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Phylogeny and Phylogenetic Nomenclature of the Campanulidae based on an Expanded Sample of Genes and Taxa

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Abstract—Previous attempts to resolve relationships among the primary lineages of Campanulidae (e.g. Apiales, Asterales, Dipsacales) have mostly been unconvincing, and the placement of a number of smaller groups (e.g. Bruniaceae, Columelliaceae, Escalloniaceae) remains uncertain. Here we build on a recent analysis of an incomplete data set that was assembled from the literature for a set of 50 campanulid taxa. To this data set we first added newly generated DNA sequence data for the same set of genes and taxa. Second, we sequenced three additional cpDNA coding regions (ca. 8,000 bp) for the same set of 50 campanulid taxa. Finally, we assembled the most comprehensive sample of campanulid diversity to date, including ca. 17,000 bp of cpDNA for 122 campanulid taxa and five outgroups. Simply filling in missing data in the 50-taxon data set (rendering it 94% complete) resulted in a topology that was similar to earlier studies, but with little additional resolution or confidence. In contrast, the addition of the ca. 8,000 bp of sequence data provided resolution and support for a number of relationships. With the addition of many more campanulid taxa we recovered a well-resolved phylogeny for Campanulidae where, with the exception of the placement of Escalloniaceae, support was high for all major clades. In addition, we were able to confidently place a number of enigmatic taxa, some whose relationships were previously unresolved and some never before included. In light of these results, we briefly expand the discussion of floral symmetry and provide a phylogenetic classification of Campanulidae with phylogenetic definitions for all well-supported major clades.

Keywords—angiosperm phylogeny, Asteridae, Campanulidae, Euasterid II, phylogenetic nomenclature.

Our understanding of plant phylogeny has increased dramatically over the past two decades and a robust hypothesis of phylogenetic relationships among the major evolutionary lineages is now emerging (e.g. Delwiche et al. 2004; Donoghue 2004; Pryer et al. 2004; Soltis et al. 2004; Qiu et al. 2005; Jansen et al. 2007; Moore et al. 2007; Graham and Iles 2009; Magallón and Castillo 2009). However, within angiosperms, phylogenetic relationships at several key nodes are still in question and these have been the focus of ongoing research (e.g. Davis et al. 2005; Jian et al. 2008; Winkworth et al. 2008b; Wang et al. 2009). Resolving evolutionary relationships within and among the lineages comprising the bulk of angiosperm diversity (e.g. campanulids, fabids, lamiids, malpighs, malvids) is central to understanding the tempo and mode of angiosperm diversification, including their morphological evolution and historical biogeography.

Although previous phylogenetic studies have consistently identified a campanulid clade (euasterids II, APG II 2003) containing Apiales, Aquifoliales, Asterales, and Dipsacales, and a number of smaller clades, (e.g. Lundberg 2001; Bremer et al. 2002; Kårehed 2002), relationships among these lineages, which together contain more than 30,000 species, have remained uncertain. The majority of previous studies that have included campanulids have focused on relationships spanning all angiosperms (e.g. Soltis et al. 2000; Qiu et al. 2005) or on the more inclusive clade, Asteridae (e.g. Bremer et al. 2002), and therefore have sampled relatively few representatives. However, several studies have focused more specifically on campanulid phylogeny (e.g. Lundberg 2001; Kårehed 2002; Winkworth et al. 2008b). Of these, Winkworth et al. (2008b) presented the most comprehensive sampling of campanulid diversity, including 50 strategically selected campanulid taxa, which, in large part, combined the asterid-wide sampling of Bremer et al. (2002) with the more focused sampling strategies of Lundberg (2001) and Kårehed (2002). In addition to a representative sample of the major campanulid lineages (e.g. Apiales, Aquifoliales, Asterales, and Dipsacales), Winkworth et al. (2008b) included members of the smaller clades, including Columelliaceae, Paracryphiaceae, Escalloniaceae, and Bruniaceae, which are placed confidently within Campanulidae but with uncertain affinities to the major lineages (Lundberg 2001; Bremer et al. 2002). Earlier phylogenetic analyses (e.g. Lundberg 2001; Bremer et al. 2002) were based on nearly complete data matrices, primarily of targeted cpDNA regions. In contrast, Winkworth et al. (2008b) assembled sequence data from the literature for seven cpDNA coding and noncoding regions, and the two nrDNA genes, 18S and 26S. Although this strategy allowed the inclusion of additional gene regions and taxa, the Winkworth et al. (2008b) dataset contained relatively large amounts of missing data (Fig. 1A; cpDNA coding regions with 13% missing data, cpDNA noncoding with 20% missing, and nrDNA with 49% missing).

Nevertheless, the results of Winkworth et al. (2008b) largely agreed with the results from previous studies. Their separate and combined Bayesian mixed model analyses of the cpDNA coding, noncoding, and nrDNA datasets supported the monophyly of the four major campanulid clades and consistently identified a clade containing Apiales and Dipsacales along with the enigmatic Paracryphiaceae (incl. *Quintinia*), the latter as sister to Dipsacales with high Bayesian posterior probability. However, the exact relationships of this well-supported lineage to the large Asterales clade, and to the smaller lineages, Bruniaceae, Columelliaceae (incl. *Desfontainia*), and an Escalloniaceae clade (contaning *Escallonia*, *Polyosma*, *Eremosyne*, and *Tribeles*) depended on the particular dataset. Furthermore, conflict in the placement of Bruniaceae and Columelliaceae between the cpDNA coding and noncoding datasets resulted in a disappointingly unresolved campanulid backbone in any analyses that combined these two data matrices. In analyses of the cpDNA coding genes, the South African Bruniaceae and South American Columelliaceae formed a clade and together joined a well-supported lineage with the *Escallonia*

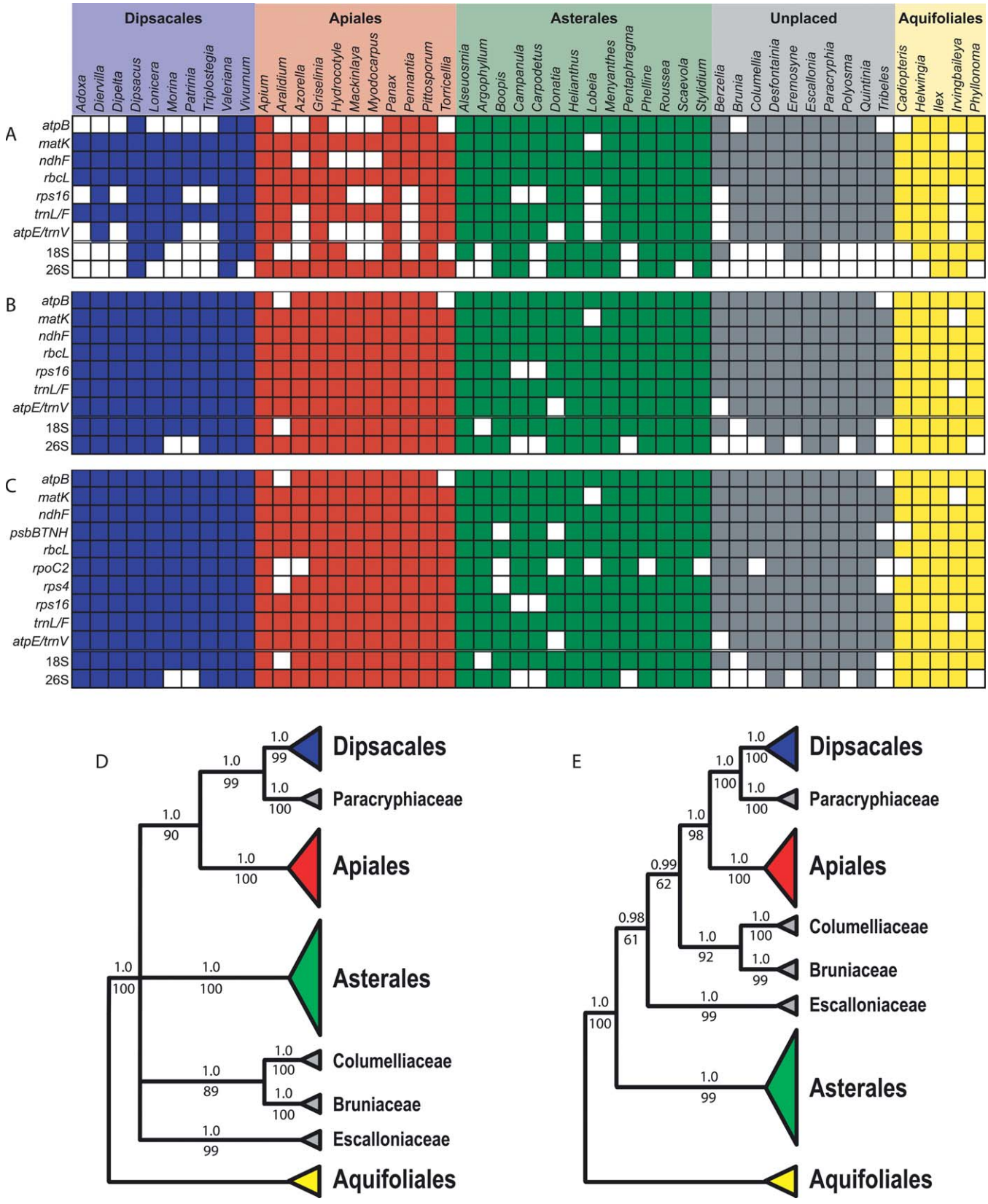

FIG. 1. Summary matrices and resulting trees for the 50-taxon datasets for Dipsacales (blue), Apiales (red), Asterales (green), Aquifoliales (yellow), and the previously unplaced campanulid lineages (gray); empty cells represent missing sequences. (A) The Winkworth et al. (2008b) matrix; (B) the Winkworth et al. (2008b) matrix but with the addition of sequence data generated in this study; and (C) the matrix in (B) but with the addition of three cpDNA coding regions (ca. 8,000 bp). (D) Summary of inferred relationships for the major lineages of Campanulidae resulting from ML and Bayesian analyses of the filled-in Winkworth et al. (2008b) data set (B). (E) Summary of inferred relationships for the major lineages of Campanulidae resulting from ML and Bayesian analyses of the data set including three additional gene regions (C). Numbers above the branches are Bayesian posterior probabilities; numbers below are ML bootstrap percentages.

clade and the Apiales plus Dipsacales-Paracryphiaceae clade. In contrast, analyses of the cpDNA noncoding matrix placed separate Bruniaceae and Columelliaceae clades in a well-supported clade along with Asterales, but without resolving relationships among these three lineages. This evident conflict between cpDNA data matrices has likely contributed to the lack of phylogenetic resolution among the major lineages of campanulids (e.g., Winkworth et al. 2008b).

Therefore, phylogenetic relationships among the primary campanulid lineages remain poorly resolved, as do, in many cases, relationships among the early branching lineages within the major clades. However, as Winkworth et al. (2008b) presented only the results of Bayesian analyses, it is difficult to compare their support values with those of earlier analyses that reported only nonparametric bootstrap and/or jackknife support (e.g. Bremer et al. 2002). Based on simulation studies comparing nonparametric bootstrap values to Bayesian posterior probability values, it has been suggested that bootstrap values are too conservative, while Bayesian posteriors may not be conservative enough (e.g. Suzuki et al. 2002; Cummings et al. 2003; Erixon et al. 2003; Simmons et al. 2004). Although it has been argued that Bayesian posterior probabilities may better approximate true clade probabilities (e.g. Wilcox et al. 2002; Alfaro et al. 2003; Erixon et al. 2003; Brandley et al. 2005), they have been criticized as being more susceptible than bootstrap proportions to erroneously assigning high probability to incorrect clades, especially those subtended by short branches (Alfaro et al. 2003). In this light, it is worrisome that several of the backbone nodes recovered with significant Bayesian posterior probabilities in the Winkworth et al. (2008b) study were marked by especially short branch lengths.

In the present study we compiled DNA sequence data for ten cpDNA regions, including seven coding and three noncoding regions for a sampling of 122 campanulid taxa (Appendix 1). This is the most comprehensive sample of campanulid diversity to date, with more than twice the number of taxa analyzed by Winkworth et al. (2008b), and, with the addition of the *psbBTNH*, *rpoC2*, and *rps4* genes, approximately 8,000 bp of additional cpDNA coding data. Furthermore, our total cpDNA matrix (122 campanulid taxa plus five outgroups by ca. 17,000 bp) is more than 95% complete. To investigate the effects of missing data and to facilitate comparison with the Winkworth et al. (2008b) analyses, we also analyzed their 50 taxon dataset, but with additional cpDNA and nrDNA sequence data (Appendix 1). We have calculated both Bayesian posterior probabilities and maximum likelihood bootstrap values, and, therefore, are able to identify any clades where these values are divergent.

