *An integrated system of classification of flowering plants*. Columbia University Press.

CRONQUIST, A. 1988. The evolution and classification of flowering plants. New York Botanical Garden, Bronx.

DAHLGREN, G. 1989. The last Dahlgrenogram. System of classification of the dicotyledons.

DE JUSSIEU, A. L. 1789. Campanulaceae, les Campanulacees. *In* Genera plantarum secundum ordines naturales disposita, 163-166. Vidua Herissant, Paris. France.

DILLON, M. O., TU, T., XIE, L., QUIPUSCOA SILVESTRE, V., & WEN, J. 2009. Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution*, *47*(5), 457-476.

DRUMMOND AJ, SUCHARD MA, XIE D & RAMBAUT A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7 *Molecular Biology And Evolution* 29: 1969-1973

DUNBAR, A., & WALLENTINUS, H. G. 1976. On pollen of Campanulaceae. III. A numerical taxonomic investigation. *Bot. Noti. ser*, *129*, 69-72.

DUNBAR, A. 1984. Pollen morphology in Campanulaceae IV. *Nordic journal of botany*, *4*(1), 1-19.

EDDIE, W. M. M., SHULKINA, T., GASKIN, J., HABERLE, R. C., & JANSEN, R. K. 2003. Phylogeny of Campanulaceae s. str. inferred from ITS sequences of nuclear ribosomal DNA. *Annals of the Missouri Botanical Garden*, 554-575.

FRAJMAN, B., & SCHNEEWEISS, G. M. 2009. A Campanulaceous fate: the Albanian stenoendemic *Asyneuma comosiforme* in fact belongs to isophyllous *Campanula*. I, 34(3), 595-601.

GAY, C. 1849. Cyphocarpo. *Historia fisica y politica de Chile.* Botanica 4: 335-337. Santiago: Museo Historia Natural.

GIVNISH, THOMAS J., KENDRA C. MILLAM, AUSTIN R. MAST, THOMAS B. PATERSON, TERRA J. THEIM, ANDREW L. HIPP, JILLIAN M. HENSS, JAMES F. SMITH, KENNETH R. WOOD, AND KENNETH J. SYTSMA. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society of London B: Biological Sciences* 276, (1656), 407-416.

GUERRERO, P. C., ROSAS, M., ARROYO, M. T., & WIENS, J. J. 2013. Evolutionary lag times and recent origin of the biota of an ancient desert (Atacama–Sechura). *Proceedings of the National Academy of Sciences*, *110*(28), 11469-11474.

GUSTAFSSON, M. H., & BREMER, K. 1995. Morphology and phylogenetic interrelationships of the Asteraceae, Calyceraceae, Campanulaceae, Goodeniaceae, and related families (Asterales). *American Journal of Botany*, 250-265.

GUSTAFSSON, M. H., BACKLUND, A., & BREMER, B. 1996. Phylogeny of the Asterales sensu lato based on rbcL sequences with particular reference to the Goodeniaceae. *Plant Systematics and Evolution*, *199*(3-4), 217-242.

GRAHAM, A. 2010. *Late Cretaceous and Cenozoic history of Latin American vegetation and terrestrial environments*. Missouri Botanical Garden Press.

HABERLE, ROSEMARIE C., ASHLEY DANG, TAMMY LEE, CYNTHIA PEÑAFLOR, HELEN CORTES-BURNS, ANDREA OESTREICH, LINDA RAUBESON ET AL. 2009. Taxonomic and biogeographic implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. *Taxon* 58(3): 715-734.

HARTLEY, A. J., CHONG, G., HOUSTON, J., & MATHER, A. E. 2005. 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. *Journal of the Geological Society*, *162*(3), 421-424.

HELED, J., & DRUMMOND, A. J. 2012. Calibrated tree priors for relaxed phylogenetics and divergence time estimation. *Systematic Biology*, *61*(1), 138-149.

HEKINIAN, R., BONTE, P., DUPLEY, P. L., BLANC, P. L., JEHANO, C., LABEYRIE, L., & DUPLEYSSIS, J. C. 1979. Volcanics from the Sierra Leone Rise. *Nature*, *257*(5680), 536-538.

HUELSENBECK, J. P., & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754-755.

HUERTAS, M. L., SCHNEIDER, J. V., & ZIZKA, G. 2007. Phylogenetic analysis of Palaua (Malveae, Malvaceae) based on plastid and nuclear sequences. *Systematic botany*, *32*(1), 157-165.

ITURRALDE-VINENT, M., & MACPHEE, R. D. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. Bulletin of the AMNH: no. 238.

KATOH, K., & STANDLEY, D. M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, *30*(4), 772-780.

KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THIERER, T., ASHTON, B., MENTJIES, P., & DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data.*Bioinformatics*, 28(12), 1647-1649.

KNOX, E. B., A. M. MUASYA, AND P. B. PHILLIPSON. 2006. The Lobeliaceae originated in southern Africa. *Taxonomy and Ecology of African Plants, their Conservation and Sustainable Use*. Proceedings of the XVIIth AETFAT Congress, Addis Ababa, Ethiopia. Royal Botanic Gardens, Kew pp. 215-227.

KNOX, E. B., MUASYA, A. M., & MUCHHALA, N. 2008. The predominantly South American clade of Lobeliaceae. *Systematic Botany*, 33(2), 462-468.

KNOX, E. B. 2014. The dynamic history of plastid genomes in the Campanulaceae *sensu lato* is unique among angiosperms. *Proceedings of the National Academy of Sciences*, 111(30), 11097-11102.

KOOPMAN, M. M., & AYERS, T. J. 2005. Nectar spur evolution in the Mexican lobelias (Campanulaceae: Lobelioideae). *American journal of botany*, 92(3), 558-562.

COSNER, M. E., JANSEN, R. K., AND LAMMERS, T. G. 1994. Phylogenetic relationships in the Campanulales based on rbcL sequences. *Plant systematics and evolution*, 190(1-2), 79-95.

LAGOMARSINO, L. P., ANTONELLI, A., MUCHHALA, N., TIMMERMANN, A., MATHEWS, S., & DAVIS, C. C. 2014. Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae). *American journal of botany*, *101*(12), 2097-2112.

LANCUCKA-SRODONIOWA, M. A. R. I. A. 1977. New herbs described from the Tertiary of Poland. *Acta Palaeobotanica*.

LANCUCKA SRODONIOWA, M. 1979. Macroscopic plant remains from the freshwater Miocene of the Nowy Sacz basin (West Carpathians, Poland). *Acta Palaeobot*, *20*, 3-117.

LAMMERS, T. G. 1992. Circumscription and phylogeny of the Campanulales. *Annals of the Missouri Botanical Garden*, 388-413.

LAMMERS, T. G. 2007a. World checklist and bibliography of Campanulaceae. Royal Botanic Gardens, Kew, UK.

LAMMERS, T. G. 2007b. Campanulaceae. *In* K. Kubitzki, J. W. Kadereit, and C. Jeffrey [eds.], The families and genera of vascular plants, 26-56. Springer-Verlag, Berlin, Germany.

LAVIN, M., WOJCIECHOWSKI, M. F., GASSON, P., HUGHES, C., & WHEELER, E. 2003. Phylogeny of robinioid legumes (Fabaceae) revisited: Coursetia and Gliricidia recircumscribed, and a biogeographical appraisal of the Caribbean endemics. *Systematic Botany*, *28*(2), 387-409.

LONSDALE, P., 1989, Geology and tectonic history of the Gulf of California, *in* Winterer, E.L., Hussong, D.M., and Decker, R.W., eds., The Eastern Pacific Ocean and Hawaii: Boulder, Colorado, Geological Society of America, Decade of North American Geology, v. N, p. 499–521.

LUEBERT, F., & WEN, J. 2008. Phylogenetic analysis and evolutionary diversification of Heliotropium sect. Cochranea (Heliotropiaceae) in the Atacama Desert. *Systematic Botany*, *33*(2), 390-402.

LUEBERT, F. 2011. Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande*, (50), 105-133.

LUNDBERG, J., AND K. BREMER. 2003. A phylogenetic study of the order Asterales using one morphological and three molecular data sets. *International Journal of Plant Sciences* 164: 553-578.

MAGALLÓN, S., GÓMEZ-ACEVEDO, S., SÁNCHEZ-REYES, L. L., & HERNÁNDEZ-HERNÁNDEZ, T. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist*.

MANSION, GUILHEM, GERALD PAROLLY, ANDREW A. CROWL, EVGENY MAVRODIEV, NICO CELLINESE, MARINE OGANESIAN, KATHARINA FRAUNHOFER ET AL. 2012. How to handle speciose clades? Mass taxon-sampling as a strategy towards illuminating the natural history of Campanula (Campanuloideae)." *PLoS One* 7, no. 11: e50076.

MARSHALL, C. J., & LIEBHERR, J. K. 2000. Cladistic biogeography of the Mexican transition zone. *Journal of biogeography*, *27*(1), 203-216.

MIERS, J. 1848. Contributions to the botany of South America. *Cyphocarpus. London Journal of Botany*, 7: 59-64.

MOORE, M. J., TYE, A., & JANSEN, R. K. 2006. Patterns of long-distance dispersal in Tiquilia subg. Tiquilia (Boraginaceae): implications for the origins of amphitropical disjuncts and Galapagos Islands endemics. *American Journal of Botany*, *93*(8), 1163-1177.

MORLEY, R. J. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics*, *6*(1), 5-20.

PARK, J. M., KOVAČIC, S., LIBER, Z., EDDIE, W. M., & SCHNEEWEISS, G. M. 2006. Phylogeny and biogeography of isophyllous species of Campanula (Campanulaceae) in the Mediterranean area. *Systematic Botany*, 31(4), 862-880.

PARRISH, J. T. 1993. Climate of the supercontinent Pangea. *The Journal of Geology*, 215-233.

PREBBLE, J. M., MEUDT, H. M., & GARNOCK-JONES, P. J. 2012. An expanded molecular phylogeny of the southern bluebells (Wahlenbergia, Campanulaceae) from Australia and New Zealand. *Australian Systematic Botany*, *25*(1), 11-30.

REICH, MARTIN, CARLOS PALACIOS, GABRIEL VARGAS, SHANGDE LUO, EION M. CAMERON, MATTHEW I. LEYBOURNE, MIGUEL A. PARADA, ALEJANDRO ZÚÑIGA, AND CHENG-FENG YOU. 2009. Supergene enrichment of copper deposits since the onset of modern hyperaridity in the Atacama Desert, Chile. *Mineralium Deposita*, *44*(5), 497-504.

ROQUET, C., SÁEZ, L., ALDASORO, J. J., SUSANNA, A., ALARCÓN, M. L., & GARCÍA-JACAS, N. 2008. Natural delineation, molecular phylogeny and floral evolution in Campanula. *Systematic Botany*, 33(1), 203-217.

ROQUET, C., SANMARTÍN, I., GARCIA-JACAS, N., SÁEZ, L., SUSANNA, A., WIKSTRÖM, N., & ALDASORO, J. J. 2009. Reconstructing the history of Campanulaceae with a Bayesian approach to molecular dating and dispersal–vicariance analyses. *Molecular Phylogenetics and Evolution*, *52*(3), 575-587.

RZEDOWSKI, J. 1966. Vegetación del Estado de San Luis Potosí.

SCHERSON, R. A., VIDAL, R., & SANDERSON, M. J. 2008. Phylogeny, biogeography, and rates of diversification of New World Astragalus (Leguminosae) with an emphasis on South American radiations. *American Journal of Botany*, *95*(8), 1030-1039.

SCHLUNEGGER, F., KOBER, F., ZEILINGER, G., & VON ROTZ, R. 2010. Sedimentology-based reconstructions of paleoclimate changes in the Central Andes in response to the uplift of the Andes, Arica region between 19 and 21 S latitude, northern Chile. *International Journal of Earth Sciences*, *99*(1), 123-137.

SCHONLAND, S. 1889-1894. Campanulaceae. ln: A. Engler & K. Prantl(editors), Die naturlichenP flanzenfamilien IV, 5: 4n70. W.Engelmann, Leipzig.

SCHULTHEIS, L. M. 2001. Systematics of Downingia (Campanulaceae) based on molecular sequence data: implications for floral and chromosome evolution. *Systematic Botany*, 26(3), 603-621.

STACE, H. M., & JAMES, S. H. 1996. Another perspective on cytoevolution in Lobelioideae (Campanulaceae). *American Journal of Botany*, 1356-1364.

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, btu033.
STEFANOVIC, S., & LAKUSIC, D. 2009. Molecular reappraisal confirms that the *Campanula* trichocalycina-pichleri complex belongs to *Asyneuma* (Campanulaceae). Bot Serb, 33, 21-31SALES, F., Ian, C., EDDIE, W., PRESTON, J., & MOELLER, M. (2004). *Jasione* L. taxonomy and phylogeny. *Turk J Bot*, 28, 253-259.