The results of our analyses are robust enough to extend the Winkworth et al. (2008b) discussion of the evolution of floral symmetry (a greatly expanded analysis of morphological evolution will be presented elsewhere). Moreover, an enhanced understanding of campanulid phylogeny now supports the phylogenetic naming of several major clades. Building upon the phylogenetic definitions provided by Donoghue et al. (2001) and Cantino et al. (2007), we provide a new classification of Campanulidae that we hope will facilitate communication and help orient future research.

Materials and Methods

Taxon Sampling and Targeted Gene Regions—Our sampling strategy was guided by two separate, but not mutually exclusive goals. Our primary focus was to select taxa that would adequately represent campanulid diversity, but we also selected taxa that were well represented by currently available sequences. Winkworth et al. (2008b) largely combined the previous sampling strategies of Bremer et al. (2002; 36 campanulids) and Lundberg (2001; 41 campanulids), identifying a sample of 50 campanulid taxa for which a significant amount of sequence data was publically available. In their sampling, Winkworth et al. (2008b) paid special attention to the representation of early-branching lineages within each of the major campanulid clades (i.e. Apiales, Aquifoliales, Asterales, and Dipsacales), as well as those taxa whose placement had remained uncertain (e.g. Bruniaceae, Columelliaceae, Escalloniaceae).

To enable comparisons between sparsely sampled and nearly complete data matrices, and to take advantage of the large body of existing sequence data, our campanulid sampling included the 50 taxa analyzed by Winkworth et al. (2008b). In addition, based largely on taxa that had been included in previous molecular phylogenetic studies of Apiales (see Kårehed 2003; Chandler and Plunkett 2004), Aquifoliales (see Kårehed 2001, 2002), Asterales (see Kårehed 2002; Lundberg 2001; Lundberg and Bremer 2003), and Dipsacales (see Donoghue et al. 2003; Moore and Donoghue 2007), we expanded this core sample to include an additional 72 campanulid taxa. This not only fleshes out the representation of several larger clades that have been under-sampled in broad-scale phylogenetic studies, but also is intended to break up long branches identified in earlier studies. In total, our expanded sample included 122 campanulid taxa – 29 Apiales, 40 Asterales, 30 Dipsacales, 9 Aquifoliales, and 14 taxa that appear to fall outside of these four major lineages (e.g. *Sphenostemon, Forgesia, Berzelia*). Winkworth et al. (2008b) simply rooted their trees along the branch subtending the Aquifoliales taxa, based on previous broader analyses that had obtained strong support for this rooting (e.g. Soltis et al. 2000; Kårehed 2001; Bremer et al. 2002). To further test the position of the root, we included five outgroup taxa selected from the genome-scale chloroplast analysis of Moore et al. (2007): *Atropa belladona* (gi: 28261696), *Nicotiana tabacum* (gi: 81238323), *Coffea arabica* (gi: 116617087), *Jasminum nudiflorum* (gi: 115391881), and *Spinacia oleracea* (gi: 11497503).

Winkworth et al. (2008b) chose to include the three coding and three noncoding cpDNA regions used by Bremer et al. (2002), the widely sequenced cpDNA gene *atpB*, and the 18S and 26S nrDNA genes. To this set we have added sequence data from three additional cpDNA coding regions: *rpoC2*, *rps4*, and *psbBTNH*. The *psbBTNH* region encodes four *psb* genes, *psbB*, *psbT*, *psbN*, and *psbH*, and we amplified and sequenced these as one unit. Although this region contains some intergenic spacer data (i.e. noncoding), this represents only a small amount of the entire region; we therefore treat this cpDNA region as part of the coding partition (see *Phylogenetic analyses*). In all, we targeted seven coding regions of cpDNA (*rbcL*, *ndhF*, *atpB*, *matK*, *rpoC2*, *rps4*, and *psbBTNH*) and three noncoding regions (*rps16* intron, *trnL* intron and *trnL-trnF* intergenic spacer (IGS), and the *atpE/trnV* IGS). In addition, from the nuclear genome we included sequences of the nrDNA 18S and 26S genes.

Whenever possible we used sequences from a single species to represent a taxon. However, building upon the data matrices assembled by Winkworth et al. (2008b) and other previous analyses, we also included a number of composite taxa, where multiple species (sometimes as many as four) were used to represent a lineage (see Appendix 1). For example, for *Hydrocotyle* (Apiales) we used existing sequence data for six of the 12 gene regions included in this study from three different *Hydrocotyle* species (*H. bowlesioides*, *H. rotundifolia*, and *H. sibthorpioides*); newly generated sequence data for the remaining six loci included in this study were obtained from a fourth species, *H. vulgaris*. Although not ideal, the use of such composite taxa allowed us to take advantage of the large amount of existing sequence data and to assemble the most complete matrices possible for each of the gene regions (e.g. Soltis et al. 2000; Bremer et al. 2002; Soltis et al. 2007; Jian et al. 2008; Wang et al. 2009). Where possible, such composite sequences were assembled from species that appear to comprise monophyletic groups based on previous studies.

Molecular Methods—Total genomic DNAs for this study were obtained either from the DNA banks of the Royal Botanic Gardens, Kew, or the South African National Biodiversity Institute, Kirstenbosch, or extracted from herbarium specimens or silica-gel dried tissue using the modified 2 × CTAB method of Doyle and Doyle (1987). All genomic DNAs were then amplified by multiple displacement amplification using the illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Piscataway, New Jersey).

DNA for newly generated sequences of the targeted gene regions was produced via polymerase chain reaction (PCR) using previously published primer sequences for all gene regions except for the cpDNA *rpoC2* and *rps4* genes (see Appendix 2). For these two genes, primers were designed to work universally across asterids based on the complete

chloroplast genome sequences of *Nicotiana tabacum* (gi: 81238323) and *Panax ginseng* (gi: 52220789). For some taxa we had difficulty amplifying larger gene regions in one fragment. In these cases, internal primers were used to amplify the fragments in multiple, overlapping pieces. Amplified PCR products were purified by precipitation from a 20% polyethylene glycol solution and washed in 70% ethanol prior to sequencing. After repeated attempts, we were unable to obtain PCR product for a limited number of taxa for some of the DNA regions (Fig. 1; Appendix 1); however, all taxa included in this study are represented by at least five of the ten cpDNA regions sequenced.

To ensure accuracy, both strands of the cleaned PCR products were sequenced. Sequencing was conducted in part at the Huck Institutes of the Life Sciences' Nucleic Acid Facility at Pennsylvania State University, and at the W. M. Keck Facility and the Science Hill DNA Analysis Facility at Yale University. All PCR and sequencing primers are listed in Appendix 2. Sequence data were assembled and edited for each gene region using Sequencher v.3.7 (Gene Codes Corporation, Ann Arbor, Michigan). One new *rbcL*, 16 new *ndhF*, 44 new *atpB*, 24 new *matK*, 92 new *rpoC2*, 110 new *rps4*, 107 new *psbBTNH*, 60 new *rps16*, 33 new *trnL/F*, 66 new *atpE/trnV*, 18 new 18S, and 17 new 26S sequences were generated for this study (Appendix 1).

Phylogenetic Analyses—Sequence alignments for both the 50-taxon dataset and the 127-taxon dataset were prepared using Muscle v.3.5 (Edgar 2004), with minor adjustments performed manually using Se-Al v.2.0a11 (Rambaut 1996). For each of the 12 gene regions used, separate alignments were made using Muscle v.3.5 (Edgar 2004) for each of the seven previously identified campanulid lineages: Apiales, Aquifoliales, Asterales, Bruniaceae-Columelliaceae, Dipsacales, Escalloniaceae s. l. (including Eremosynaceae and Polyosmaceae), and Paracryphiaceae s. l. (including Quintiniaceae and Sphenostemaceae) (Lundberg 2001; Bremer et al. 2002; Winkworth et al. 2008b). Each alignment was visually inspected and manually adjusted using Se-Al v.2.0a11 (Rambaut 1996). To take advantage of this previous knowledge of campanulid clades and to obtain data matrices with fewer missing data due to alignment ambiguities, the individual clade alignments were iteratively aligned to one another using the group-to-group profile alignment method implemented in Muscle v.3.5 (Edgar 2004). This approach has been successfully employed for large-scale phylogenetic analyses in angiosperms (e.g. Smith and Donoghue 2008) and does not force the monophyly of profiled groups in subsequent phylogenetic analyses (Smith et al. 2009). The resulting campanulid-wide multiple sequence alignments were visually inspected and adjusted using Se-Al v.2.0a11 (Rambaut 1996).

When considering missing data in the alignments, including both within-frame length mutations and missing data due to alignment ambiguity or incomplete sequences, a threshold value of 50% representation was used. We used Phyutility v.2.2 (Smith and Dunn 2008) to exclude sites where a gap was inferred in more than half of the sequences; otherwise, gaps were treated as missing data.

To directly compare our results to those of Winkworth et al. (2008b), we compiled two primary data matrices for the 50-taxon dataset. The first included the four cpDNA coding regions *atpB*, *ndhF*, *matK*, and *rbcL*, the three noncoding cpDNA regions *rps16*, *trnL/F*, and *atpE/trnV*, and the nrDNA 18S and 26S genes. This matrix corresponds to the combined matrix assembled by Winkworth et al. (2008b), but differs in having far fewer missing data (see Results; Fig. 1B). To this data matrix we added the three cpDNA regions *psbBTNH*, *rpoC2*, and *rps4*, which boosted the amount of cpDNA coding data by ca. 8,000 bp (Fig. 1C). For our 127-taxon dataset (122 campanulids and five outgroups), we assembled a combined cpDNA dataset that included all 10 cpDNA regions (the seven cpDNA coding genes; *atpB*, *ndhF*, *matK*, *psbBTNH*, *rbcL*, *rpoC2*, and *rps4*, and the three cpDNA noncoding regions; *atpE/trnV*, *rps16*, and *trnL/F*). In view of the low return in terms of resolving relationships in campanulids compared to the sequencing effort, we chose not to employ the nrDNA 18S and 26S genes in this analysis. For the assembly of all of the data matrices, we used Phyutility v.2.2 (Smith and Dunn 2008) to concatenate gene regions and convert between file types (e.g. FASTA to NEXUS).

The program Modeltest v.3.6 (Posada and Crandall 1998) was used to determine the model of sequence evolution best fit to the data according to the Akaike information criterion (AIC). Maximum likelihood (ML) analyses were conducted using RAxML v.7.04 (Stamatakis 2006; Stamatakis et al. 2008), and consisted of 500 rapid bootstrap replicates with each gene region treated as a separate partition. Every fifth bootstrap tree generated by the rapid bootstrap analyses was used as a starting tree for full ML searches and the trees with the highest ML scores were chosen. Bayesian phylogenetic analyses were conducted using MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003), again with each gene region treated as a separate partition. Each analysis was conducted using the same models used for the ML analyses and consisted of two runs of 20,000,000 generations from a random starting tree using a variable rate prior, six discrete rate categories to estimate the gamma distribution, and four Markov chains (using the default heating values) sampled every 1,000 generations. Convergence of the chains was determined by examining the plot of all parameter values and the $-\ln L$ against generation time using the program Tracer v.1.4 (Rambaut and Drummond 2004). Stationarity was assumed when all parameter values and the $-\ln L$ had stabilized. Burn-in trees were then discarded and the remaining trees, and their associated parameter values, were saved. To explore more tree space and to decrease the chance of obtaining stationarity on local optima, two independent analyses were performed for each data set.

Results

Data Matrices—Figure 1 shows a graphical representation of the Winkworth et al. (2008b) 50-taxon dataset along with the two 50-taxon datasets compiled for this study. To the Winkworth et al. (2008b) combined genome dataset (Fig. 1A) we added newly generated sequence data for all gene regions except *rbcL* (which was already complete), bringing the cpDNA coding and cpDNA noncoding data partitions to 98% and 97% complete, respectively, and the nrDNA partition to 84% complete (Fig. 1B). With the addition of the three cpDNA coding regions, *psbBTNH*, *rpoC2*, and *rps4*, the cpDNA coding partition was increased by ca. 8,000 bp and was 94% complete (Fig. 1C). Our dataset with 122 campanulid taxa and five outgroups comprised the same ten cpDNA regions and was 93% and 91% complete for the coding and noncoding partitions, respectively.

Multiple sequence alignments for the seven cpDNA coding regions and the two nrDNA regions were relatively straight forward. However, in each of these nine gene regions, the alignments resulted in several single base and small insertions and deletions, many of these involving previously published sequences and likely reflecting sequencing errors (as noted by Winkworth et al. 2008b). Alignment of the three noncoding cpDNA regions required the introduction of numerous short gaps throughout the alignment. All data matrices used in this study are available in TreeBASE (study number S2493).