STRAUB, SHANNON CK, MARK FISHBEIN, TATYANA LIVSHULTZ, ZACHARY FOSTER, MATTHEW PARKS, KEVIN WEITEMIER, RICHARD C. CRONN, AND AARON LISTON. 2011. Building a model: developing genomic resources for common milkweed (Asclepias syriaca) with low coverage genome sequencing. *BMC genomics* 12(1): 1.

ŠTORCHOVÁ, H., HRDLIČKOVÁ, R., CHRTEK JR, J., TETERA, M., FITZE, D., & FEHRER, J. 2000. An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. *Taxon*, 79-84.

SWOFFORD, D. L. 2002. PAUP 4.0 b10: Phylogenetic analysis using parsimony.
Hall, T. A. (1999, January). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, pp. 95-98).

TAKHTAJAN, A. L. 1980. Outline of the classification of flowering plants (Magnoliophyta). *The botanical review*, *46*(3), 225-359.

TAKHTAJAN, A.L. 1983. The systematic arrangement of dicotyledonous families. *Anatomy of the dicotyledons*, *2*(2), 180-201.

TAKHTAJAN, A.L. 1987. Systema magnoliophytorum. Nauka.

TAKHTAJAN, A.L. 1997. Diversity and classification of flowering plants. New York: Columbia University Press.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., & KUMAR, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, mst197.

TANK, DAVID C., JONATHAN M. EASTMAN, MATTHEW W. PENNELL, PAMELA S. SOLTIS, DOUGLAS E. SOLTIS, CODY E. HINCHLIFF, JOSEPH W. BROWN, EMILY B. SESSA, AND LUKE J. HARMON. 2015. Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. *New Phytologist*, *207*(2), 454-467.

THORNE. R.F. 1983. Proposed new realignments in angsiosperms. *Nordic Journal of Botany*. 3:85-117.

WANG, Q., ZHOU, S. L., & HONG, D. Y. 2013. Molecular phylogeny of the platycodonoid group (Campanulaceae s. str.) with special reference to the circumscription of Codonopsis. *Taxon*, *62*(3), 498-504.

WENDLING, B. M., GALBREATH, K. E., & DECHAINE, E. G. 2011. Resolving the evolutionary history of Campanula (Campanulaceae) in western North America. *PloS one*, *6*(9), e23559.

WIGGINS, I. L. 1980. *Flora of Baja California*. Stanford University Press.

WIKSTRÖM, N., SAVOLAINEN, V., & CHASE, M. W. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1482), 2211-2220.

WIMMER, F. E., DIELS, L., ENGLER, A., & STUBBE, H. 1968. *Campanulaceae, Lobelioideae Supplementum et Campanulaceae, Cyphioideae*. Engelmann.

Table 1. Comparative morphology among subfamilies of Campanulaceae. Asterisks denote traits that are the predominant condition but with a few exceptions.

	Campanuloideae	Cyphioideae	Lobelioideae	Nemacladoideae	Cyphocarpoideae
habit	variable	perennial	variable	annual*	annual
resupination	non-resupinate	non-resupinate	resupinate*	variable	non-resupinate
symmetry	actinomorphic	zygomorphic 3:2	zygomorphic 2:3	variable	zygomorphic 1:4
filament fusion	free	variable	partially fused	partially fused	free
anthers	connivent	connivent	connate	connivent	connivent
pollen surface sculpture	variable	nanogranulate	reticulate*	spinulate	reticulate
styles at maturity	straight	straight	straight	curved	curved
number of stigma lobes	variable	one	two	two	two
capsule dehiscence	apical valves*	apical valves	apical valves*	apical valves	longitudinal slits
placentation at maturity	axile*	apical	axile*	axile	free central

Table 2. Historical classification of Campanulaceae.

Taxa recognized	Author
Cyphioideae *s.l.*	
Cyphia	Bremer 1876
Pseudonemacladus	Schonland 1889
Nemacladus	Wimmer 1968
Cyphocarpus	Takhtajan 1980, 1983
	Lammers 1992
5 distinct clades	
Campanuloideae	
Lobelioideae	Cronquist 1980, 1987
Cyphioideae	Tahktajan 1987, 1996
Nemacladoideae	Lammers 1998, 2007
Cyphocarpoideae	
Lobelioideae *s.l.*	
Lobeliodieae	
Nemacladoideae	Thorne 1983
Cyphocarpoideae	

Table 3. Classification and references for taxa analyzed in this study *Indicates taxa included in both data sets. † Indicates taxa included only in cpDNA data set. ‡ Indicates taxa included only in nrDNA dataset.

Classification	Literature Reference	Specimen Reference
Campanuliidae		
Asterales		
Campanulaceae		
Lobelioideae		
* *Dialypetalum floribundum*	this study	Koopman 160 (MO)
* *Diastatea micrantha*	this study	Ayers 1775 (ASC)
* *Diastatea tenera*	this study	Ayers 1777 (ASC)
* *Downingia cuspidata*	this study	Ayers 969 (ASC)
* *Isotoma fluviatilis*	this study	Hansen s.n. (ASC)
† *Lobelia anceps*	Knox, 2014	Knox 2370 (IND) TreeBase 15797
† *Lobelia baumannii*	Knox, 2014	Knox 3609 (IND) TreeBase 15797
† *Lobelia malowensis*	Knox, 2014	Knox 4814 (IND) TreeBase 15797
* *Lobelia morogoroensis*	this study	Knox 619 (IND)
* *Lobelia laxiflora*	this study	Ayers 413 (TEX)
* *Lobelia erinus*	this study	Hansen s.n. (ASC)
† *Lobelia heterophylla*	Knox, 2014	Archer 1101021 TreeBase 15797
* *Lobelia jalicensis*	this study	Ayers 1776 (ASC)
* *Lobelia polyphylla*	this study	Ayers 1570 (ASC)
* *Lobelia volcanica*	this study	Ayers 1765 (ASC)
* *Lobelia xalapensis*	this study	Ayers 1790 (ASC)
† *Lobelia sonderiana*	Knox, 2014	Knox 4662 (IND) TreeBase 15797
* *Lysipomia laciniata* subsp. *linearifolia*	this study	Dotti 119 (ASC)
‡ *Lysipomia lehmannii*	this study	
* *Lysipomia sparrei*	this study	Ayers 1417 (ASC)
* *Lysipomia multiflora*	this study	
* *Lysipomia muscoides* subsp. *delicatula*	this study	Sanchez-Vega 8713 (ASC)
† *Lysipomia sp. nov.*	this study	Sanchez-Vega 8728 (ASC)
* *Monopsis lutea*	this study	Ayers 610 (BH)

Table 3. continued

Classification	Literature Reference	Specimen Reference
Campanuliidae		
Asterales		
Campanulaceae		
Lobelioideae		
* *Palmerella debilis*	this study	Johnson 22 (ASC)
* *Siphocampylus jelskii*	this study	Sanchez-Vega 8725 (ASC)
Campanuloideae		
† *Adenophora remotiflora*	Kim, 2015	GenBank NC026999
* *Campanula armena*	this study	Hansen s.n. (ASC)
* *Campanula garganica*	this study	Hansen s.n. (ASC)
* *Campanula glomerata*	this study	Ayers s.n. (ASC)
* *Campanula medium*	this study	Hansen s.n. (ASC)
* *Campanula parryi*	this study	Rink 9836 (ASC)
* *Campanula punctata*	this study	Hansen s.n. (ASC)
* *Campanula rotundifolia*	this study	Hansen s.n. (ASC)
* *Campanula sibirica* subsp. *taurica*	this study	Hansen s.n. (ASC)
* *Codonopsis clematidea*	this study	Hansen s.n. (ASC)
* *Cyananthus lobatus*	this study	living accession DBG
† *Hanabusaya asiatica*	unpublished	GenBank NC024732
* *Musschia aurea*	this study	Ayers s.n (ASC)
* *Phyteuma scheuzeri*	this study	Hansen s.n. (ASC)
* *Platycodon grandiflorus*	this study	Hansen s.n. (ASC)
† *Trachelium caeruleum*	Haberle, 2008	GenBank NC010442
* *Wahlenbergia pygmaea*	this study	Rominger 2765 (ASC)
Cyphioideae		
† *Cyphia angustiloba*	Knox, 2014	Knox 5134F (IND) TreeBase 15797
† *Cyphia banksiana*	Knox, 2014	Knox 5108H (IND) TreeBase 15797
† *Cyphia belfastica*	Knox, 2014	Knox 5164 (IND) TreeBase 15797
† *Cyphia bulbosa*	Knox, 2014	Knox 5106A (IND) TreeBase 15797
* *Cyphia bulbosa*	this study	Raven 28882 (MO)

Table 3. continued

Classification	Literature Reference	Specimen Reference
Campanuliidae		
Asterales		
Campanulaceae		
Cyphioideae		
† *Cyphia crenata*	Knox, 2014	Knox 5110A (IND) TreeBase 15797
† *Cyphia dentariifolia*	Knox, 2014	Knox 5126 (IND) TreeBase 15797
† *Cyphia elata*	Knox, 2014	Knox 5165 (IND) TreeBase 15797
† *Cyphia glandulifera*	Knox, 2014	Knox 3706C (IND) TreeBase 15797
* *Cyphia longipetala*	this study	Raven 28883 (MO)
† *Cyphia phyteuma*	Knox, 2014	Knox 5155A (IND) TreeBase 15797
† *Cyphia schlechteri*	Knox, 2014	Knox 5115E (IND) TreeBase 15797
† *Cyphia tortilis*	Knox, 2014	Knox 5141B (IND) TreeBase 15797
Nemacladoideae		
* *Nemacladus glanduliferus*	this study	Ayers 1413 (ASC)
* *Nemacladus gracilis*	this study	Morin 635
* *Nemacladus interior*	this study	Morin 671
* *Nemacladus pinnatifidus*	this study	Morin 583
‡ *Nemacladus tenuis*	this study	Morin 601
* *Pseudonemacladus oppositifolia*	this study	Ayers 1751 (ASC)
Cyphocarpoideae		
* *Cyphocarpus innocuus*	this study	Ayers 1514 (SGO)
* *Cyphocarpus psammophilus*	this study	Ayers 1553 (SGO)
* *Cyphocarpus rigescens*	this study	Ayers 1581 (SGO)
Asterales		
Rousseaceae		
† *Carpodetus serratus*	Knox, 2014	Knox 5211 (IND) TreeBase 15797

Table 3. continued

Classification	Literature Reference	Specimen Reference
Campanuliidae		
Asteraceae		
* *Acourtia wrightii*	this study	Goodwin 3429 (ASC)
† *Helianthus annuus*	Timme, 2007	GenBank DQ383815
† *Praxelis clematidea*	Zhang, 2014	GenBank NC023833
† *Silybum marianum*	unpublished	GenBank NC028027
Goodeniaceae		
* *Scaevola aemula*	this study	Hansen s.n. (ASC)
Dipsacales		
Caprifoliaceae		
† *Lonicera japonica*	unpublished	GenBank NC026839
Lamiidae		
Solanales		
Convolvulaceae		
† *Ipomoea batatas*	Yan, 2015	GenBank NC026703
Solanaceae		
† *Cuscuta exaltata*	McNeal, 2007	GenBank NC009963
Gentianales		
Rubiaceae		
† *Coffea arabica*	Samson, 2007	GenBank NC008535
Garryales		
Apocynaceae		
† *Asclepias syriaca*	Straub, 2013	GenBank NC022432
Lamiales		
Gesneriaceae		
† *Boea hygrometrica*	Zhang, 2012	GenBank NC016468
Oleaceae		
† *Olea europaea*	unpublished	GenBank NC013707
Basal Euasterids		
Ericales		
Primulaceae		
† *Primula poissonii*	Yang, 2014	GenBank NC024543
Theaceae		
† *Camellia sinensis*	Yang, 2013	GenBank NC020019

Table 4. Optimal models of evolution used for BEAST divergence dating analysis.