Phylogenetic Analyses—Because trees resulting from preliminary analyses of both the individual genes and the separate data partitions (i.e. cpDNA coding, cpDNA noncoding, and nrDNA) were largely consistent with one another, only differing in regions that received relatively low ML bootstrap values and/or < 0.95 Bayesian posterior probabilities (trees not shown), we present only the results from the combined phylogenetic analyses representing the largest possible data matrices for the two 50-taxon datasets and the expanded 127-taxon dataset. To account for the inherent differences that exist between loci in base composition and among site rate variation, both ML and Bayesian phylogenetic analyses were fully partitioned, with each gene region treated as a separate partition. For each partition, model selection, as implemented in Modeltest v.3.6 (Posada and Crandall 1998), resulted in the GTR + I + G model of sequence evolution. Following the recommendation of Ronquist and Huelsenbeck (2003), the parameter for the proportion of invariable sites (I) was not implemented, but rather, the GTR + G model was implemented and the number of discrete rate categories used to estimate the gamma shape parameter was increased from four to six. Although runtime is increased linearly with an increase in the number of rate categories, this modification from the default parameters has been shown to improve convergence properties, especially with large datasets like the ones being

analyzed here (Ronquist and Huelsenbeck 2003). Likewise, Stamatakis (2006) discourages the use of the I parameter, and, therefore, our ML analyses used the GTR + G model of sequence evolution as implemented by the rapid bootstrap algorithm in RAxML (Stamatakis et al. 2008).

Figure 1D and 1E show a comparison of the summary trees resulting from both ML and Bayesian phylogenetic analyses of the 50-taxon datasets. The tree in Fig. 1D was obtained from the dataset represented in Fig. 1B, which differs from Winkworth et al. (2008b; Fig. 1A) only by filling in missing data. The tree in Fig. 1E was obtained from the dataset in Fig. 1C, in which three cpDNA regions were added to the dataset in Fig. 1B. For both datasets, Bayesian and ML analyses resulted in identical topologies. Bayesian posterior probabilities and bootstrap percentages are shown for the major lineages comprising the campanulid clade, and it is clear that both analyses recovered the major clades with strong support. However, using only the genes used by Winkworth et al. (2008b) yielded a topology (Fig. 1D) that was largely unresolved with respect to relationships among the major campanulid lineages (Bayesian posterior probabilities < 0.50 and ML bootstrap values < 50%; Fig. 1D). In contrast, analyses including the additional cpDNA regions provided significant Bayesian posterior probabilities for the successive sister group relationships of the Asterales clade, the Escalloniaceae clade, and the Columelliaceae-Bruniaceae clade to the well-supported Apiales-Paracryphyiaceae-Dipsacales clade (Fig. 1E). However, ML bootstrap percentages for these backbone relationships were still marginal.

Figure 2 shows the majority rule consensus tree resulting from the partitioned Bayesian analysis of the expanded gene sampling for the 50-taxon dataset (Fig. 1C). The ML topology was identical, and the ML bootstrap values are shown on the tree. We note that there are several minor differences between this result and the result highlighted by Winkworth et al. (2008b; see their Fig. 1). Regarding relationships among the major lineages, in our analysis the Escalloniaceae clade appears as sister to a clade including Columelliaceae plus Bruniaceae, Apiales, and Dipsacales plus Paracryphiaceae. The position of the Escalloniaceae clade was unresolved in Winkworth et al. (2008b). Unfortunately, although this clade is supported here by a high posterior probability (0.98), bootstrap support is low (61%). Likewise, although our addition of genes favors the placement of the Bruniaceae-Columelliaceae

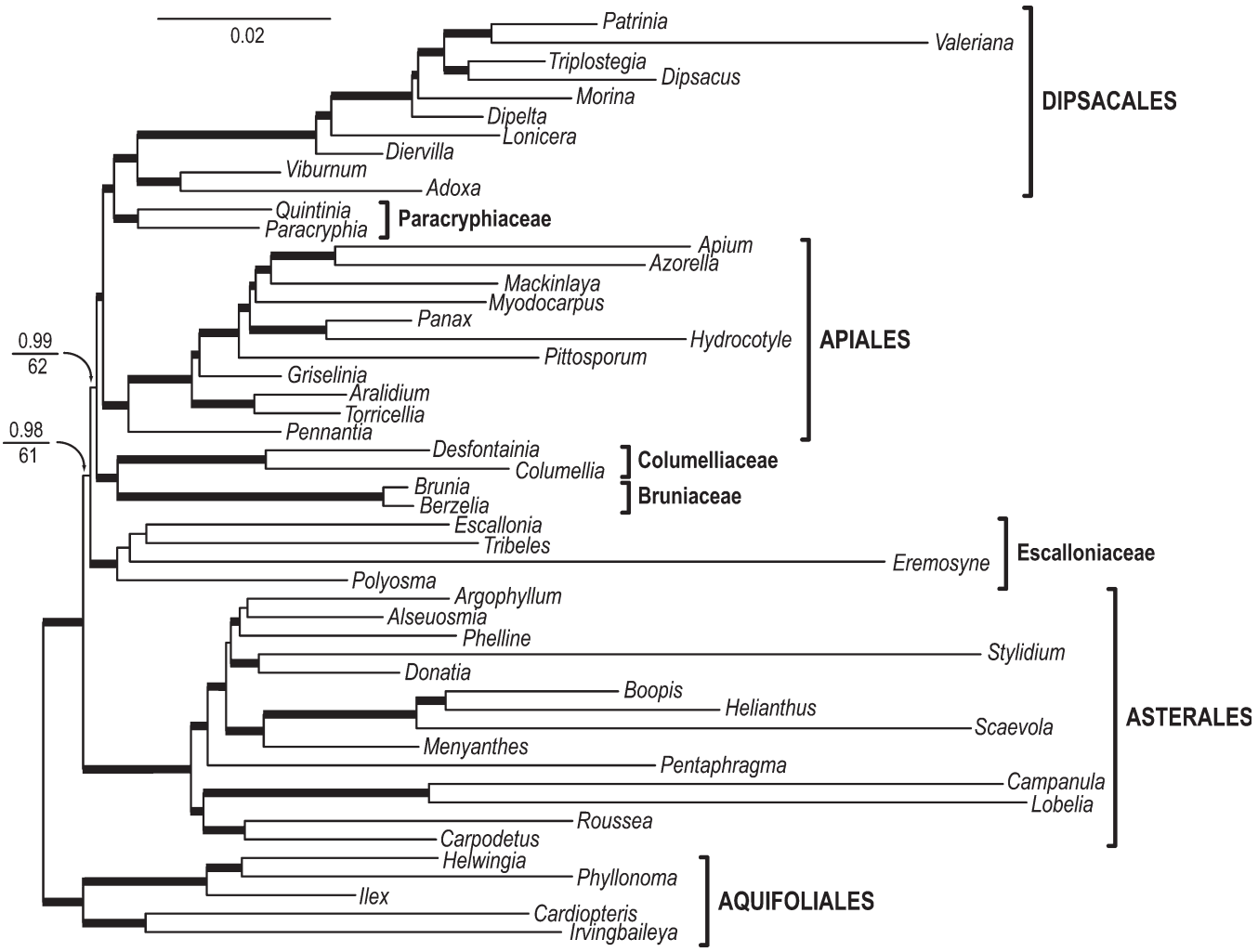

FIG. 2. Majority rule consensus tree with mean branch lengths from the partitioned Bayesian analysis of the expanded sample of genes for the 50-taxon dataset (Fig. 1C). Branch lengths are proportional to the mean number of substitutions per site as measured by the scale bar. Thickened branches received Bayesian posterior probabilities ≥ 0.95 and ML bootstrap percentages ≥ 80%. Bayesian posterior probabilities and ML bootstrap values (above and below the branches, respectively) are indicated for the two backbone nodes that received significant Bayesian posterior probabilities but low ML bootstrap support.

clade with the Apiales-Paracryphiaceae-Dipsacales clade (as opposed to a weak link with Asterales, as in Winkworth et al. 2008b), the support for the new placement remains somewhat uncertain (posterior probability of 0.99, bootstrap of 62%). The addition of gene regions boosts support in several cases within the major clades. We note, for example, that our expanded analysis favors the placement of *Morina* with the Dipsacaceae plus Valerianaceae clade (i.e. recognition of the Valerina clade of Donoghue et al. 2001; 2003).

Figure 3 shows the majority rule consensus tree resulting from the partitioned Bayesian analysis of the dataset including 122 campanulid taxa and five outgroups. Again, the ML topology was identical, and ML bootstrap values are shown on the tree. Regarding relationships among the major lineages along the backbone of the tree, several results are noteworthy. First, our analysis strongly supports previous studies that have rooted the campanulid clade between the Aquifoliaceae and a clade containing the rest of Campanulidae, which is supported by a prior probability of 1.0 and a bootstrap value of 100% (Fig. 3; outgroups not shown). Second, this much larger tree agrees with our expanded 50-taxon tree in placing Asterales as the sister group of a clade containing Escalloniaceae, Columelliaceae-Bruniaceae, Apiales, and Dipsacales-Paracryphiaceae. However, this relationship remains poorly supported; indeed, it receives less support in the larger tree (Bayesian posterior probability = 0.85, ML bootstrap = 47%). Third, this analysis more strongly supports the placement of the Columelliaceae-Bruniaceae clade as the sister group of the Apiales-Dipsacales-Paracryphiaceae clade (Bayesian posterior probability = 1.0, ML bootstrap = 90%). Fourth, Apiales continues to appear as the sister group of Dipsacales plus Paracryphiaceae with strong support (Bayesian posterior probability = 1.0, ML bootstrap = 99%). Likewise, we continue to recover a strong link between Paracryphiaceae and Dipsacales (Bayesian posterior probability = 1.0, ML bootstrap = 100%). Overall, expanded taxon sampling has increased support for the backbone relationships, which are now uniformly high with the exception of the placement of the Escalloniaceae.

Relationships within the major clades in Fig. 3 largely correspond with those obtained in previous analyses. For example, the major relationships within Dipsacales are the same as those obtained in previous studies (reviewed in Donoghue et al. 2003; Winkworth et al. 2008a). One significant addition concerns the placement of *Zabelia* with the Morinaceae, as opposed to its expected placement with *Abelia* in the Linnaeeae based on prior taxonomic treatments (e.g. Hara 1983). Within Asterales it is noteworthy that the results of Winkworth et al. (2008b; also Kårehed 2002; Lundberg and Bremer 2003) are upheld in the placement of Campanulaceae with Roussaceae, *Donatia* with Stylidiaceae, and the position of *Pentaphragma* as the sister group to the remainder of Asterales minus the Campanulaceae-Roussaceae clade (in contrast to Bremer et al. 2002). However, our results, which agree with those of Kårehed (2002) and Lundberg and Bremer (2003) with respect to the position of Alseuosmiaceae and its relatives, differ from those of Winkworth et al. (2008b) who found significant support for a link with Stylidiaceae.

Figure 3 also shows the placement of several additional taxa that have not previously been sampled in analyses that have included a broad sampling of campanulid lineages. In agreement with Lundberg (2001), *Sphenostemon* was placed with confidence in the Paracryphiaceae clade (with *Paracryphia* and *Quintinia*). Likewise, we confirmed the placement of *Platyspermation* with *Alseuosmia, Wittsteinia*, and *Crispiloba* in the Alseuosmiaceae clade (e.g. Lundberg and Bremer 2003). We were also able to confirm the placement of several other rarely sampled campanulid taxa (see Lundberg 2001) in the strongly supported Escalloniaceae clade, including *Anopterus*, *Forgesia*, and *Valdivia*.

Discussion

***Filling in and Expanding the 50-taxon Dataset*—**Phylogenetic studies prior to Winkworth et al. (2008b), which focused on broader relationships within angiosperms or on the placement of enigmatic groups, consistently obtained two major results. First, they demonstrated the existence of a well-supported campanulid clade that includes the Apiales, Aquifoliales, Asterales, and Dipsacales, along with several other less diverse and historically problematical lineages (e.g. Escalloniaceae, Bruniaceae, Columelliaceae, and Paracryphiceae) (e.g. Soltis et al. 2000; Lundberg 2001; Bremer et al. 2002; Kårehed 2002, 2003; Lundberg and Bremer 2003). Second, they showed Aquifoliales to be the sister group of the rest of the campanulids (e.g. Soltis et al. 2000; Kårehed 2001; Bremer et al. 2002). Otherwise, these studies either differed from one another with respect to the relationships among the major campanulid lineages, or were unable to provide confident resolution.