Gene	Model	Gene	Model	Gene	Model
atpA	GTR+G+I	petN	JC+G	rpl33	GTR+I
atpB	GTR+G+I	psaA	GTR+G+I	rpl36	JC+G
atpE	GTR+G	psaB	GTR+G+I	rpoA	GTR+G+I
atpF	GTR+G	psaC	HKY+G	rpoB	GTR+G+I
atpH	GTR+G	psaI	HKY+G	rpoC1	GTR+G+I
atpI	GTR+G+I	psaJ	HKY+G	rpoC2	GTR+G+I
cemA	GTR+G	psbA	GTR+G+I	rps2	GTR+G
matK	GTR+G	psbC	GTR+G+I	rps4	GTR+G
rbcL	GTR+G+I	psbD	GTR+G	rps7	GTR+G
ycf4	GTR+G	psbE	HKY+G	rps8	GTR+G
ndhB	GTR+G	psbF	JC+G	rps11	GTR+G+I
ndhC	GTR+G	psbH	HKY+G	rps14	GTR+G
ndhI	HKY+G	psbI	JC+G	rps18	JC+G
ndhJ	GTR+G	psbK	GTR+G	rps19	HKY+G
petA	GTR+G	psbZ	GTR+G	psbL	HKY+G
petB	HKY+G	rpl2	GTR+G	psbM	JC+G
petD	GTR+G+I	rpl14	GTR+G	psbN	JC+G+I
petG	HKY+G	rpl16	GTR+G	psbT	JC+G+I
petL	JC+G	rpl20	GTR+G+I	ccsA	GTR+G

Table 5. Summary of short-reads and assembly statistics.

	unfiltered reads	clean reads	reads mapped to cpDNA reference	% of cpDNA reference covered (162,321 bp)	mean coverage cpDNA	reads mapped to nrDNA reference	% of nrDNA reference covered (5353 bp)	mean coverage nrDNA
Acourtia wrightii	1060588	849124	73366	72.8	57.4	20868	95.4	168.8
Campanula armena	1986678	1723596	118449	96.8	105.6	70711	100	753.9
Campanula garganica	2042308	3689045	152328	82.4	425.5	69348	100	1140
Campanula glomerata	2303612	1955403	35121	93.3	28.3	60863	100	572
Campanula medium	363424	265257	46352	99.8	42.6	2071	100	34.9
Campanula parryi	1282996	1039319	16186	60.3	29.2	8245	100	119.9
Campanula punctata	542348	477782	9472	95.9	14.9	6160	100	72.9
Campanula rotundifolia	2410832	1842137	235446	99.8	206	152571	100	4183.4
Campanula sibirica subsp. taurica	3284162	2764819	308918	99.8	271.8	44477	100	668.2
Codonopsis clematidea	3401274	2237679	253556	99.9	261.2	238700	100	4236.4
Cyananthus lobatus	925010	823353	8701	96.8	14.3	9600	100	259.4
Cyphia bulbosa Raven 28882	3355348	2708454	175255	90.8	246.5	27648	100	644.4
Cyphia longipetala	2599876	2208565	43197	82.2	21.8	33620	100	432
Cyphocarpus innocuus	2102392	1774859	172939	93.4	142.8	296919	100	245.5
Cyphocarpus psammophilus	1474048	1240001	137440	91.2	119.3	36738	100	330.2
Cyphocarpus rigescens	2468788	1740488	179441	94.6	193.7	34519	100	589.5
Dialypetalum floribundum	1542076	1137704	81963	92.2	105.2	15049	100	247.3
Diastatea micrantha	2171336	1456907	333062	100	379.2	15160	99.8	286.6
Diastatea tenera	1346604	1136822	66114	96.7	62.3	17950	100	114.6
Downingia cuspidata	1590338	1398095	100348	77.4	96.6	46627	99.9	613.3
Isotoma fluviatavilis	534556	415246	38892	99.2	41.3	3175	100	44.5
Lobelia erinus	657638	554694	16599	97.7	19.5	5217	100	108.6
Lobelia jalicensis	2936864	2459700	94303	91.3	122.3	1813	90.2	35.2
Lobelia laxiflora	209348	164177	8934	96.2	9.3	23554	98.9	431.2
Lobelia morogoroensis	357090	311798	9868	93	11.2	780	84.2	12.9
Lobelia polyphylla	947536	688309	74517	98.9	84.5	25322	100	180.7
Lobelia volcanica	1564022	1231331	122870	93	149.6	19036	100	695
Lobelia xalapensis	2171538	1860419	34620	83.1	35.1	21888	100	392.7
Lysipomia sp. nov SV8728	1082912	902580	95769	76.2	96.9	42848	100	338.1
Lysipomia laciniata subsp. linearifolia	1873730	1602971	288290	92.7	305.8	22586	100	398.1
Lysipomia lehmannii	419460	334104	13606	77.7	16.4	8887	100	22.8
Lysipomia multiflora	1067422	702554	147847	87.8	158.7	13140	100	176.8
Lysipomia muscoides subsp. delicatula	1271392	1006402	53555	80.4	44.6	7317	91	149.3
Lysipomia sparrei	1814236	1520523	66803	84.7	61.4	84158	99.7	3296
Monopsis lutea	2468866	1724992	246707	92.2	309.7	24926	100	433.6

Table 5. continued

	unfiltered reads	clean reads	reads mapped to cpDNA reference	% of cpDNA reference covered (162,321 bp)	mean coverage cpDNA	reads mapped to nrDNA reference	% of nrDNA reference covered (5353 bp)	mean coverage nrDNA
Musschia aurea	812908	573568	105875	99.5	157.9	4279	100	101.3
Nemacladus glanduliferus	2735036	1997887	131068	92.5	138.2	49405	100	2022.4
Nemacladus gracilis	2355360	1649935	125302	84.8	132.7	42996	100	721.7
Nemacladus interior	1206642	887131	63985	83.4	73.1	26240	100	364.1
Nemacladus pinnatifidus	1182554	913183	60864	84.4	57.9	14974	100	294
Nemacladus tenuis	1351338	890829				20221	100	442.7
Palmerella debilis	1102922	2301402	150124	90.3	96.3	62679	100	1183.7
Phyteuma sheuzeri	2858984	2360450	131251	90	70.3	88046	100	1634.3
Platycodon grandiflorus	2508554	2155608	149517	89.9	170.5	78803	92	472.5
Pseudonemacladus oppositifolius	761446	565735	43443	98.8	46	4699	100	86.4
Scaevola aemula	3336494	2923350	49223	78.3	39.3	26238	100	503.2
Siphocampylus jelskii	1351608	992531	130871	93.7	147.4	29415	100	363
Wahlenbergia pygmaea	758744	664881	36650	99.6	60.4	2692	100	55.8

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

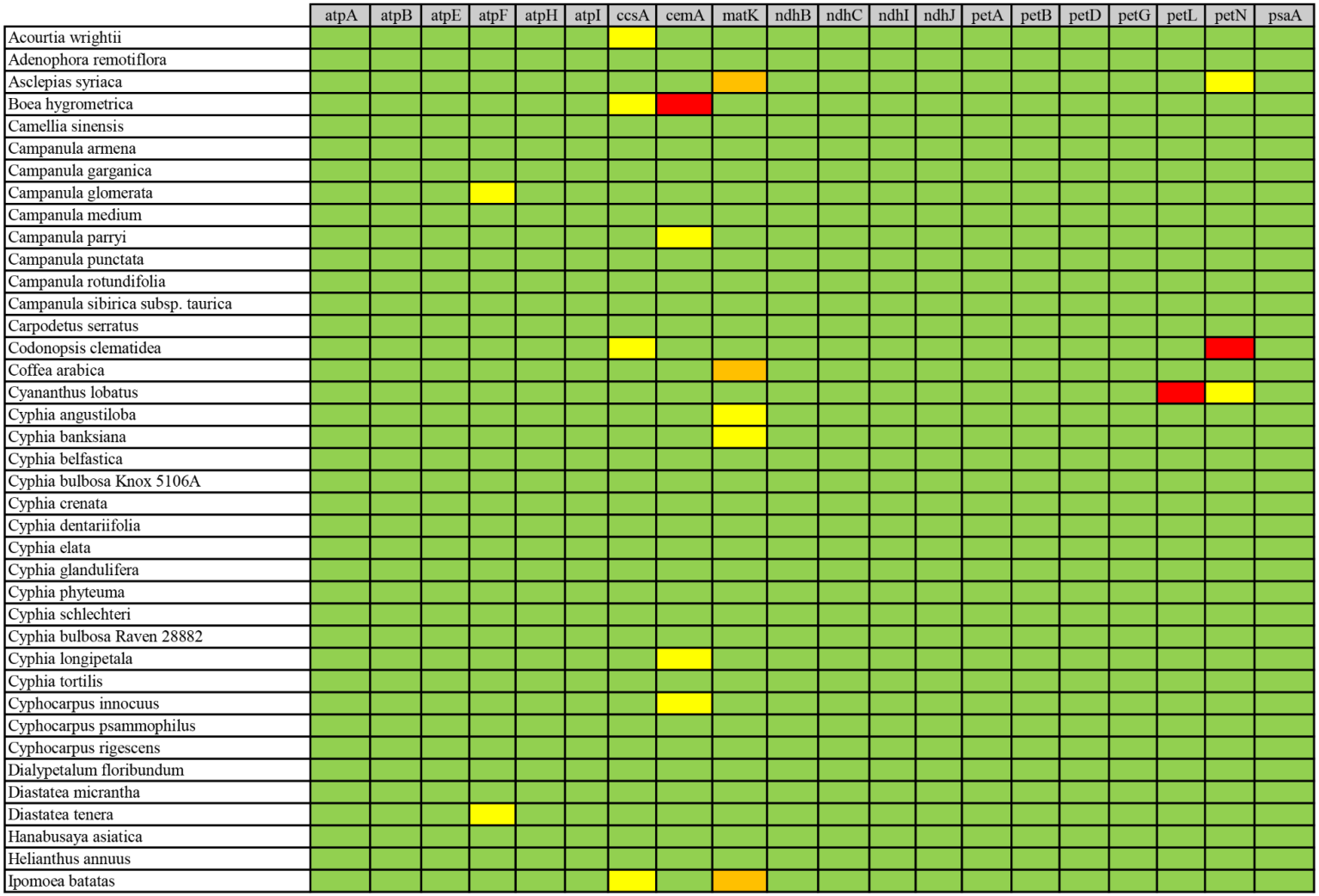

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

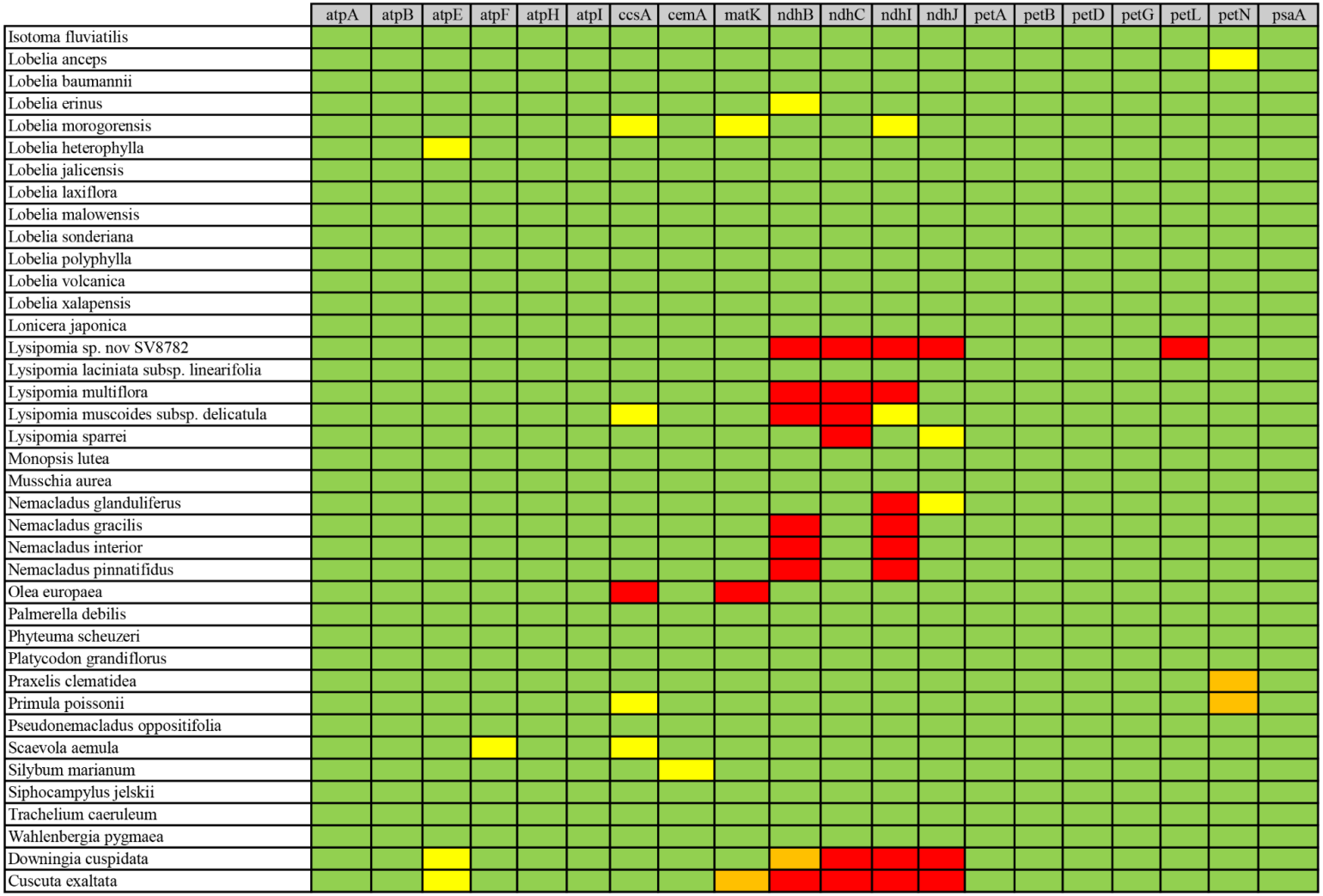

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

	psaB	psaC	psaI	psaJ	psbA	psbC	psbD	psbE	psbF	psbH	psbI	psbK	psbL	psbM	psbN	psbT	psbZ	rbcL	rpl2	rpl14
Acourtia wrightii																				
Adenophora remotiflora																				
Asclepias syriaca																				
Boea hygrometrica																				
Camellia sinensis																				
Campanula armena																				
Campanula garganica																				
Campanula glomerata																				
Campanula medium																				
Campanula parryi																				
Campanula punctata																				
Campanula rotundifolia																				
Campanula sibirica subsp. taurica																				
Carpodetus serratus																				
Codonopsis clematidea																				
Coffea arabica																				
Cyananthus lobatus																				
Cyphia angustiloba																				
Cyphia banksiana																				
Cyphia belfastica																				
Cyphia bulbosa Knox 5106A																				
Cyphia crenata																				
Cyphia dentariifolia																				
Cyphia elata																				
Cyphia glandulifera																				
Cyphia phyteuma																				
Cyphia schlechteri																				
Cyphia bulbosa Raven 28882																				
Cyphia longipetala																				
Cyphia tortilis																				
Cyphocarpus innocuus																				
Cyphocarpus psammophilus																				
Cyphocarpus rigescens																				
Dialypetalum floribundum																				
Diastatea micrantha																				
Diastatea tenera																				
Hanabusaya asiatica																				
Helianthus annuus																				
Ipomoea batatas																				