Winkworth et al. (2008b) compiled matrices of existing sequence data for seven cpDNA and two nrDNA regions in the hope that these data would yield a more resolved phylogeny, and, indeed, they made some progress. For example, they were able to confidently resolve the position of the Paracryphiaceae (including *Quintinia*, sensu Lundberg 2001) as the sister group to the Dipsacales, and showed that the Dipsacales-Paracryphiaceae clade is sister to the Apiales. These results alone had important implications for flower evolution, confirming the suspicion (e.g. Donoghue et al. 2003) that small, rotate, radially symmetrical corollas were probably ancestral in the Dipsacales-Paracryphiaceae-Apiales clade, and that large, tubular, bilaterally symmetrical flowers were most likely derived with the origin of the Caprifoliaceae (sensu Donoghue et al. 2001) within the Dipsacales (Winkworth et al. 2008b). However, the analyses of Winkworth et al. (2008b) provided little additional resolution of backbone relationships among the major campanulid lineages.

Part of the problem with Winkworth et al. (2008b) might have been the fact that their data matrices included a significant amount of missing data (Fig. 1A; cpDNA coding regions with 11% missing data, cpDNA noncoding with 20% missing, and nrDNA with 49% missing). However, our 94% complete dataset for the same genes used by Winkworth et al. (2008b) resulted in an overall topology that was similar to the Winkworth et al. (2008b) tree. This filled-in dataset provides increased confidence in the Apilaes-Dipsacales-Paracryphiaceae clade. In addition, several clades that were only weakly supported in the Winkworth et al. (2008b) study were recovered with stronger support. However, several other relationships remain only weakly supported (tree not shown), including (1) the unresolved position of *Myodocarpus* with respect to core Apiales (i.e. Apiaceae and Araliaceae), (2) the position of both the *Alseuosmia-Argophyllum-Phelline* clade and the Stylidiaceae clade (*Stylidium* and *Donatia*) within

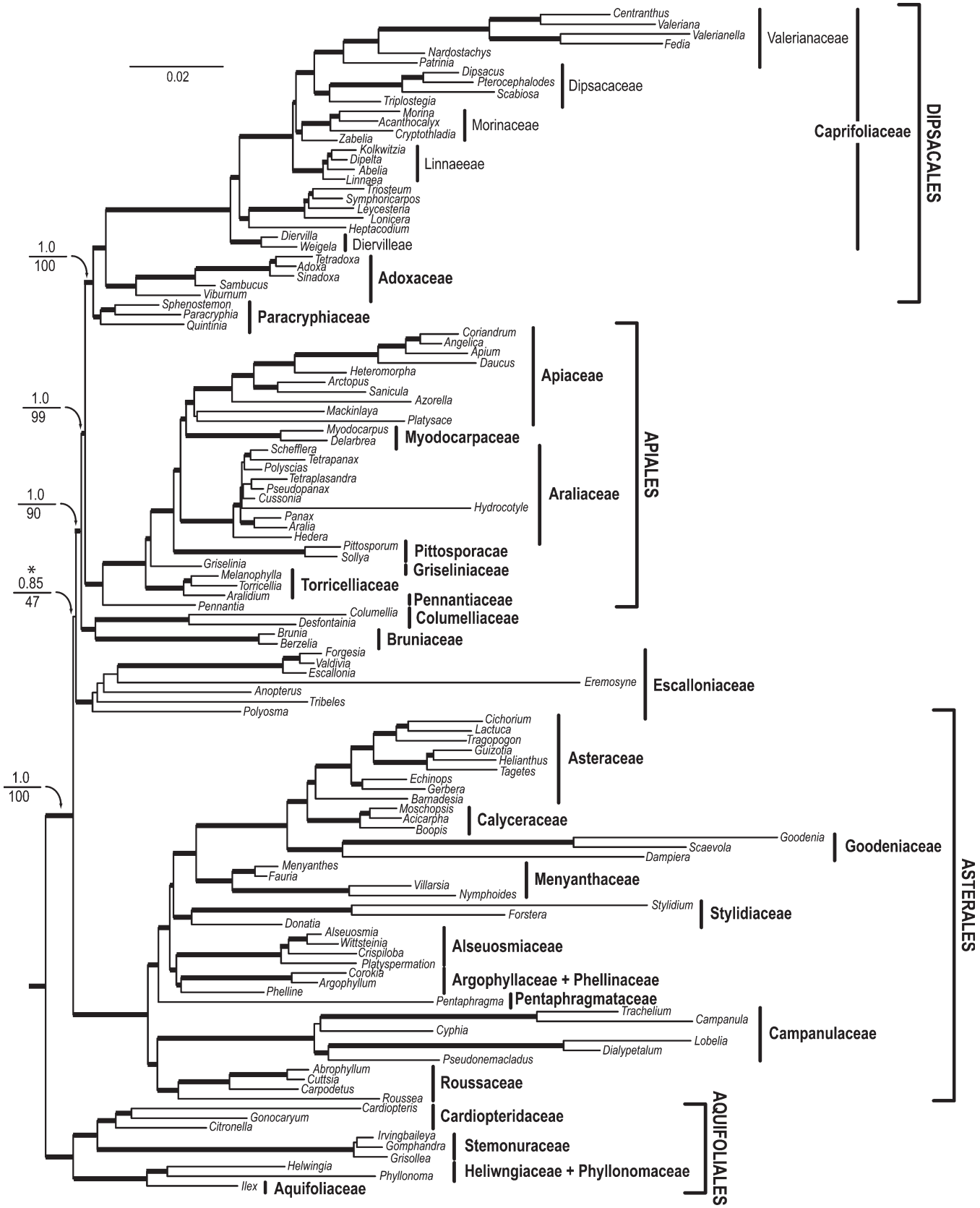

FIG. 3. Majority rule consensus tree with mean branch lengths from the partitioned Bayesian analysis of ten chloroplast genes for 122 campanulid taxa and five outgroups (removed). Branch lengths are proportional to the mean number of substitutions per site as measured by the scale bar. Thickened branches received Bayesian posterior probabilities ≥ 0.95 and ML bootstrap percentages ≥ 75%. Bayesian posterior probabilities and ML bootstrap percentages (above and below the line, respectively) are indicated along the backbone; asterisk marks the low statistical support for the position of the Escalloniaceae clade.

the core Asterales, (3) the relationships among *Alseuosmia*, *Argophyllum,* and *Phelline*, and (4) the relationships among the lineages comprising the Escalloniaceae clade. Overall, we conclude that filling in much of the missing data in the Winkworth et al. (2008b) data matrix provided little additional resolution of the campanulids.

In contrast, the addition of the *psbBTNH*, *rpoC2*, and *rps4* gene regions, ca. 8,000 bp of cpDNA coding data, did help to resolve the campanulid backbone, and provided support for many of the relationships left unresolved even in the filled-in analysis of the Winkworth et al. (2008b) genes (compare Figs. 1D and 1E). In the analysis of this expanded dataset we obtained increased support for the successive sister group relationships of the Asterales clade, the Escalloniaceae clade, and the Bruniaceae-Columelliaceae clade, to the well-supported Apiales-Dipsacales-Paracryphiaceae clade (Figs. 1E and 2). However, while these relationships received significant Bayesian posterior probability values, it is noteworthy that ML bootstrap values were not high.

In addition to the backbone, most other relationships are resolved with greater confidence with the addition of the ca. 8,000 bp of cpDNA coding data (Fig. 2). With the exception of the relationships within the Escalloniaceae clade, the relationships among *Alseuosmia*, *Argophyllum,* and *Phelline* within the Asterales, and the sister group relationship of Stylidiaceae (*Stylidium* and *Donatia*) and the *Alseuosmia-Argophyllum-Phelline* clade, all other relationships recovered within the major campanulid lineages were supported by Bayesian posterior probabilities greater than 0.95 and ML bootstrap values greater than 80% (Fig. 2). For example, *Myodocarpus* is resolved as sister to Apiaceae with strong support. This agrees with the majority of earlier studies (but see Chandler and Plunkett 2004), but this is the first analysis in which this result has received high levels of statistical support.

A noteworthy case concerns the relationships among *Helwingia*, *Ilex*, and *Phyllonoma* within the Aquifoliales. In broad scale angiosperm or asterid studies, *Helwingia* was consistently identified as more closely related to *Ilex* than to *Phyllonoma*, with varying levels of support (e.g. Morgan and Soltis 1993; Soltis and Soltis 1997; Olmstead et al. 2000; Lundberg 2001; Kårehed 2002). Winkworth et al. (2008b) obtained this same result, as did we when we simply filled in the Winkworth et al. data matrix (albeit with only moderate ML bootstrap support [66%], tree not shown). However, with the three additional cpDNA coding regions added, we found strong support for a *Phyllonoma-Helwingia* clade to the exclusion of *Ilex*. This result makes sense from the standpoint of morphological characters, as *Helwingia* and *Phyllonoma* share several apomorphies, including epiphyllous inflorescences, fimbriate stipules, and brochidodromous leaf venation (see Stevens 2001 onward).

Increased Taxon Sampling—Increased taxon sampling can have a significant effect on both topology and statistical support (e.g. Hillis 1996, 1998; Zwickl and Hillis 2002), and this may be especially the case in sparsely sampled lineages like the campanulids. In adding taxa to the 50-taxon dataset our aim was to increase the sampling of large and undersampled groups (e.g. Apiaceae, Asteraceae), to break up long branches (e.g. *Forstera* in the Stylidiaceae), and to include enigmatic taxa that have not yet been included in phylogenetic studies (e.g. *Zabelia, Valdivia, Gomphandra*). These additions yielded a number of important results. Regarding the backbone phylogeny, support is now high for all major clades except for the position of Escalloniaceae, where support is even weaker in this larger study. The short branches subtending a number of these early diverging clades suggest a rapid radiation early in campanulid phylogeny. We have been successful in confidently resolving the phylogenetic relationships of several enigmatic groups, including *Zabelia* (sister to Morinaceae in the Dipsacales), *Sphenostemon* (Paracryphiaceae), *Valdivia*, *Forgesia*, and *Anopterus* (in the expanded Escalloniaceae, sensu Lundberg 2001), *Platyspermation* (Alseuosmiaceae in the Asterales), *Gonocaryum* and *Gomphandra* (Cardiopteridaceae and Stemonuraceae, repectively, in the Aquifoliales).

Taken together, our analyses demonstrate the efficacy of the now standard, brute-force approach (e.g. underlying most NSF-AToL projects) of simply adding both genes and taxa to resolve difficult phylogenetic problems. However, a number of weakly supported results (e.g. relationships within the Araliaceae and Escalloniaceae clades), and inconsistencies with previous studies, caution that further increases will be necessary to obtain uniformly satisfying results. For example, in the Dipsacales, the ((*Pterocephalodes, Dipsacus*) *Scabiosa*) relationship seen in Fig. 3 (also see Moore et al. 2006) is at odds with a richly sampled analysis of Dipsacaceae (Carlson et al. 2009), which instead strongly supports (*Pterocephalodes* (*Dipsacus*, *Scabiosa*)). Likewise, the ((*Acanthocalyx*, *Morina*) *Cryptothladia*) result shown in Fig. 3 conflicts with the earlier, more detailed analysis of Morinaceae (Bell and Donoghue 2003), which instead obtained (*Acanthocalyx* (*Morina*, *Cryptothladia*)). In the Asterales, *Echinops* and *Gerbera* form a strongly supported clade in the Asteraceae (Bayesian posterior probability = 1.0, ML bootstrap = 100%; Fig. 3), a result that contradicts detailed studies of phylogenetic relationships in Asteraceae, which place *Gerbera* and *Echinops* in the separate, early-diverging Asteraceae lineages Mutisioideae and Carduoideae, respectively (e.g. Panero and Funk 2008).

Implications for Flower Evolution—A detailed analysis of morphological evolution is beyond the scope of the present paper, but is being pursued elsewhere. However, it is worth noting that our much-expanded analyses support the conclusions of Winkworth et al. (2008b) regarding flower evolution in the Dipsacales. That is, we concur that radially symmetrical flowers were probably ancestral at the level of the Apiales-Dipsacales-Paracryphiaceae clade, and that the evolution of bilaterally symmetrical corollas probably coincided with the origin of the Caprifoliaceae (sensu Donoghue et al. 2001, 2003) within the Dipsacales (Fig. 3). The location of this particular shift in symmetry appears to correlate with duplications in genes that are involved in the development of flower symmetry (both the CYCLOIDEA and DIVARICATA gene families; Howarth and Donoghue 2005; 2009).

Looking more broadly across the Campanulidae, the Aquifoliales, Apiales, Escalloniaceae, Bruniaceae, and Columelliaceae clades are all characterized by radially symmetrical flowers, except in rare circumstances (e.g. the enlarged corollas of marginal flowers in the inflorescences of some Apiaceae; the bilateral androecium of *Cheirantheira* of the Pittosporaceae). Asterales exhibit both conditions and it is likely that bilateral flowers evolved multiple times independently within this clade.