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

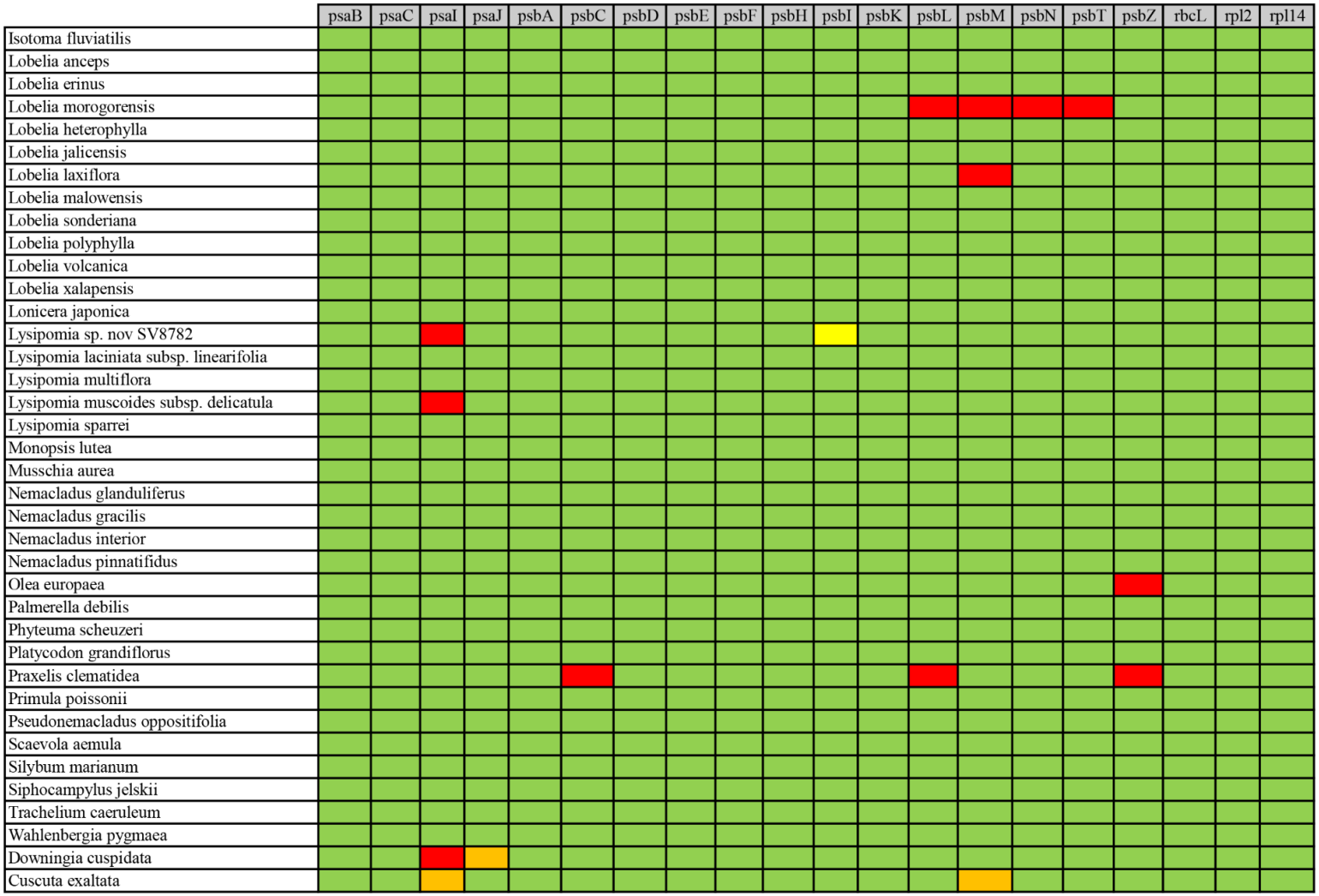

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

	rpl16	rpl20	rpl33	rpl36	rpoA	rpoB	rpoC1	rpoC2	rps2	rps4	rps7	rps8	rps11	rps14	rps18	rps19	ycf4
Acourtia wrightii																	
Adenophora remotiflora																	
Asclepias syriaca																	
Boea hygrometrica																	
Camellia sinensis																	
Campanula armena																	
Campanula garganica																	
Campanula glomerata																	
Campanula medium																	
Campanula parryi																	
Campanula punctata																	
Campanula rotundifolia																	
Campanula sibirica subsp. taurica																	
Carpodetus serratus																	
Codonopsis clematidea																	
Coffea arabica																	
Cyananthus lobatus																	
Cyphia angustiloba																	
Cyphia banksiana																	
Cyphia belfastica																	
Cyphia bulbosa Knox 5106A																	
Cyphia crenata																	
Cyphia dentariifolia																	
Cyphia elata																	
Cyphia glandulifera																	
Cyphia phyteuma																	
Cyphia schlechteri																	
Cyphia bulbosa Raven 28882																	
Cyphia longipetala																	
Cyphia tortilis																	
Cyphocarpus innocuus																	
Cyphocarpus psammophilus																	
Cyphocarpus rigescens																	
Dialypetalum floribundum																	
Diastatea micrantha																	
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Hanabusaya asiatica																	
Helianthus annuus																	
Ipomoea batatas																	

Table 6. Percentage of missing data from cpDNA dataset. Red = 90-100% , orange = 51-89%, yellow = 11-50%, green 0-10%.

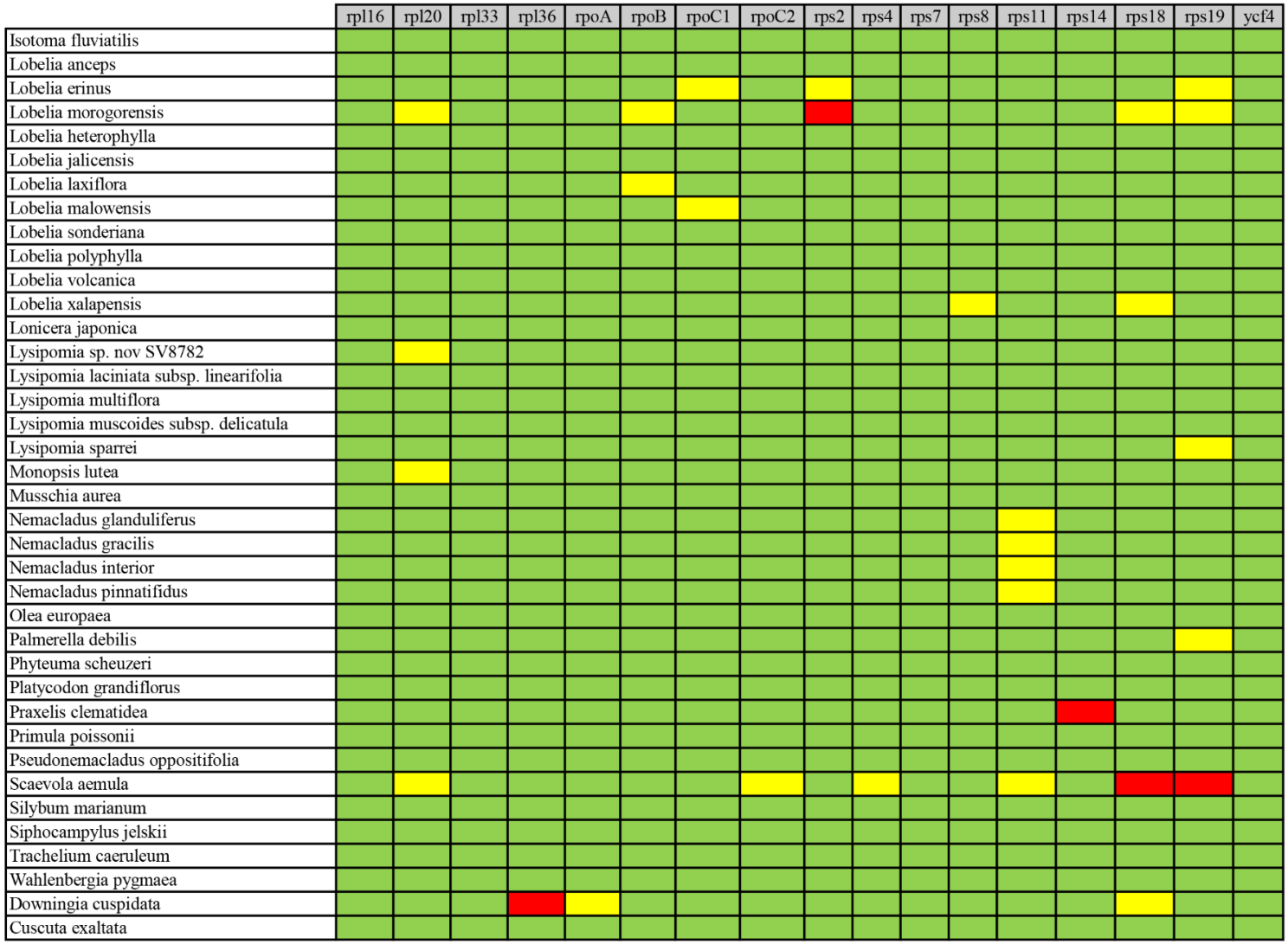

Table 7. Percentage of missing data in the nrDNA dataset.

	28s	5.8s	18s
Acourtia wrightii			
Campanula armena	1.2		
Campanula garganica			
Campanula glomerata			
Campanula medium			
Campanula parryi			
Campanula punctata			
Campanula rotundifolia			
Campanula sibirica subsp. taurica			
Codonopsis clematidea			
Cyananthus lobatus			
Cyphia bulbosa Raven 28882			
Cyphia longipetala			
Cyphocarpus innocuus			
Cyphocarpus psammophilus			
Cyphocarpus rigescens	2.9		
Dialypetalum floribundum			
Diastatea micrantha			
Diastatea tenera			
Downingia cuspidata			
Isotoma fluvitavilis			
Lobelia morogorensis			
Lobelia erinus			
Lobelia jalicensis			
Lobelia laxiflora			
Lobelia polyphylla			
Lobelia volcanica			
Lobelia xalapensis			
Lysipomia laciniata subsp. linearifolia			
Lysipomia lehmannii			
Lysipomia multiflora			
Lysipomia muscoides subsp. delicatula			
Lysipomia sparrei			
Monopsis lutea	1.7		
Musschia aurea			
Nemacladus glanduliferus			
Nemacladus gracilis			
Nemacladus interior			
Nemacladus pinnatifidus			
Nemacladus tenuis			
Palmerella debilis			
Phyteuma scheuzeri			
Platycodon grandiflorus			
Pseudonemacladus oppositifolia			
Scaevola aemula			
Siphocampylus jelskii			
Wahlenbergia pygmaea			

Table 8. Summary of support values from phylogenetic analyses. MLBV = Maximum Likelihood Bootstrap Values, MPBV = Maximum Parsimony Bootrap Values, BIPP = Bayesian Inference Posterior Probabilites

	cpDNA				nrDNA		
	MLBV	**BIPP**	**MPBV**		**MLBV**	**BIPP**	**MPBV**
Campanulaceae	100	1	50		100	1	100
Campanuloideae	100	1	100		58	0.5432	N/A
Lobelioideae	100	1	99		100	1	100
Cyphioideae	100	1	100		100	1	100
Nemacladoideae	94	1	N/A		51	0.6991	N/A
Cyphocarpoideae	100	1	100		100	0.9993	100
Nemacladus	100	1	100		100	1	100
[Nemacladoideae + Cyphocarpoideae]	100	1	65		68	0.996	50

Table 9. Divergence time estimates for clades of interest from the BEAST analysis. Ages are estimated in millions of years before present. Mean age is provided, followed by the 95% highest posterior density in