Although earlier authors interpreted bilateral corollas as ancestral in Asteraceae (e.g. Jeffrey 1977), a more thorough understanding of phylogenetic relationships (including the connection with Calyceraceae) suggests that radially symmetric corollas are likely the ancestral condition (e.g. Bremer

1994; Urtubey and Stuessy 2001; Panero and Funk 2008). Within each of the early diverging lineages of Asteraceae (e.g. Barnadesioideae, Mutisioideae, Stifftioideae, Wunderlichioideae, Gochnatioideae, Carduoideae sensu Panero and Funk 2008) one finds both radial and bilateral flowers (Anderberg et al. 2007). In most species, capitula are isomorphic, producing only radial flowers, or only bilateral flowers (ranging from bilabiate, e.g. 2 + 3, to ligulate, e.g. 0 + 5) but not both (e.g. all members of Stifftiodeae, Wunderlichioideae, Gochnatioideae). However, dimorphic capitula with both central radial flowers and marginal bilateral flowers are found in both the Barnadesioideae and Mutisioideae. In the Barnadesiodeae – the sister lineage to the remainder of Asteraceae – the majority of species have monomorphic capitula with either radial or bilateral flowers, however, dimorphic capitula are found in several species of *Barnadesia* and the monotypic genus *Huarpea* (Gustafsson et al. 2001; Urtubey and Stuessy 2001). In the Mutisioideae there are several genera with large dimorphic capitula with central radial flowers and marginal ligulate flowers resembling those common in the Asteroideae (e.g. *Brachyclados*, *Chaptalia*, *Gerbera*, *Trichocline*; Panero and Funk 2008). Although we are unable to fully analyze the phylogenetic distribution of these states, the overall distribution does appear to favor multiple origins of bilateral flowers, and possibly also the loss of bilateral flowers from capitula in some lineages.

In addition to the bilaterally symmetrical flowers found in Asteraceae (alongside radial flowers in many lineages), another shift to bilateral corolla symmetry occurred in Goodeniaceae. With the exception of the radially symmetrical *Brunonia* (nested within Goodeniaceae), corollas in Goodeniaceae are either bilabiate (2 + 3) or unilabiate (0 + 5). The fan-shaped, unilabiate flowers of Goodeniaceae have an adaxial slit that forms the basis for the 0 + 5 arrangement of the corolla lobes; this differs from the ligulate (0 + 5) flowers of Asteraceae (Gustafsson 1996). However, without a detailed comparative analysis of floral symmetry in Asteraceae, there is still some possibility that the Asteraceae-Calyceraceae-Goodeniaceae clade was ancestrally bilateral, with multiple reversions to radial symmetry (e.g. in Calyceraceae, *Brunonia* in Goodeniaceae, and throughout the lineages of Asteraceae).

Bilateral flowers also appear to have evolved within the Campanulaceae sensu lato, with the number of shifts depending on the phylogenetic placement of the bilateral *Cyphia* and *Cyphocarpus* in relation to the radial Campanuloideae and the bilateral Lobelioideae-Nemacladoideae lineage (sensu Lammers 2007). Our expanded analysis shows *Cyphia* as sister to the Campanuloideae, supporting the possibility of at least two shifts in symmetry, but this placement is only weakly supported.

Finally, within Stylidiaceae, both *Stylidium* and *Levenhookia* are bilaterally symmetrical, while the remaining genera (*Donatia*, *Forstera*, and *Phyllachne*) exhibit radial symmetry. Our expanded analysis, in agreement with earlier analyses that included all five genera of Stylidiaceae (e.g. Laurent et al. 1999; Wagstaff and Wege 2002; Lundberg and Bremer 2003), places *Donatia* as the well-supported sister group to the remainder of the clade. In the analyses of Lundberg and Bremer (2003), the bilateral genera, *Stylidium* and *Levenhookia*, were recovered as successive sister groups to a clade containing the two other radially symmetrical groups (*Forstera* and *Phyllachne*) with strong statistical support. In contrast, the analyses of both Laurent et al. (1999) and Wagstaff and Wege (2002) placed *Stylidium* and *Levenhookia* in a clade. These results suggest that a shift in corolla symmetry either evolved once in Stylidiaceae, or the bilaterally symmetrical corollas of *Stylidium* and *Levenhookia* evolved independently, or the *Stylidium-Levenhookia-Forstera-Phyllachne* clade was ancestrally bilateral, with a reversion to radial symmetry in *Forstera* and *Phyllachne*. Whichever the case, it is important to note that the bilateral symmetry of *Stylidium* and *Levenhookia* is different from the bilateral symmetry exhibited throughout the rest of the Asterales, and the two are actually different from one another. This reflects their highly specialized pollination mechanisms (Wagstaff and Wege 2002; Armbruster and Muchhala 2009). In *Stylidium*, bilateral symmetry is created by the much reduced ventral corolla lobe and the column that is held under tension until triggered by an insect pollinator. In *Levenhookia*, bilateral symmetry is the result of the ventral corolla lobe forming a hood over the column, which is released when stimulated by a pollinator.

In summary, within the Campanulidae, bilateral (monosymmetric) corollas appear to have originated in the Caprifoliaceae of the Dipsacales, and at least three times (e.g. Asteraceae/Goodeniaceae, Campanulaceae s. l., and Stylidiaceae) in the Asterales. However, it appears more likely that there were multiple origins in Asterales: several instances within Asteraceae, independently in Goodeniaceae, and possibly twice within both Campanulaceae sensu lato and Stylidiaceae. More detailed morphological analyses and better taxon sampling are necessary to pin this down more exactly. It will be especially interesting to determine whether gene duplications similar to those correlated with the shift to bilateral flowers within Dipsacales (Howarth and Donoghue 2005, 2009) are associated with any of the independent shifts within Asterales.

Phylogenetic Nomenclature—Phylogenetic nomenclature provides definitions of taxon names that refer directly to inferred phylogenetic relationships among taxa (de Queiroz and Gauthier 1990, 1992, 1994; de Queiroz 1992), and an explicit evolutionary framework for the communication, storage, and retrieval of taxonomic information (Cantino et al. 2007). In view of the repeated appearance of a number of strongly supported campanulid clades, in our analyses and in prior studies, we believe that it is now appropriate and useful to provide formal phylogenetic definitions for several of these major lineages.

Phylogenetic definitions have already been provided for several of the major clades covered here. Cantino et al. (2007) defined Campanulidae to formalize the use of the informal name "campanulids" (sensu Bremer et al. 2002; Judd and Olmstead 2004) for the clade containing Aquifoliales, Asterales, Apiales, and Dipsacales (the "asterid II" clade of Chase et al. [1993]; the "euasterid II" clade of APG [1998]). Likewise, Cantino et al. (2007) defined the name Apiidae for the clade including Asterales, Apiales, and Dipsacales. This choice of names emphasized the asterid relationships of Apiales, a lineage previously considered part of the Rosidae (e.g. Cronquist 1981). Dipsacales was one of the first plant clades to receive a formal treatment following early formulations of phylogenetic nomenclature (Donoghue et al. 2001; see Donoghue et al. 2003). In addition to defining the name Dipsacales itself, Donoghue et al. (2001) provided node-based phylogenetic definitions for Adoxaceae, Adoxoideae, Adoxina, Caprifoliaceae, Caprifolieae, Diervilleae, Linnina, Linnaeeae, and Valerina. Other major campanulid clades, including some

that have long-standing names associated with them (e.g. Apiales, Aquifoliales, and Asterales), lack the phylogenetic definitions necessary to standardize their usage in angiosperm phylogenetics (and beyond).

Here we provide phylogenetic definitions for eight additional clade names within Campanulidae, marked by the dark circles in Fig. 4. Note that with these additional names, all of the major clades of campanulids are given phylogenetic definitions with the exception of the two that are still too weakly supported, linking Escalloniaceae and Bruniaceae-Columelliaceae, in turn, with the Apiales-Paracyphiaceae-Dipsacales clade.

The phylogenetic definitions given here follow the draft PhyloCode (Cantino and de Queiroz 2007; http://www.phylocode.org/). For five clade names, where there has been sufficient sampling and relationships among subclades are well-resolved and well-supported, we provide node-based definitions using two or more specifiers. Branch-modified node based definitions are used for three clade names owing to uncertainty about relationships, due to incomplete taxon sampling and/or a lack of resolution of early-branching lineages within an otherwise well-supported clade. Our eight new phylogenetic definitions are presented in Table 1, and discussed briefly in the following section. Table 1 also includes the previously published definitions of Campanulidae, Apiidae, and Dipsacales.

Dipsapiidae D. C. Tank & M. J. Donoghue, new clade name. Definition (node based) – The least inclusive clade containing *Apium graveolens* L. 1753 (Apiales), *Paracryphia alticola* Shltr. (Steenis) 1950 (Paracryphiaceae), and *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales).

There is no preexisting scientific name for this clade. The name Dipsapiidae is given to emphasize the close relationship of the Apiales to the Dipsacales. This basic result appeared in several earlier studies, notably in Bremer et al. (2002) and in Winkworth et al. (2008b), and is strongly supported in the analyses presented here.

Dipsidae D. C. Tank & M. J. Donoghue, new clade name. Definition (node based) – The least inclusive clade containing *Paracryphia alticola* Shltr. (Steenis) 1950 (Paracryphiaceae) and *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales).

There is no preexisting scientific name for this clade. The name Dipsidae recognizes the sister group relationship between Paracryphiaceae and Dipsacales. This result was recovered with strong statistical support by Winkworth et al. (2008b) and is strongly supported in the analyses presented here. The recognition of this clade is important for the interpretation of floral evolution and biogeography (see Winkworth 2008b).

Paracryphiaceae Airy Shaw 1964: 265 [D. C. Tank and M. J. Donoghue], converted clade name. Definition (Branch-modified node based) – The most inclusive crown clade containing *Paracryphia alticola* Shltr. (Steenis) 1950 (*Paracryphia*) but not *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales) or *Apium graveolens* L. 1753 (Apiales).

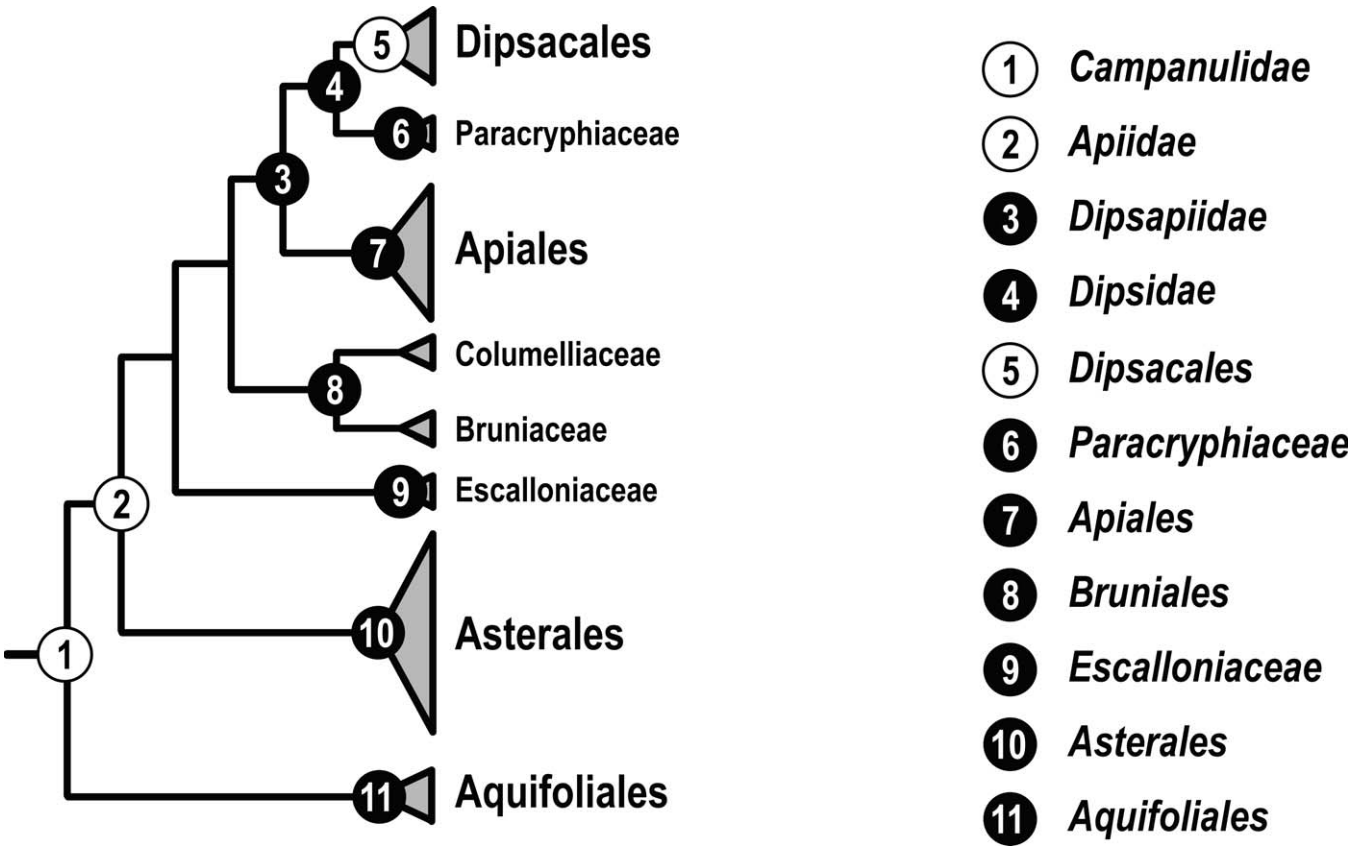

FIG. 4. Summary tree showing inferred phylogenetic relationships of the major lineages of Campanulidae. Numbered circles denote named clades with phylogenetic definitions as given in Table 1. Dark circles mark clades that are defined in this paper; white circles mark clades that were defined elsewhere.