Node	Age
[Campanulaceae + Rousseaceae]	76.8 (67.0-85.9)
[[Campanuloideae , Cyphioideae] + [Lobeliodeae, Nemacladoideae, Cyphocarpoideae]]	62.5 (55.5-69.5)
[Campanuloideae + Cyphioideae]	60.5 (53.3-67.9)
[Lobelioideae + [Nemacladoideae, Cyphocarpoideae]]	60.0 (52.9-66.8)
[Nemacladoideae + Cyphocarpoideae]	57.0 (47.9-65.0)
crown Campanuloideae	51.5 (43.2-59.4)
crown Cyphioideae	23.2 (15.6-31.1)
crown Lobelioideae	55.7 (48.6-62.7)
crown Nemacladoideae	52.5 (41.1-61.6)
crown Cyphocarpoideae	12.8 (2.8-21.8)
crown *Nemacladus*	27.1 (17.3-37.2)

Figure 1. Distribution map of Cyphocarpoideae

Figure 2. Maximum likelihood tree reconstruction from plastid DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

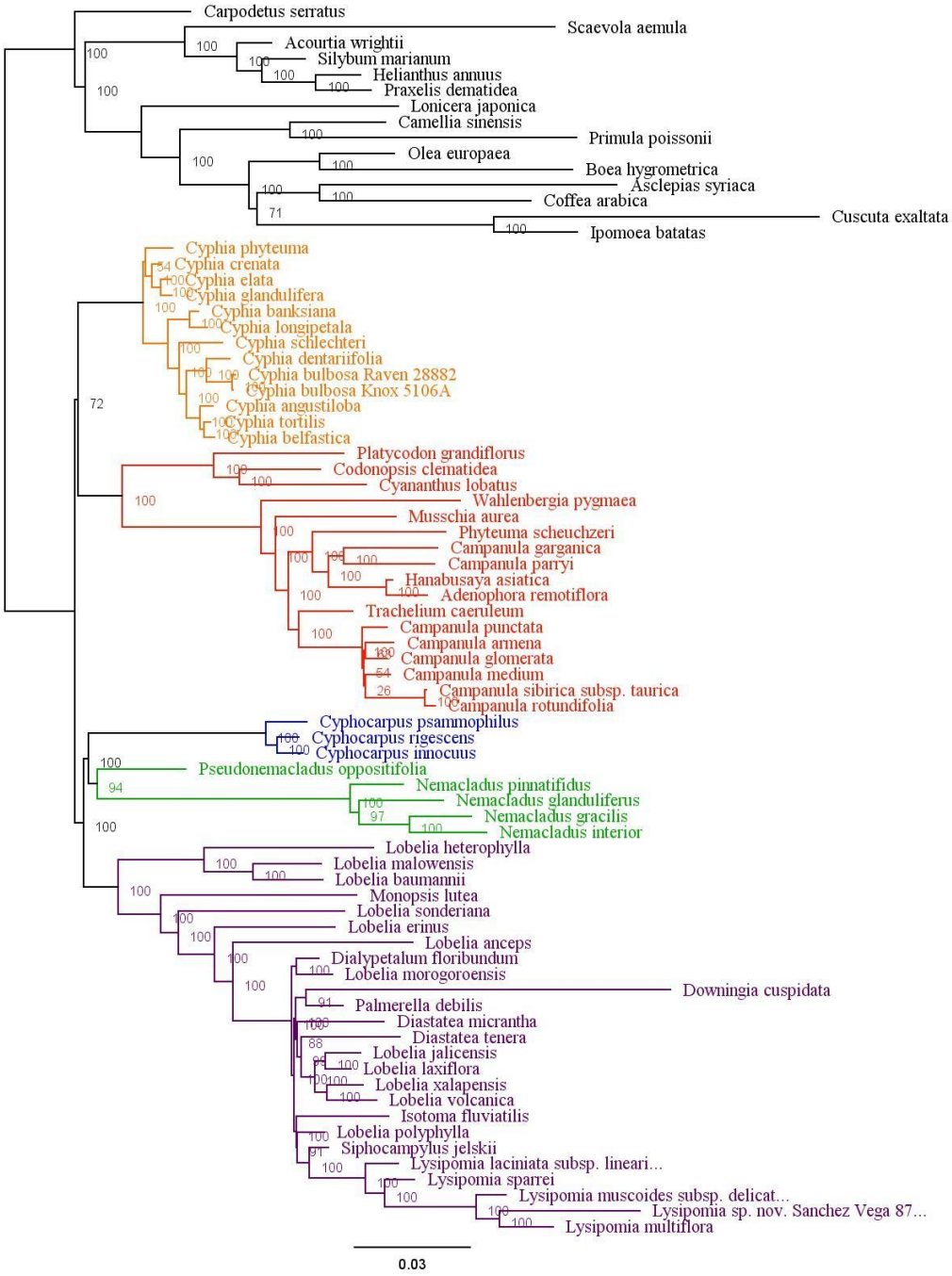

Figure 3. Bayesian inference tree reconstruction from plastid DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

Figure 4. Maximum parsimony tree reconstructed from plastid DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

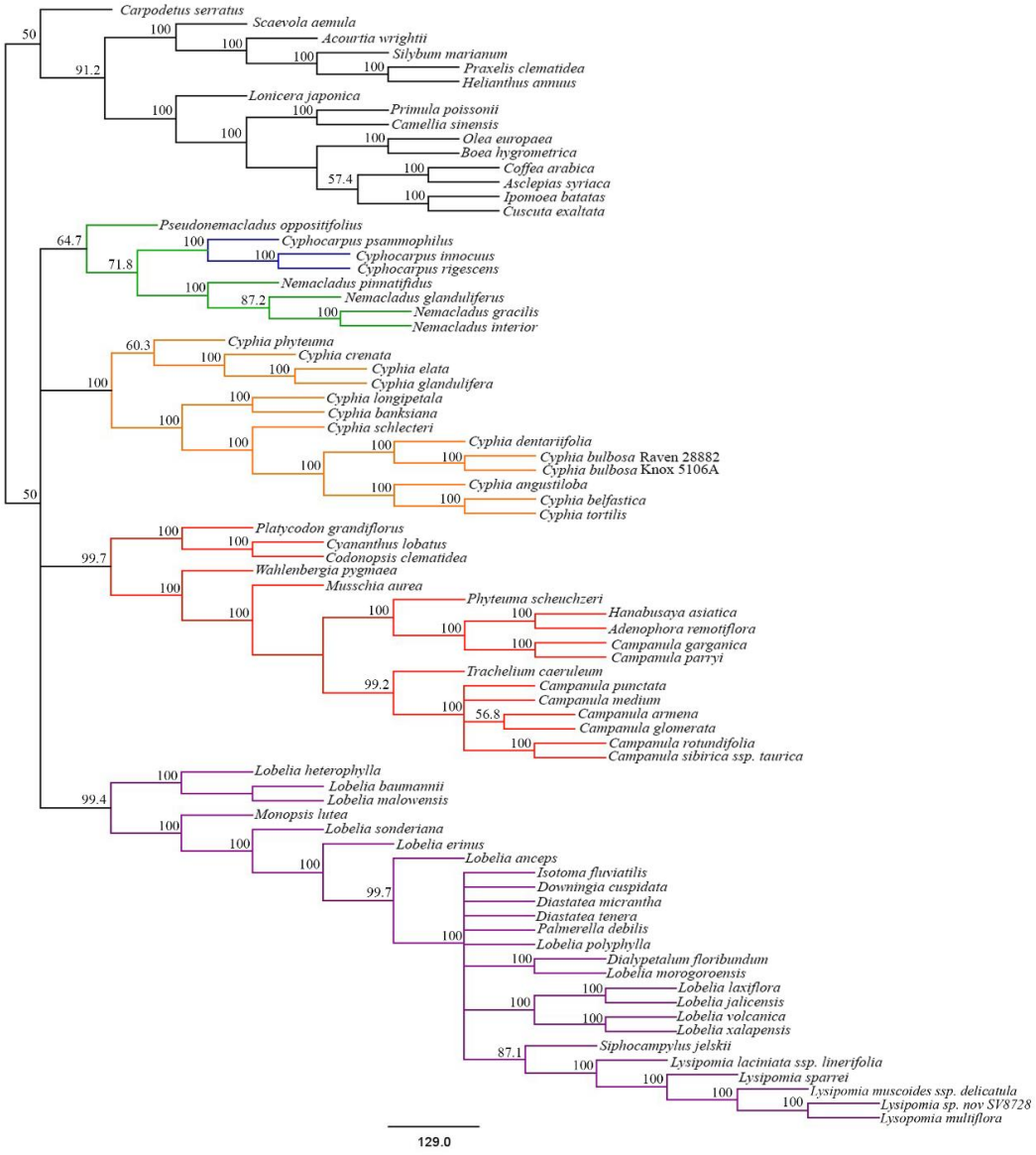

Figure 5. Maximum likelihood tree reconstruction from nuclear ribosomal DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

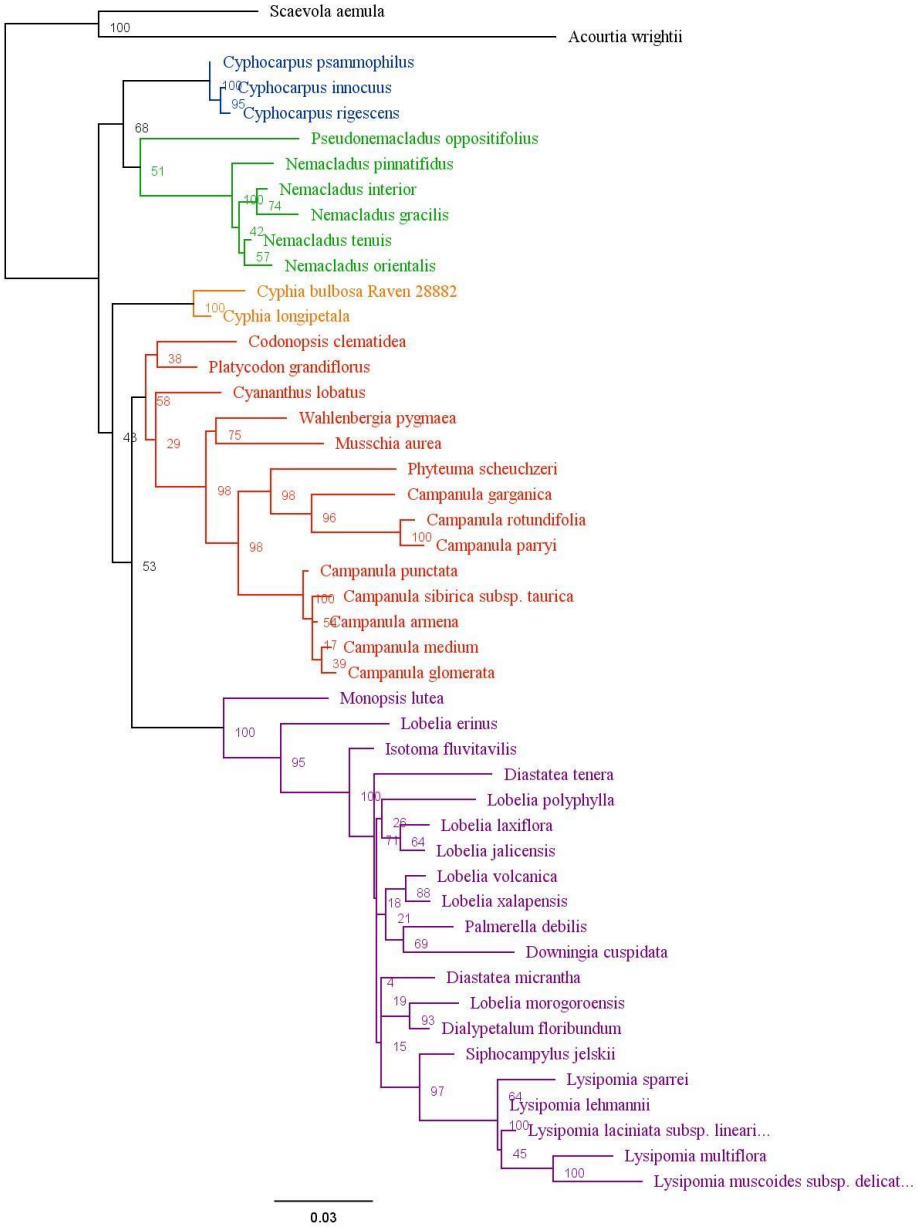

Figure 6. Bayesian inference tree reconstruction from nuclear ribosomal DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