TABLE 1. Phylogenetic definitions for major campanulid clades as shown in Fig. 4. # refers to the numbered clades indicated on the campanulid summary tree shown in Fig. 4. Phylogenetic definitions follow the draft PhyloCode (Cantino and de Queiroz 2007).

#	Clade	Definition	Notes
1	Campanulidae	The most inclusive crown clade containing *Campanula latifolia* L. 1753 (Asterales) but not *Garrya eliptica* Douglas ex Lindl. 1834 (Garryidae) or *Lamium purpureum* L. 1753 (Lamiidae) or *Cornus mas* L. 1753 (Cornales) or *Erica carnea* L. 1753 (Ericales).	Phylogenetic definition from Cantino et al. (2007); euasterid II sensu APG II (2003)
2	Apiidae	The most inclusive crown clade including *Apium graveolens* L. 1753 (Apiales), *Helianthus annuus* L. 1753 (Asterales), and *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales), but not *Ilex crenata* Thunb. 1784 (Aquifoliales) or *Cardiopteris quinqueloba* Hassk. 1855 (Aquifoliales) or *Garrya elliptica* Douglas ex Lindl. 1834 (Garryidae) or *Lamium purpureum* L. 1753 (Lamiidae).	Phylogenetic definition from Cantino et al. (2007)
3	Dipsapiidae	The least inclusive clade containing *Apium graveolens* L. 1753 (Apiales), *Paracryphia alticola* Shltr. (Steenis) 1950 (Paracryphiaceae), and *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales).	New clade name; Bremer et al. (2002); Winkworth et al. 2008b
4	Dipsidae	The least inclusive clade containing *Paracryphia alticola* Shltr. (Steenis) 1950 (Paracryphiaceae) and *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales).	New clade name; Bremer et al. (2002); Winkworth et al. (2008b)
5	Dipsacales	The least inclusive clade containing *Dipsacus sativus* (L.) Honck. 1782 (Dipsacaceae), *Linnaea borealis* L. 1753 (Linnaeeae), *Lonicera caprifolium* L. 1753 (Caprifolieae), and *Viburnum lantana* L. 1753 (Adoxaceae).	Phylogenetic definition from Donoghue et al. (2001); Dipsacales sensu APG II (2003)
6	Paracryphiaceae	The most inclusive crown clade containing *Paracryphia alticola* Shltr. (Steenis) 1950 (*Paracryphia*) but not *Dipsacus sativus* (L.) Honck. 1782 (Dipsacales) or *Apium graveolens* L. 1753 (Apiales).	Converted clade name; Paracryphiaceae sensu Stevens (2001 onwards), Winkworth et al. (2008b), Lundberg (2001)
7	Apiales	The least inclusive clade containing *Pennantia corymbosa* J. R. Forst. & G. Forst. 1775 (Pennantiaceae) and *Apium graveolens* L. 1735 (Apiaceae).	Converted clade name; Apiales sensu APG II (2003), Kårehed (2001, 2003), Chandler and Plunkett (2004)
8	Bruniales	The most inclusive crown clade containing *Berzelia lanuginosa* Brongn. 1826 (Bruniaceae) but not *Dipsacus sativus* (L.) Honck. 1782 (Dipsapiidae) or *Escallonia myrtilloides* L. f. 1782 (Escalloniaceae) or *Helianthus annuus* L. 1753 (Asterales).	Converted clade name; Bremer et al. (2002), Winkworth et al. (2008b)
9	Escalloniaceae	The most inclusive crown clade containing *Escallonia myrtilloides* L. f. 1782 (*Escallonia*) but not *Berzelia lanuginosa* Brongn. 1826 (Bruniaceae) or *Columellia oblonga* Ruiz & Pav. 1798 (Columelliaceae) or *Dipsacus sativus* (L.) Honck. 1782 (Dipsapiidae) or *Helianthus annuus* L. 1753 (Asterales).	Escalloniaceae sensu Stevens (2001 onwards), Lundberg (2001)
10	Asterales	The least inclusive clade containing *Roussea simplex* Sm. 1789 (Roussaceae), *Campanula latifolia* L. 1753 (Campanulaceae), *Pentaphragma begoniifolium* (Roxb.) G. Don 1834 (Pentaphragmataceae), *Alseuosmia macrophylla* A. Cunn. 1838 (Alseuosmiaceae), *Stylidium graminifolium* Sw. ex Willd. 1805 (Stylidiaceae), and *Helianthus annuus* L. 1753 (Asteraceae).	Asterales sensu APG I (1998) and APG II (2003), Lundberg and Bremer (2002)
11	Aquifoliales	The least inclusive clade containing *Ilex crenata* Thunb. 1784 (Aquifoliaceae) and *Cardiopteris quinqueloba* Hassk. 1855 (Cardiopteridaceae).	Aquifoliales sensu APG II (2003); Kårehed (2001)

The name Paracryphiaceae has traditionally been restricted to the monotypic genus *Paracryphia*, but is here expanded to include the two other southeast Asian genera *Sphenostemon* and *Quintinia*. Lundberg (2001) was the first to include the three genera in a phylogenetic analysis where a relationship between the three genera was recognized, and Winkworth et al. (2008b) confirmed the close relationship between *Paracryphia* and *Quintinia* (see Stevens 2001 onwards for a discussion of possible synapomorphies). However, neither *Quintinia* (25 spp.) nor *Sphenostemon* (ten spp.) have been widely sampled in any analyses, including those presented here. Therefore, a branch-modified node based definition is used to maintain the intended scope for Paracryphiaceae in case future detailed phylogenetic studies reveal a different pattern of relationships among the three genera. For example, if a standard node based definition was used for this clade name, in the unlikely event that *Sphenostemon* and/or *Quintinia* were placed elsewhere in Campanulidae, or either genus was found to be grossly paraphyletic, this would result in the name Paracryphiaceae referring to a much larger or much smaller clade, respectively, than originally intended.

Apiales Nakai 1930: 58. [D. C. Tank and M. J. Donoghue], converted clade name. Definition (node based) – The least inclusive clade containing *Pennantia corymbosa* J. R. Forst. & G. Forst. 1775 (Pennantiaceae) and *Apium graveolens* L. 1735 (Apiaceae).

The name Apiales was applied to this clade by APG II (2003). Apiales received strong statistical support in broad-scale phylogenetic studies of angiosperms and asterids (e.g. Olmstead et al. 2000; Soltis et al. 2000; Bremer et al. 2002). Kårehed (2002, 2003) recovered a close relationship of *Pennantia* and Apiales and Chandler and Plunkett (2004) confirmed the placement of *Pennantia* as the sister group to the remainder of Apiales. Our analyses confirm these earlier studies and provide resolution and support for relationships among the subclades of Apiales.

Bruniales Dumort. 1829: 33 [D. C. Tank and M. J. Donoghue], converted clade name. Definition (branch-modified node based) – The most inclusive crown clade containing *Berzelia lanuginosa* Brongn. 1826 (Bruniaceae) but not *Dipsacus sativus* (L.) Honck. 1782 (Dipsapiidae) or *Escallonia myrtilloides* L. f. 1782 (Escalloniaceae) or *Helianthus annuus* L. 1753 (Asterales).

Bruniales is intended to include the Bruniaceae and Columelliaceae. Bremer et al. (2002) recovered Bruniales, albeit

weakly; this clade was subsequently recovered by Winkworth et al. (2008b) with strong support, and our results confirm this relationship. Although this clade has appeared in multiple studies, and relationships within each of the two included lineages are well-resolved and well-supported, because Lundberg (2001) placed Columelliaceae with Dipsacales, we have chosen to use a branch-modified node based definition. The name Bruniales is based on Bruniaceae, the larger of the two clades comprising Bruniales. This choice also coincides with the usage by Stevens (2001 onwards), and will alleviate confusion if the contents of the clade were to change. Thus, if Columelliaceae is determined to be more closely related to another lineage of Campanulidae than it is to Bruniaceae (e.g. within Dipsapiidae), then the name Bruniales will become synonymous with Bruniaceae.

Escalloniaceae R. Br. ex Dumort 1829: 35, 37 [D. C. Tank and M. J. Donoghue], converted clade name. Definition (branch-modified node based) – The most inclusive crown clade containing *Escallonia myrtilloides* L.f. 1782 (*Escallonia*) but not *Berzelia lanuginosa* Brongn. 1826 (Bruniaceae) or *Columellia oblonga* Ruiz & Pav. 1798 (Columelliaceae) or *Dipsacus sativus* (L.) Honck. 1782 (Dipsapiidae) or *Helianthus annuus* L. 1753 (Asterales).

This clade brings together a group of heterogeneous taxa that have been linked in various molecular systematic studies. A relationship between Escalloniaceae and Eremosynaceae was recovered with strong support by Soltis et al. (2000). Lundberg (2001) and Winkworth et al. (2008b) also recovered this relationship and added several other lineages to this clade including, *Tribeles*, *Anopterus*, and *Polyosma*. Although this is a well-supported clade, relationships within Escalloniaceae are still unresolved in our analyses, and therefore, we have used a branch-modified node based definition to accommodate this uncertainty.

Asterales Link 1829: 731 [D. C. Tank and M. J. Donoghue], converted clade name. Definition (node based) – The least inclusive clade containing *Roussea simplex* Sm. 1789 (Roussaceae), *Campanula latifolia* L. 1753 (Campanulaceae), *Pentaphragma begoniifolium* (Roxb.) G. Don 1834 (Pentaphragmataceae), *Alseuosmia macrophylla* A. Cunn. 1838 (Alseuosmiaceae), *Stylidium graminifolium* Sw. ex Willd. 1805 (Stylidiaceae), and *Helianthus annuus* L. 1753 (Asteraceae).

The name Asterales was applied to this clade by APG (1998) and APG II (2003), and has a long association with this group in traditional classifications (e.g. Cronquist 1981). Numerous studies have repeatedly recovered this clade with strong statistical support (e.g. Olmstead et al. 2000; Soltis et al. 2000; Albach et al. 2001; Bremer et al. 2002; Lundberg and Bremer 2003). Relationships among the early branching lineages of Asterales were mostly poorly resolved prior to Winkworth et al. (2008b) and the analyses presented here.

Aquifoliales Senft 1856: 118 [D. C. Tank and M. J. Donoghue], converted clade name. Definition (node based) – The least inclusive clade containing *Ilex crenata* Thunb. 1784 (Aquifoliaceae) and *Cardiopteris quinqueloba* Hassk. 1855 (Cardiopteridaceae).

The name Aquifoliales was applied to this clade by APG II (2003). Aquifoliales are the strongly supported sister group to the remainder of Campanulidae and have been recovered by numerous studies (e.g. Soltis et al. 2000; Bremer et al. 2002). Winkworth et al. (2008b) did not include sampling outside of Campanulidae, so it was not until our study that a focused analysis with a broad sampling of campanulid lineages recovered this same result. This clade includes multiple genera that were once in the largely dismantled Icacinaceae in an expanded Cardiopteridaceae clade and the newly established Stemonuraceae (Kårehed 2001).

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Appendix 1. Taxa, source/voucher, and GenBank information for sequences used in this study. Taxa in bold were included in the 50-taxon, core campanulid analyses, as discussed in the text. GenBank GI or accession numbers are given for all sequences, and accession numbers in bold are for sequences that were newly generated for this study,–indicates a sequence not available. Order for each clade is taxon, source/voucher, *atpB, matK, ndhF, psbBTNH, rbcL, rpoC2, rps4, rps16 intron, trnL-trnF, atpE-trnV*, 18S, 26S.