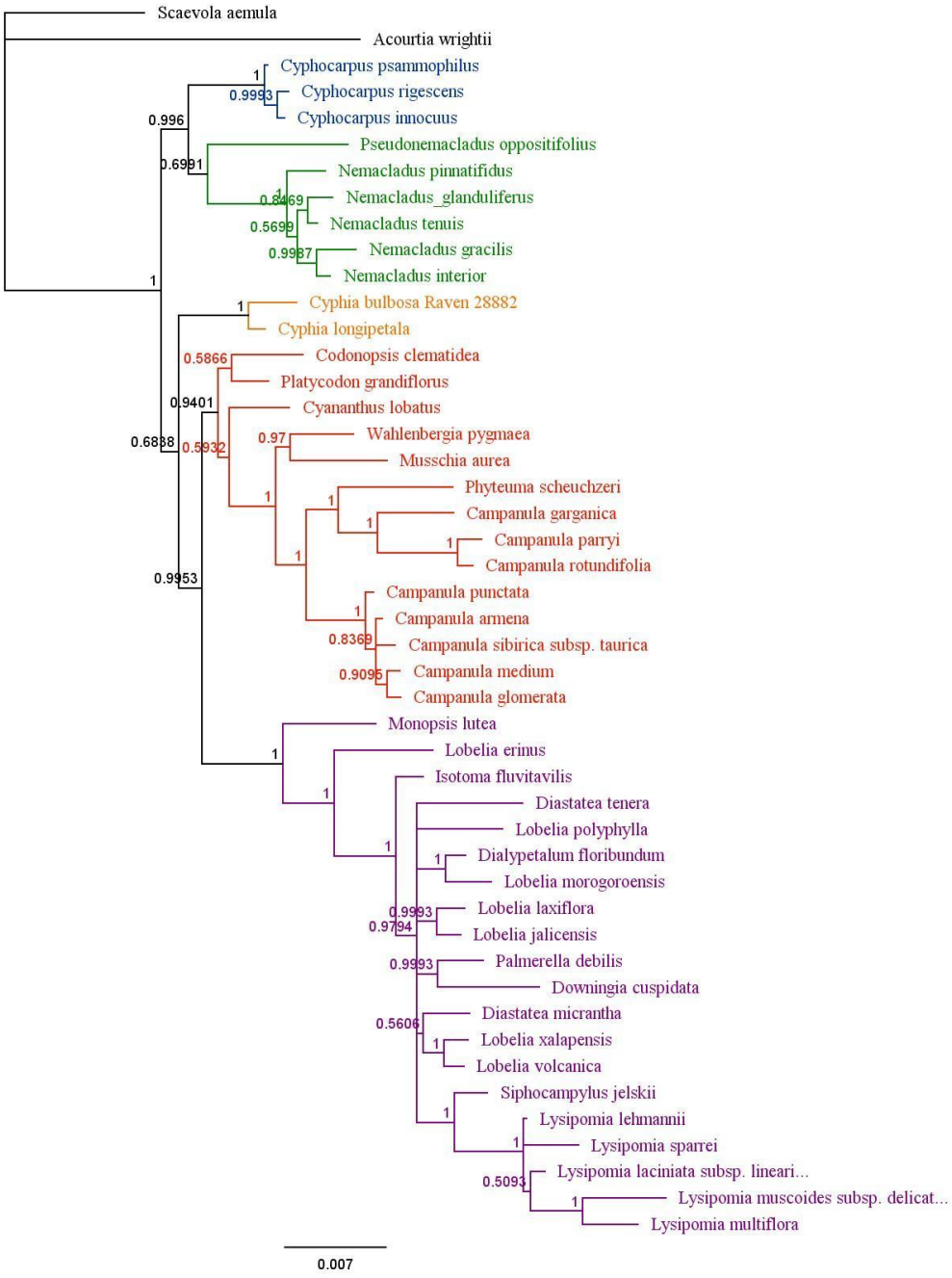

Figure 7. Maximum parsimony tree reconstructed from nuclear ribosomal DNA. Numbers are bootstrap support values. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

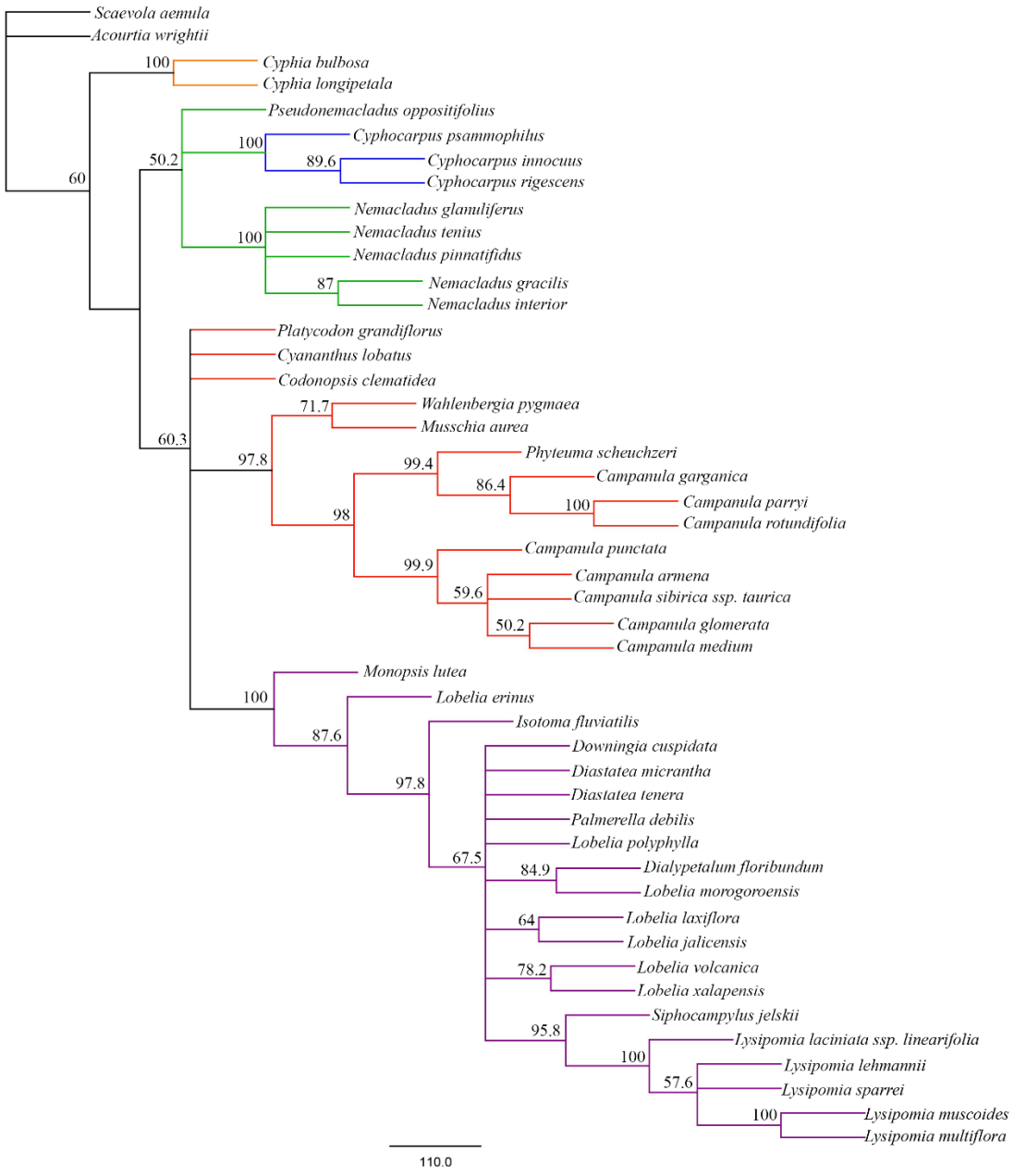

Figure 8. Backbone topologies of 8 most parsimonious trees from PAUP analysis of cpDNA.

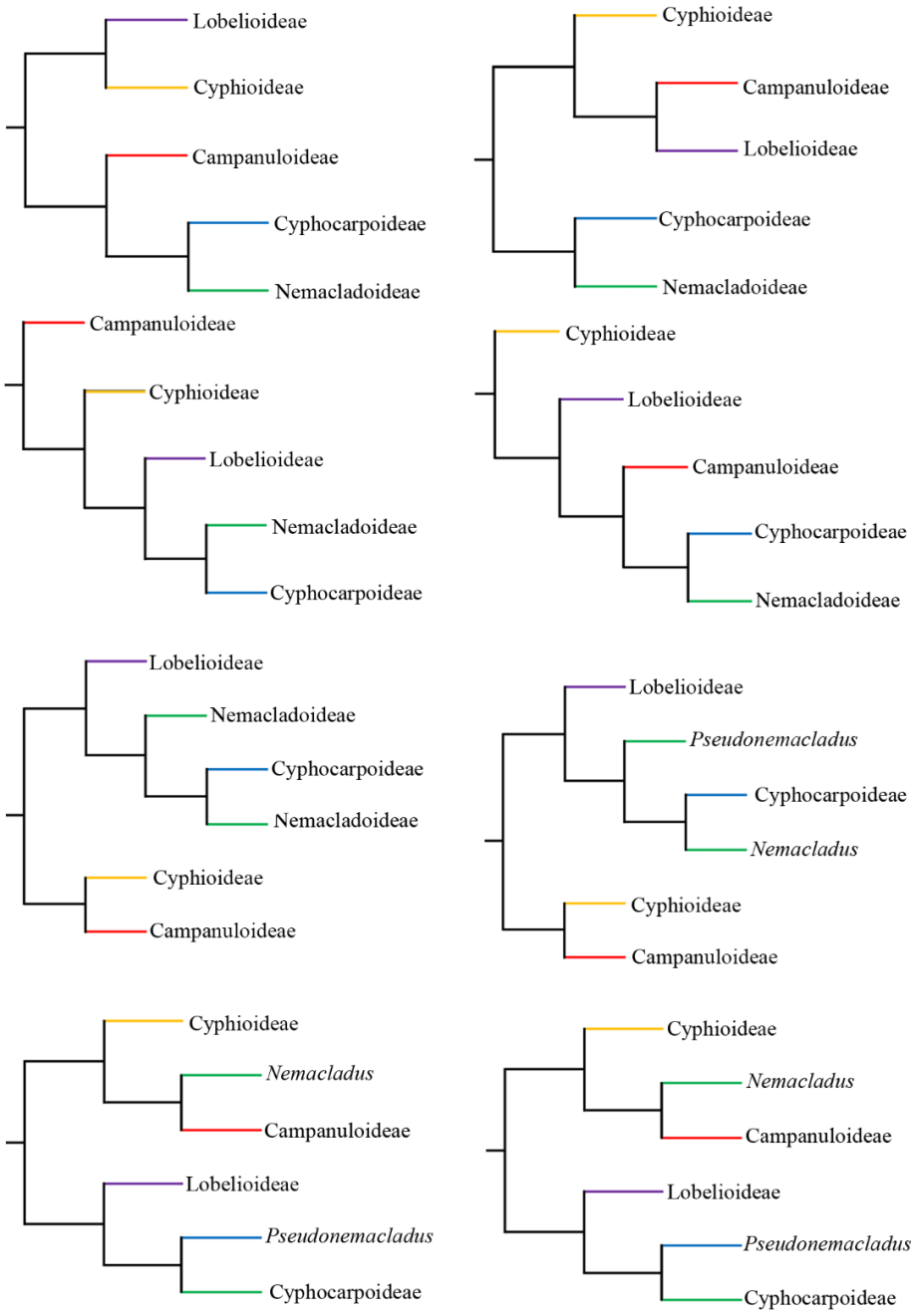

Figure 9. Divergence times estimated by BEAST. Numbers are divergence times in millions of years before prsent. Subfamilies are denoted by color (Cyphioideae = orange, Campanuloideae = red, Lobelioideae = purple, Nemacladoideae = green, Cyphocarpoideae = blue).

Figure 10. Ancestral character mapping showing evolution of a curved style is derived within Campanulaceae. Black lines indicate the presence of a curved style.

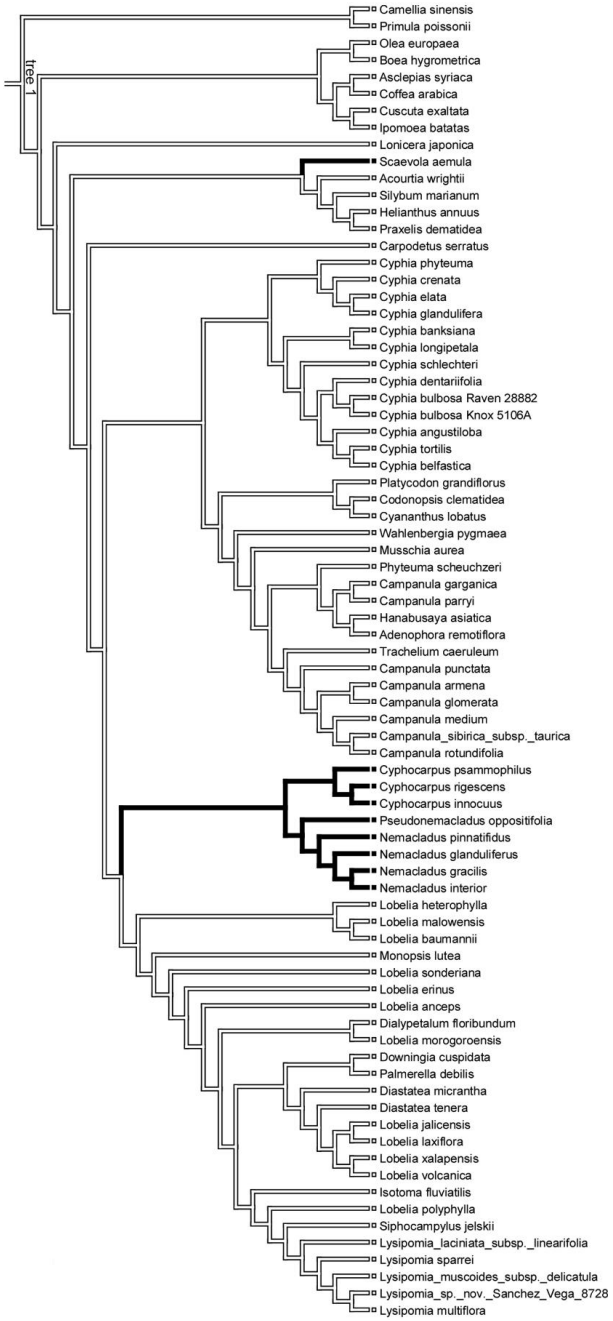

Figure 11. Ancestral character mapping showing evolution of a two-lobed stigma is derived within Campanulaceae. Black lines indicate the presence of a two-lobed stigma.

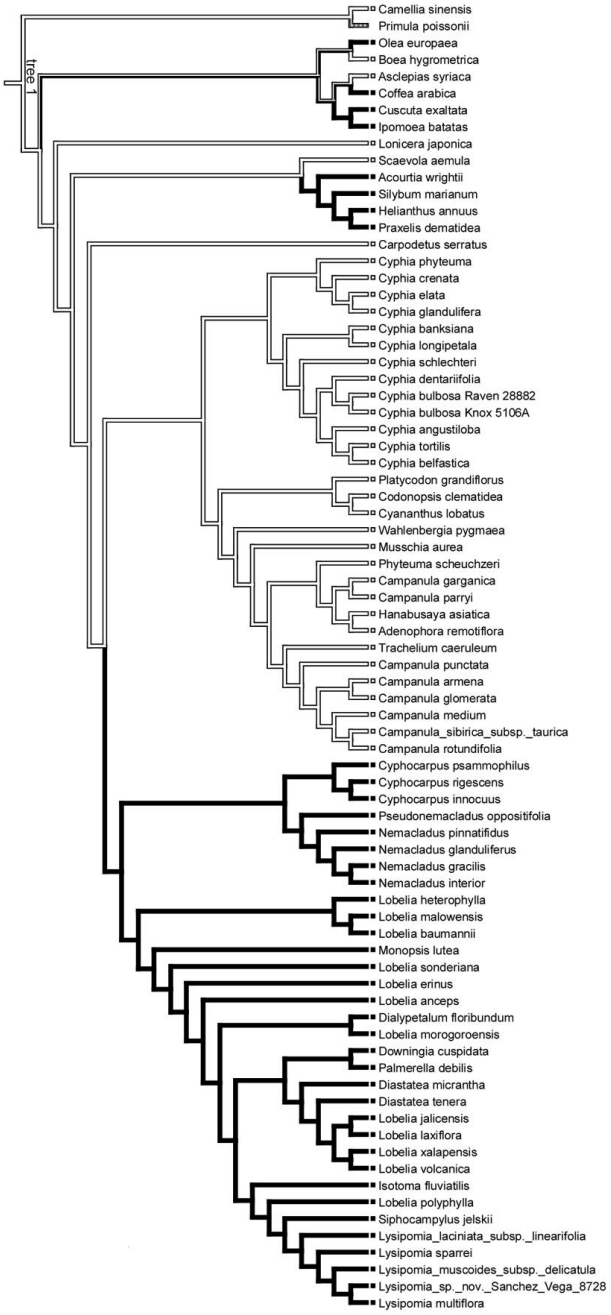

Figure 12. The Rio-Grande Rise and Walvis Ridge complex of submerged islands. A putative migration route of the MRCA of Cyphocarpoideae and Nemacladoideae from South Africa to the Neotropics during a time when the continents were closer together and the islands were exposed.

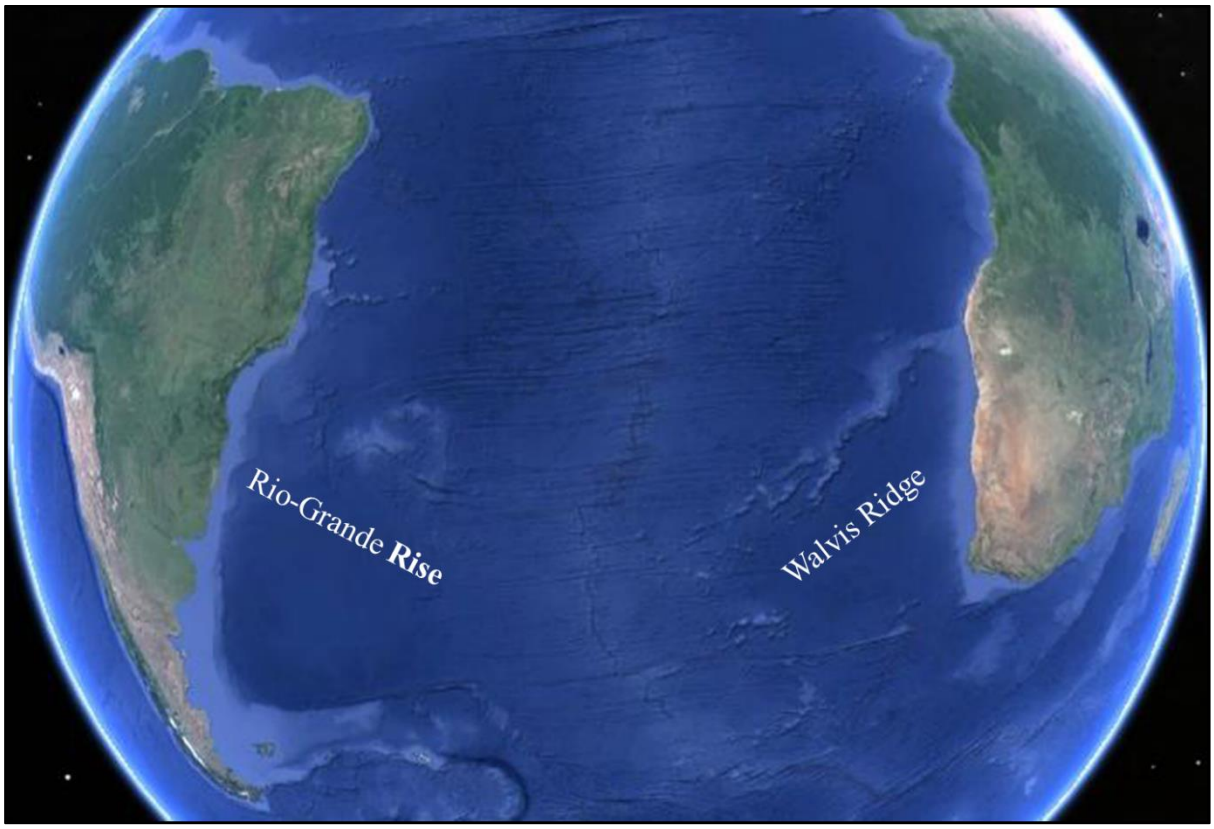

Figure 13. GAARlandia. A putative migration route of the MRCA of Nemacladoideae from the Neotropics to the Sierra Madre Oriental during a time the islands were exposed.

Figure 14. Alternative scenarios for the dispersal history of Nemacladoideae and Cyphocarpoideae. Black = MRCA of Nemacladoideae and Cyphocarpoideae, Yellow = MRCA of Nemacladoideae, RED = MRCA of Cyphocarpoideae.

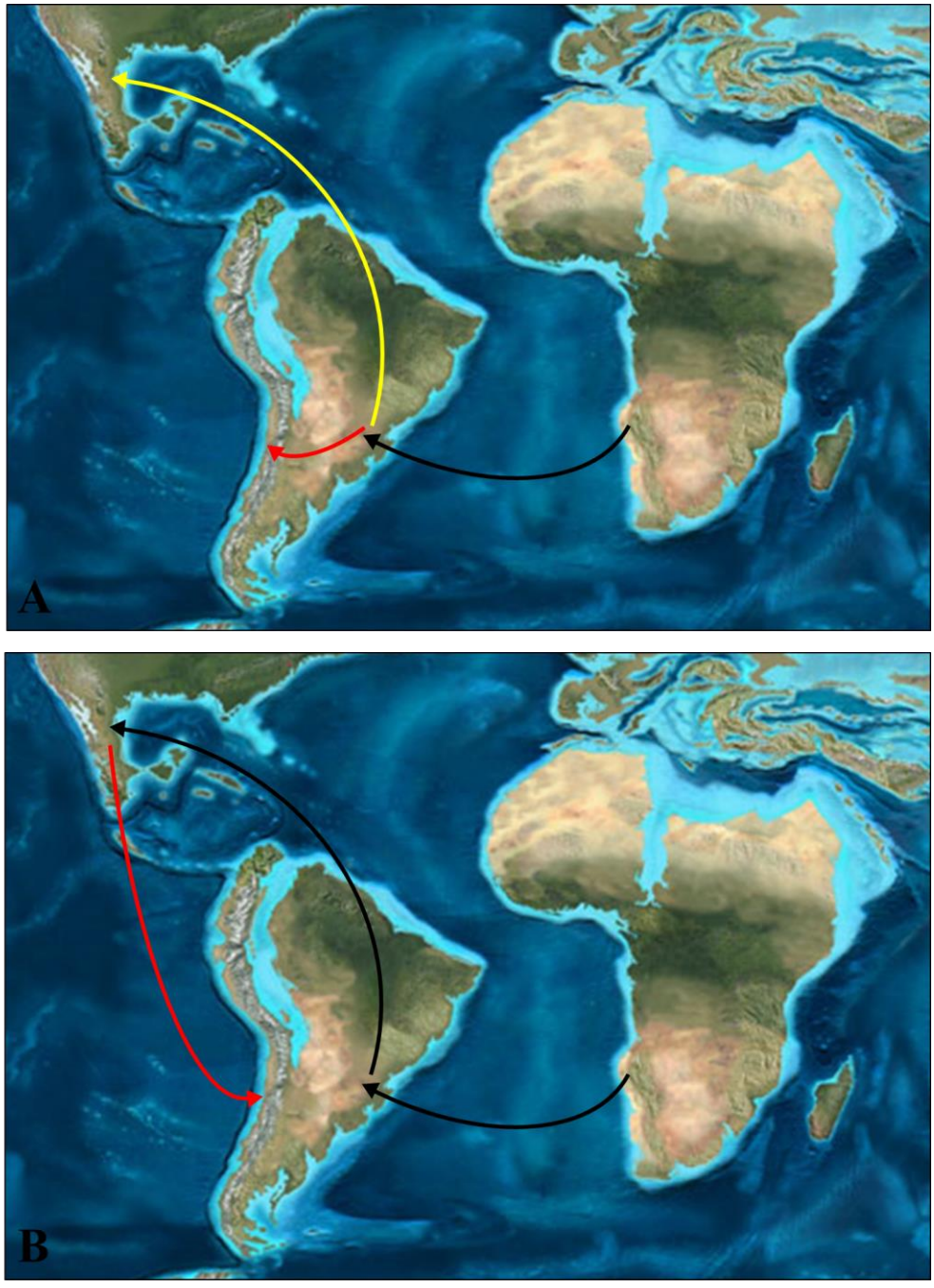

Plate 1. Botanical illustration of *Cyphocarpus innocuus.* A. Habit. B. Flower, opened longitudinally, calyx removed. C. Flower, front view. D. Upper leaf. E. Lower leaf. F. Mature fruit. G. Flower, lateral view.