APIALES: *Angelica acutiloba* (Siebold & Zucc.) Kitag., N/A,–,–,–,–,–,–,–,–, 9309345,–,–,–; *Angelica gigas* Nakai, N/A,–,–, 6424733,–,–,–,–,–,–,–,–,–; *Angelica lucida* L., N/A,–, 2281130,–,–, 1292957,–,–,–,–,–,–,–; *Angelica sylvestris* L., *Fay 651* (K), **GQ983632,**–,–, **GQ983736,**–, **GQ983866, GQ983916, GQ983998,**–, **GQ984093,**–,–; ***Apium graveolens* L.,** *Chase 2523* (K), 8467928, 22795488, 22795478, **GQ983681,** 7239781, **GQ983848, GQ983883,** 22795509, 22795504, 22795498, 7595372, 19919636; *Aralia spinosa* L., *Plunkett 1371* (WS), **GQ983605,** 22795866, 22795801, **GQ983696,** 289043, **GQ983801, GQ983963, GQ983999,** 22795982, **GQ984102,**–,–; ***Aralidium pinnatifidum* Miq.,** *Soltis 5981* (WS),–, 2281230, 22795803, **GQ983731,** 10945632,–,–, 22796018, 22795950, 22795936,–, 37778828; *Arctopus dregei* Sond., *Goldblatt 11880* (MO), **GQ983621,**–, **GQ983671, GQ983688,**–, **GQ983818, GQ983933, GQ983987, GQ984047, GQ984124,**–,–; *Arctopus echinatus* L., N/A,–, 14276782,–,–, 33669419,–,–,–,–,–,–,–; ***Azorella caespitosa* Cav.,** *Lowry II 6560* (MO), **GQ983635,**–, **GQ983673, GQ983680,**–,–, **GQ983897,**–, **GQ984059, GQ984099, GQ983561,**–; ***Azorella selago* Hook.f.,** N/A,–, 14276784,–,–, 34559276,–,–,–,–,–,–, 37778838; ***Azorella trifurcata* Pers.,** N/A,–,–,–,–,–,–,–, 6692890,–,–,–,–; *Coriandrum sativum* L., *Plunkett 1479* (VCU), **GQ983603,** 2281158, 6424734, **GQ983685,** 336618, **GQ983795, GQ983929, GQ984010, GQ984062, GQ984120,**–,–; *Cussonia holstii* Harms ex Engl., N/A,–,–,–,–,–,–,–,–, 41087186,–,–,–; *Cussonia spicata* Thunb., *Goldblatt 10490* (MO), **GQ983596,** 2281238, **GQ983676, GQ983748,** 1292997, **GQ983836, GQ983905, GQ983995,**–, **GQ984107,**–,–; *Daucus carota* L., N/A, 113200887, 2281160, 113200887, 113200887, 1374996, 113200887, 113200887, 113200887, 113200887, 113200887,–,–; *Delarbrea harmsii* R. Vig., *Lowry II 4732* (MO),–,–,–, **GQ983715,**–, **GQ983823, GQ983884, GQ984008, GQ984040, GQ984121,**–,–; *Delarbrea michieana* (F. Muell.) F. Muell., N/A, 6687376, 2281244, 6687380,–, 1293003,–,–,–,–,–,–,–; ***Griselinia littoralis* Raoul,** Cult. Strybing Arboretum xy-2609,–, 22796281,–, **GQ983737,**–, **GQ983858, GQ983944,** 22796311, 22796304, 22796295,–,–; ***Griselinia lucida* G. Forst.,** N/A, 14718067,–, 6424740,–, 7239801,–,–,–,–,–, 7595442, 19919638; *Hedera helix* L., *Chase 2743* (K), 8452660, 18073960, 6424738, **GQ983726,** 7240253, 90403732, 32401821, **GQ983991,** 41087201, **GQ984126,**–,–; *Herteromorpha trifoliata* Eckl. & Zeyh., *Chase 5802* (K), **GQ983611,** 2281176, **GQ983664, GQ983721,** 1292983, **GQ983790, GQ983966, GQ984006,**–, **GQ984134,**–,–; ***Hydrocotyle bowlesioides* Mathias & Constance,** N/A,–, 2281252,–,–, 1292981,–,–,–, 41087169,–,–, 37778868; ***Hydrocotyle rotundifolia* Roxb.,** N/A,–,–,–,–,–,–,–,–, 6692898,–,–,–,–; ***Hydrocotyle sibthorpioides* Lam.,** N/A,–,–,–,–,–,–,–,–,–,–, 18854,–; ***Hydrocotyle vulgaris* L.,** *Fay 231* (K), **GQ983599,**–, **GQ983666, GQ983702,**–, **GQ983834, GQ983976,**–,–, **GQ984128,**–,–; ***Mackinlaya confusa* Hemsl.,** *Plunkett 1549* (VCU), **GQ983615,**–, **GQ983672, GQ983756,**–, **GQ983850, GQ983948, GQ983990,**–,–, **GQ983568,**–; ***Mackinlaya macrosciadia* (F. Muell.) F. Muell.,** *Plunkett 1365* (WS),–, 2281262,–,–, 1293011,–,–,–, 41087171, **GQ984097,**–, 37778875; *Melanophylla alnifolia* Baker, N/A, 14718123,–, 6688637,–, 1293009,–,–,–,–,–,–,–; *Melanophylla perrieri* Keraudren, *Ratovoson 5* (MO),–,–,–, **GQ983683,**–,–,–, **GQ984024,**–, **GQ984117,**–,–; *Melanophylla sp.* Baker, N/A,–, 22797126,–,–,–,–,–,–, 22797169,–,–,–; ***Myodocarpus crassifolius* Dubard & Viguier,** *Plunkett 1825* (VCU),–,–,–,–,–,–, **GQ983954,**–,–,–,–,–; ***Myodocarpus fraxinifolius* Brongn. & Gris,** *McPherson & Munzinger 18258* (MO), **GQ983630,**–, **GQ983678, GQ983770,**–, **GQ983830,**–, **GQ984015, GQ984072, GQ984090, GQ983570,**–; ***Myodocarpus involucratus* Dubard & Viguier,** N/A,–, 14276758,–,–, 33669449,–,–,–,–,–,–, 37778887; ***Panax ginseng* C. A. Mey.,** N/A, 51235292, 51235292, 51235292, 51235292, 51235292, 51235292, 51235292, 51235292, 28172968, 51235292, 2645178,–; ***Panax quinquefolius* L.,** N/A,–,–,–,–,–,–,–,–,–,–,–, 37778890; ***Pennantia corymbosa* J. R. Forst. & G. Forst,** *Gemmill s. n.* (WS),–,–,–,–,–,–,–, **GQ984002, GQ984043,**–,–, 37778891; ***Pennantia cunninghamii* Miers,** *Telford 8827* (CBG), 32526542, 32526550, 17932826, **GQ983775,** 32526546, **GQ983817, GQ983908,**–,–, **GQ984122, GQ983573,**–; ***Pittosporum fairchildii* Cheeseman,** N/A, 8517654,–,–,–,–,–,–,–,–,–,–,–; ***Pittosporum japonicum* Hort. Ex C. Presl,** N/A,–,–,–,–,–,–,–,–,–,–, 532222,–; ***Pittosporum tobira* [Dryand.],** N/A,–,–, 6424736,–, 1293037,–,–,–,–,–,–, 37778912; ***Pittosporum undulatum* Vent.,** *Chase 15890* (K),–, 22797482,–, **GQ983729,**–, **GQ983829, GQ983886,** 22797627, 22797595, 22797546,–,–; *Platysace lanceolata* (Labill.) Druce, *Davies 422* (CBG), **GQ983628, GQ983657, GQ983665, GQ983735,** 33669457, **GQ983849, GQ983956, GQ984034, GQ984055, GQ984087,**–,–; *Polyscias amplifolia* Harms, N/A,–,–,–,–,–,–,–,–, 22212626,–,–,–; *Polyscias guilfoylei* L. H. Bailey, *Plunkett 1322* (WS), **GQ983620,** 2281270, **GQ983674, GQ983710,** 1293019, **GQ983820, GQ983893, GQ983997,**–, **GQ984110,**–,–; *Pseudopanax arboreus* (L. f.) Philipson, N/A,–, 2281268,–,–, 1293017,–,–,–, 41087229,–,–,–; *Pseudopanax colensoi* (Hook. f.) Philipson, *Bayer NZ-01002* (CANB), **GQ983637,**–, **GQ983677, GQ983703,**–, **GQ983792, GQ983951, GQ984028,**–, **GQ984089,**–,–; *Sanicula europaea* L., *Chase 10281*

(K),–,–,–, **GQ983742,**–, **GQ983851,**–,–, **GQ984058,**–,–,–; *Sanicula gregari* Bicknell, N/A,–, 2281214,–,–, 295090,–,–,–,–,–,–,–; *Schefflera actinophylla* Harms, N/A,–,–,–,–,–,–,–,–, 21305246,–,–,–; *Schefflera arboricola* (Hayata) Merr., N/A,–, 2281278,–,–, 1293025,–,–,–,–,–,–,–; *Schefflera delavayi* (Franch.) Harms, *Chase 14856* (K), **GQ983613,**–, **GQ983669, GQ983778,**–, **GQ983809,**–, **GQ984020,**–,–,–,–; *Schefflera monticola* Ridl., *Barker 90* (K),–,–,–,–,–,–, **GQ983887,**–,–, **GQ984088,**–,–; *Sollya heterophylla* Lindl., *Chase 123* (NCU), 6689308, 2281280, 6689310, **GQ983694,** 1293039, **GQ983800, GQ983904, GQ984035, GQ984042,**–,–,–; *Tetrapanax papyriferus* (Hook.) K. Koch, *Plunkett 1344* (WS), **GQ983624,** 2281284, **GQ983670, GQ983772,** 1293031, **GQ983870, GQ983889, GQ983988,** 41087253, **GQ984118,**–,–; *Tetraplasandra hawaiensis* A. Gray, N/A,–, 2281224,–,–, 1293029,–,–,–,–,–,–,–; *Tetraplasandra oahuensis* Harms, Cult. Honolulu Botanical Garden; Plunkett 92-0220 (WS), **GQ983616,**–, **GQ983667, GQ983773,**–, **GQ983869, GQ983896, GQ983996,** 22212663, **GQ984077,**–,–; ***Torricellia tiliifolia* DC.,** *Soltis 3784* (WS),–, 22797813, 22797773, **GQ983700,** 10945636, **GQ983856, GQ983936,** 22797861, 22797851, 22797833,–, 37778905

AQUIFOLIALES: ***Cardiopteris moluccana* Blume,** *Regalado & Katik 1181* (GH),–,–,–,–,–,–, **GQ983971,**–,–,–,–,–; ***Cardiopteris quinqueloba* Hassk.,** N/A,–, 22795834, 17932816,–, 9909610,–,–, 22796002, 22795967, 22795908,–,–; ***Cardiopteris sp.* Wall. ex Royle,** *van der Werff et al. 17270* (MO), **GQ983618,**–,–,–,–,–,–,–,–,–, **GQ983562, GQ983581**; *Citronella moorei* (Benth.) R. A. Howard, N/A,–,–, 17932828,–,–,–,–,–,–,–,–,–; *Citronella suaveolens* (Blume) R. A. Howard, *Chase 1327* (K), **GQ983606, GQ983648,**–, **GQ983733,**–, **GQ983852, GQ983882, GQ984030, GQ984050, GQ984125,**–,–; *Gomphandra javanica* Valeton., *Chase 1293* (K), **GQ983598, GQ983651, GQ983663, GQ983741,** 9909646, **GQ983876, GQ983924,**–, **GQ984063, GQ984095,**–,–; *Gonocaryum litorale* (Blume) Sleumer, *Chase 1294* (K), 8439291, **GQ983654,** 11558467, **GQ983783,** 7413863, **GQ983811, GQ983949,**–, **GQ984057, GQ984078,**–,–; *Grisollea myrianthea* Baill., *McPherson & Ratiana 722* (MO),–, **–,** 17932802, **GQ983699,**–, **–, GQ983935, GQ984031,–, GQ984115,**–,–; ***Helwingia japonica* (Thunb.) F. Dietr.,** N/A, 14718069, 22796639, 6424742,–, 7240257,–,–, 22796677, 22796657, 22796628, 1777668,–; ***Helwingia sp.* Willd.,** *Peng et al. 17408* (S),–,–,–, **GQ983706,**–, **GQ983808, GQ983964,**–,–,–,–, **GQ983586**; ***Ilex crenata* Thunb.,** *Chase 119* (NCU), 8452684,–,–, **GQ983747,**–, **GQ983841, GQ983972,**–,–,–,–,–; ***Ilex mitis* Radlk.,** N/A,–,–,–,–, 3445319,–,–,–,–,–,–,–,–; ***Ilex opaca* [Soland.],** N/A,–,–,–,–,–,–,–,–,–,–, 7595458, 19919644; ***Ilex sp.* L.,** N/A,–, 22796586, 22796548,–,–,–,–, 22796676, 22796656, 22796626,–,–; ***Irvingbaileya australis* (C. T. White) R. A. Howard,** *White 1232* (S),–,–,–, **GQ983782,**–, **GQ983822, GQ983880, GQ984022,**–, **GQ984084,**–,–; ***Irvingbaileya sp.* R. A. Howard,** N/A, 6688493,–, 6688425,–, 6651256,–,–,–,–,–, 6688423, 19919643; ***Phyllonoma laticuspis* Engl.,** *Salazar et al. s. n.* (MEXU), 6689005,–, 6424743, **GQ983759,** 7240423,–, **GQ983895,**–,–,–, 1777687,–; ***Phyllonoma ruscifolia* Willd. ex Schult.,** *Maldonado et al. 2955* (MO),–, 22797484,–,–,–, **GQ983807,**–, 22797628, 22797596, 22797548,–,–