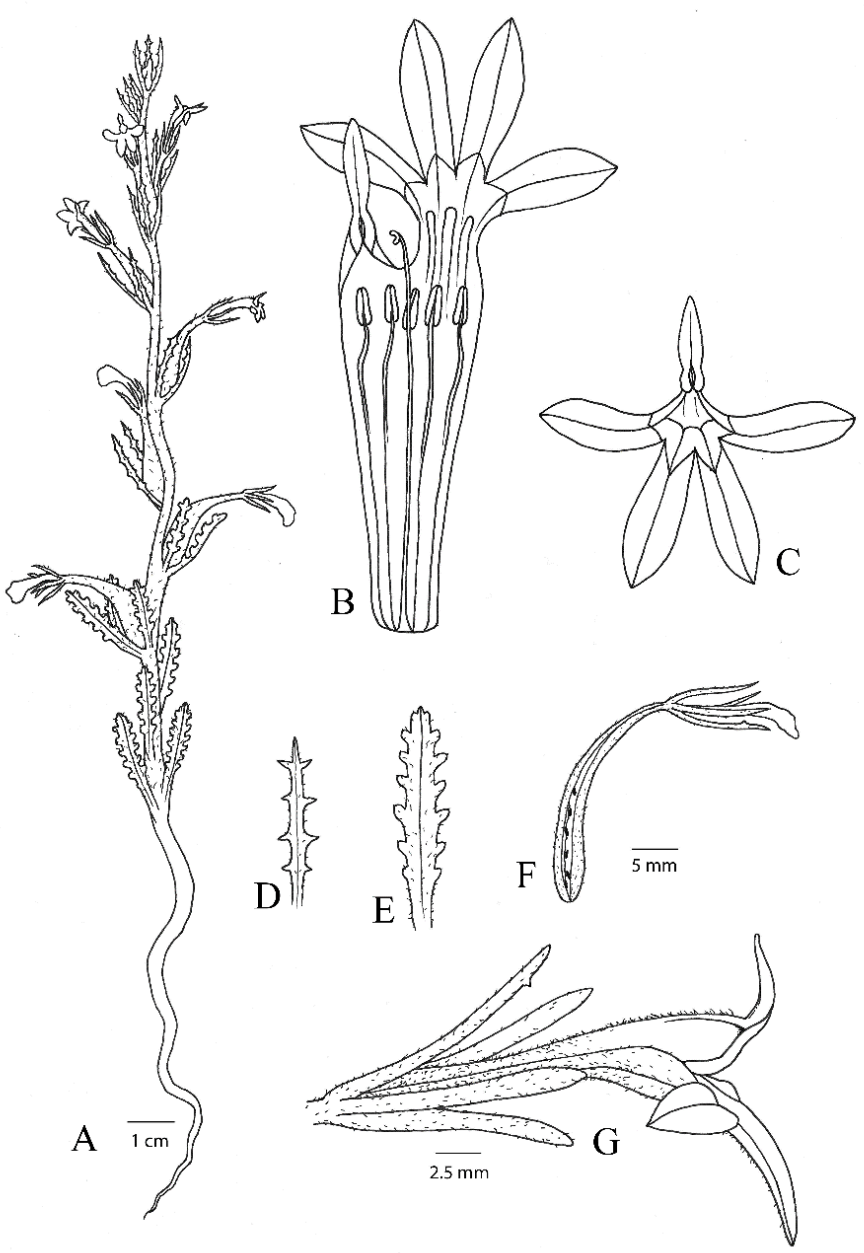

Plate 2. Botanical illustration of *Cyphocarpus psammophilus.* A. Flower, lateral view. B. Flower, opened longitudinally, calyx removed. C. Flower, front view. D.Habit. E. Leaf. F. Mature fruit.

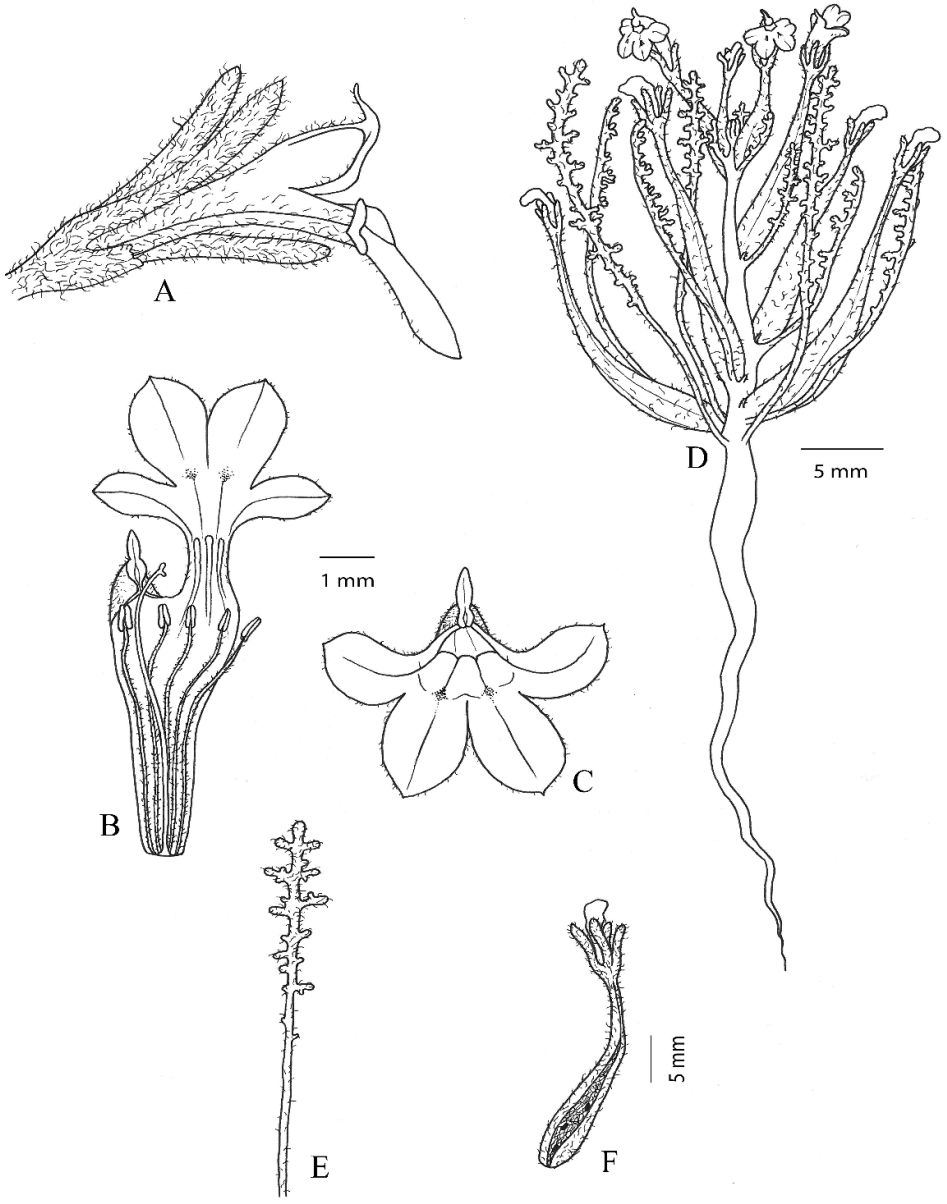

Plate 3. Botanical illustration of *Cyphocarpus rigescens.* A. Flower, front view. B. Flower, lateral view. C. Flower, opened longitudinally, calyx removed. D.Upper leaf. E. Lower leaf. F. Mature fruit.

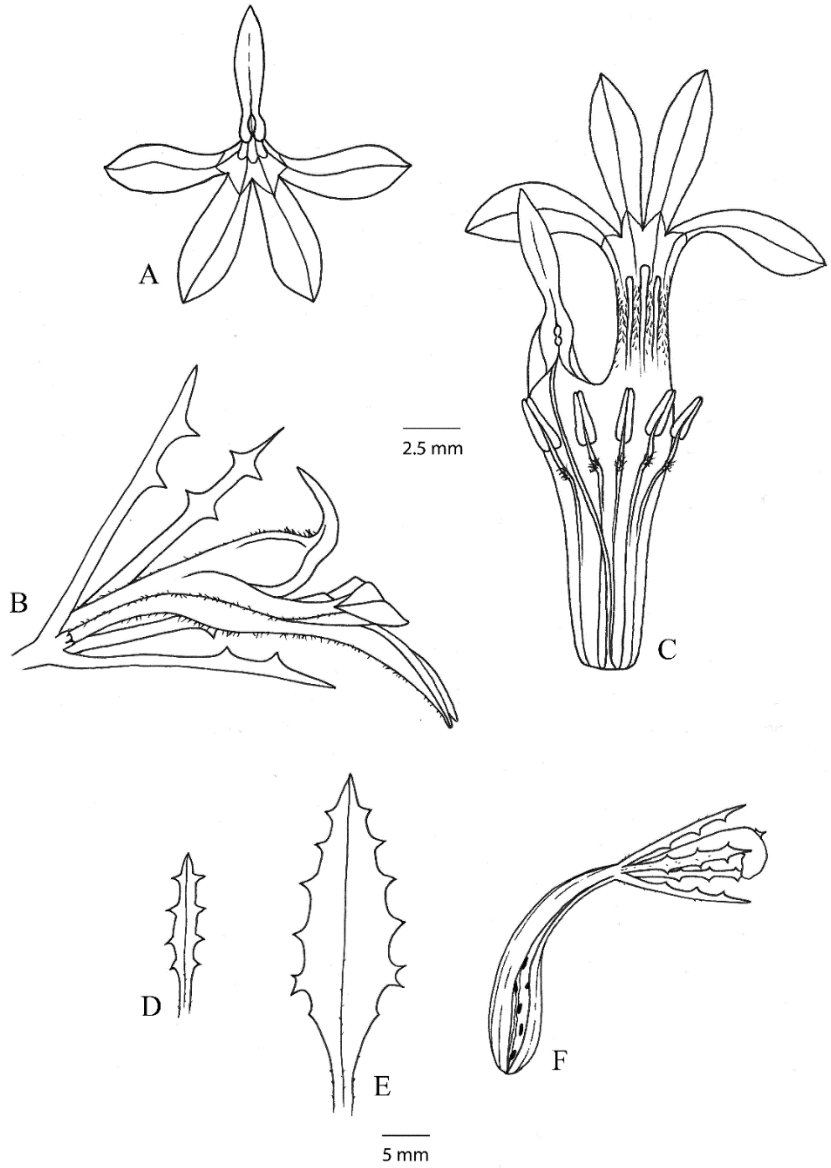

Plate 4. Photos of *Cyphocarpus* species illustrating unique flower morphology. A. *Cyphocarpus psammophilus*, B. *Cyphocarpus rigescens*, lateral view, C. *Cyphocarpus innocuus*, D. *Cyphocarpus rigescens.*

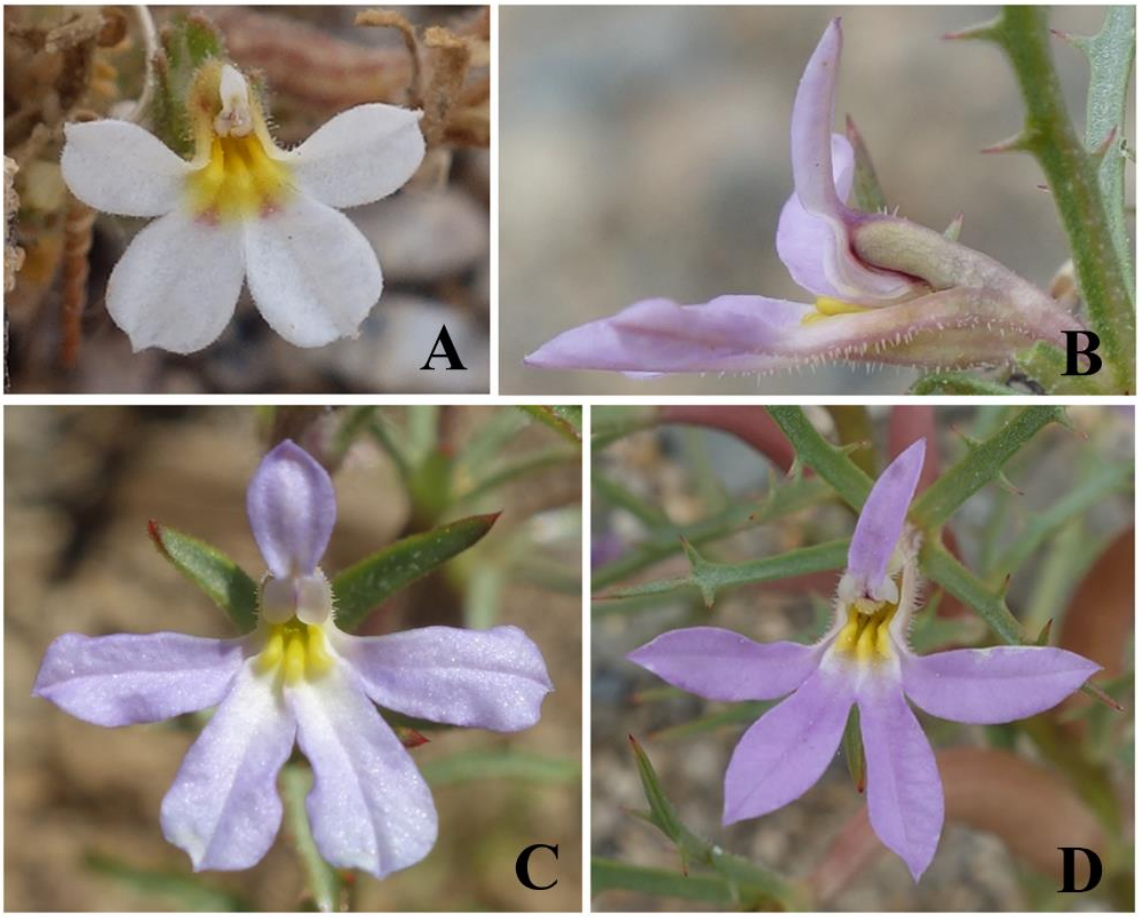

Plate 5. Scanning electron microscopy images of *Cyphocarpus* species. A. *Cyphocarpus innocuus* lower lip of corolla showing pubescent filaments adnate to corolla. B. *Cyphocarpus psammophilus* pollen. C. *Cyphocarpus rigescens* stylar hairs. D. *Cyphocarpus rigescens* mature style and stigma.