ASTERALES: *Abrophyllum ornans* (F. Muell.) Benth., *Forster 29801* (GH), 15422201, **GQ983653,** 7413609, **GQ983739,** 1304273, **GQ983859, GQ983928,**–, **GQ984068, GQ984083,**–,–; *Acicarpha tribuloides* Juss., *Gustafsson 207* (UPS),–, 22795872, 22795805, **GQ983757,** 1304279, **GQ983839, GQ983962,** 22796021, 22795985, 22795942,–,–; ***Alseuosmia macrophylla* A. Cunn.,** *de Lange 5178* (AK), 6686770, 22795868, 3764064, **GQ983684,** 1304271,–, **GQ983894,** 22796019, 22795983, 22795938, 7595364,–; ***Alseuosmia sp.* A. Cunn.,** no voucher (Soltis lab DNA collection, University of Florida),–,–,–,–,–, **GQ983871,**–,–,–,–,–, **GQ983579**; ***Argophyllum laxum* Schltr.,** *Pillon et al. 144* (NOU),–,–,–, **GQ983758,**–, **GQ983844, GQ983958,**–,–,–,–, **GQ983580**; ***Argophyllum sp.* J. R. Forst. & G. Forst.,** N/A, 15422207, 22795870, 7413613,–, 1304277,–,–, 22796020, 22795984, 22795940,–,–; *Barnadesia arborea* Kunth, *Chase 2470* (K),–, **GQ983656,**–, **GQ983717,**–, **GQ983828, GQ983937, GQ984016,**–, **GQ984132,**–,–; *Barnadesia caryophylla* S. F. Blake, N/A, 14717953,–, 845245,–, 289491,–,–,–, 46102431,–,–,–; ***Boopis anthemoides* Juss.,** N/A,–,–, 845241,–, 289455,–,–,–,–,–,–,–; ***Boopis graminea* Phil.,** N/A, 6686913, 22795874,–,–,–,–,–, 22796022, 22795986, 22795944, 4558894, 19919625; ***Campanula elatines* L.,** N/A,–, 22795953,–,–,–,–,–,–, 22795987, 22795955,–,–; ***Campanula ramosa,*** N/A,–,–, 7240507,–, 289893,–,–,–,–,–,–,–; ***Campanula ramulosa* Wall.,** N/A,–,–,–,–,–,–,–,–,–,–, 1777643,–; ***Campanula trachelium* L.,** *Chase 2546* (K),–,–,–, **GQ983777,**–, **GQ983821, GQ983978,**–,–,–,–,–; ***Carpodetus serratus* J. R. Forst. & G. Forst.,** N/A, 15425561, 22795876, 7413838,–, 2385356,–,–,–, 22795988, 22795892,–,–; ***Carpodetus sp. J. R. Forst. & G. Forst.,*** *Johns 9563* (K),–,–,–, **GQ983728,**–, **GQ983837, GQ983932,**–,–,–, **GQ983563,**–; *Cichorium intybus* L., *Chase 2511* (K), 8452630, 54021386, 845275, **GQ983707,** 289848, **GQ983835, GQ983879, GQ983984,** 46102439, **GQ984112,**–,–; *Corokia cotoneaster* Raoul, N/A, 8452633, 45758353, 6424717,–, 7240147,–,–,–,–,–,–,–; *Corokia macrocarpa* Kirk, *Chase 2472* (K),–,–,–, **GQ983762,**–, **GQ983813, GQ983927, GQ984005, GQ984067, GQ984130,**–,–; *Crispiloba disperma* (S. Moore) Steenis, *Gray 03964* (GH), 15425563, **GQ983650,** 7413666, **GQ983754,** 1304285, **GQ983810, GQ983931, GQ984012, GQ984064, GQ984111,**–,–; *Cuttsia viburnea* F. Muell., *Lepschi & Mowatt 1496* (GH), 15425565, **GQ983640,** 7572951, **GQ983764,** 2385361, **GQ983803, GQ983957,**–, **GQ984071, GQ984094,**–,–; *Cyphia decora* Thulin, no voucher (Bremer lab DNA collection, Uppsala University),–, **GQ983652,**–, **GQ983730,**–, **GQ983857, GQ983910,**–, **GQ984060, GQ984137,**–,–; *Cyphia rogersii* S. Moore, N/A, 15425567,–, 7413840,–, 18075942,–,–,–,–,–,–,–; *Dampiera diversifolia* de Vriese, *Chase 2979* (K),–, **GQ983658,** 845321, **GQ983725,**–, **GQ983867,**–,–,–, **GQ984080,**–,–; *Dampiera spicigera* Benth., N/A, 15425569,–,–,–, 1304295,–,–,–, 16566361,–,–,–; *Dialypetalum sp.* Benth., *Koopman et al. s. n.* (MO), 15425571, **GQ983649,** 15425603, **GQ983691,** 15425607, **GQ983842, GQ983955,**–, **GQ984048, GQ984100,**–,–; ***Donatia fascicularis* J. R. Forst. & G .Forst.,** no voucher (Soltis lab DNA collection, University of Florida),–, 22795878, 4538559,–, 1304291,–, **GQ983902,** 22796023, 22795989,–,–,–; ***Donatia sp.* J. R. Forst. & G. Forst.,** N/A, 6687446,–,–,–,–,–,–,–,–,–,–, 6687445, 19919630; *Echinops bannaticus* Rochel ex Schrad., *Chase 423* (K), 15425573,–,–, **GQ983687,**–, **GQ983831, GQ983975, GQ984003,**–, **GQ984113,**–,–; *Echinops exaltatus* Schrad., N/A,–, 32364845, 845339,–, 290607,–,–,–,–,–,–,–,–; *Echinops humilis* M. Bieb., N/A,–,–,–,–,–,–,–,–, 4154117,–,–,–; *Fauria crista-galli* Makino, no voucher (Bremer lab DNA collection, University of Florida), 15425577, **GQ983644,** 7413842, **GQ983752,** 1304327, **GQ983853, GQ983909, GQ983986, GQ984049, GQ984096,**–,–; *Forstera bellidifolia* Hook., N/A, 15425579,–, 4538579,–, 4538585,–,–,–,–,–,–,–; *Forstera bidwillii* Hook. f., no voucher (Bremer lab DNA collection, Uppsala University),–, **GQ983645,**–, **GQ983744,**–, **GQ983816, GQ983974, GQ983982, GQ984051, GQ984104,**–,–; *Gerbera jamesonii* Adlam, N/A, 6706177, 32364851, 845360,–, 290869,–,–,–, 4154126,–,–,–; *Goodenia mimuloides* S. Moore, no voucher (Bremer lab DNA collection, Uppsala University),–,–,–, **GQ983750,**–,–,–,–,–, **GQ984075,**–,–; *Goodenia ovata* Sm., *Clarke 2645* (GH), 15425581, **GQ983643,** 15425605,–, 1304309,–, **GQ983891,**–,–,–,–,–; *Goodenia scapigera* R. Br., N/A,–,–,–,–,–,–,–,–, 16566367,–,–,–; *Guizotia abyssinica* (L. f.) Cass., N/A, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719, 183217719,–,–; ***Helianthus annuus* L.,** N/A, 6687661, 22796588, 845417, 94502469, 4249678, 94502469, 22796678, 94502469, 22796658, 22796630, 4558888, 19919624; *Lactuca sativa* L., N/A, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147, 78675147,–,–; ***Lobelia angulata* G. Forst.,** *Chase 2540* (K), 8517626,–,–, **GQ983738,**–,–, **GQ983950,**–, **GQ984053, GQ984129,**–,–; ***Lobelia cardinalis* L.,** N/A,–,–, 6424722,–, 6649958,–,–,–,–,–,–,–; ***Lobelia erinus* L.,** N/A,–,–,–,–,–,–,–,–,–,–, 1777694, 6941722; ***Lobelia puberula* Michx.,** N/A,–,–,–,–,–,–,–,–,–,–,–; ***Lobelia sp.* L.,** *Chase 2172* (K),–,–,–,–,–,–,–, **GQ983992,**–,–,–,–; ***Menyanthes trifoliata* L.,** *Fay 345* (K), 8452697, 22797128, 845605, **GQ983705,** 12651656, **GQ983840, GQ983925,** 22797185, 22797170, 22797150, 6688827, **GQ983588**; *Moschopsis rosulata* (N. E. Brown) Dusén, *Pisano V. 5579* (GH), 15425585, **GQ983662,** 15425601, **GQ983720,** 1304325, **GQ983863, GQ983903, GQ984009, GQ984056, GQ984073,**–,–; *Nymphoides geminata* (R. Br.) Kuntze, N/A, 6688862,–,–,–,–,–,–,–,–,–,–,–; *Nymphoides indica* (L.) Kuntze, N/A,–,–, 6424716,–,–,–,–,–,–,–,–,–; *Nymphoides peltata* (S. G. Gmel.) Kuntze, *Fay 364* (K),–, **GQ983659,**–, **GQ983771,** 1304329, **GQ983862, GQ983970, GQ983980, GQ984069, GQ984138,**–,–; ***Pentaphragma ellipticum* Poulsen,** N/A, 15425587, 22797130, 6424718,–, 405537,–,–, 22797186, 22797171, 22797152,–,–; ***Pentaphragma sp.* Wall. ex G. Don,** *Duangjai 49* (BRUN),–,–,–, **GQ983761,**–, **GQ983812, GQ983969,**–,–,–, **GQ983574,**–; ***Phelline billardieri* Pancher ex Loes.,** N/A,–,–,–,–,–,–,–,–,–, 7595509,–; ***Phelline comosa* Labill.,** *Ziesing 289* (CBG), 8517652,–, 7414408, **GQ983714,**–,–, **GQ983941,**–,–,–,–, 19919629; ***Phelline lucida* Vieill. ex Baill.,** N/A,–, 22797488,–,–, 7414414,–,–, 22797630, 22797598, 22797552,–,–; *Platyspermation crassifolium* Guillaumin, *McPherson 4733* (MO), 18077608,–, 21953245, **GQ983723,** 18077612,–,–, **GQ984011, GQ984065,**–,–,–; *Pseudonemacladus oppositifolius* (B. L. Rob.) McVaugh, *Moore s. n.* (HUH), 15425591, **GQ983646,** 9864436, **GQ983701,** 15425609, **GQ983797, GQ983914,**–, **GQ984066, GQ984105,**–,–; ***Roussea simplex* Sm.,** no voucher (Soltis lab DNA collection, University of Florida), 8452741, 22797490, 7573262, **GQ983686,** 4530134, **GQ983806, GQ983960,** 22797631, 22797599, 22797554, 1777689, 19919684; ***Scaevola aemula* R. Br.,** N/A, 14718211,–,–,–,–, 156598709,–,–,–,–, 6689107,–; ***Scaevola auriculata* Benth.,** *Chase 2982* (K),–,–,–, **GQ983734,**–,–, **GQ983934,**–,–,–,–, **GQ983591**; ***Scaevola frutescens* K. Krause,** N/A,–,–, 847669,–, 295253,–,–,–,–,–,–,–; ***Scaevola sp.* L.,** N/A,–, 22797486,–,–,–,–, 22797629, 22797597, 22797550,–,–; ***Stylidium bulbiferum* Benth.,** N/A,–, 22797492, 4538632,–, 4538634,–,–, 22797632, 22797600, 22797556,–,–; ***Stylidium graminifolium* Sw.,** *Smith 89/41* (GH), 6689232,–,–, **GQ983693,**–,–, **GQ983973,**–,–,–, 6689228, **GQ983592**; *Tagetes erecta* L., N/A,–,–, 847678,–, 295291,–,–,–, 45169924,–,–,–; *Tagetes patula* L., N/A,–, 21629875,–,–,–,–,–,–,–,–,–,–; *Tagetes sp.* L., N/A, 6689409,–,–,–,–,–,–,–,–,–,–,–; *Trachelium caeruleum* L., N/A, 170784721, 170784721, 170784721, 170784721, 170784721, 170784721, 170784721,–, 170784721, 170784721,–,–; *Tragopogon*

dubius Scop., *Soltis 2472-2* (WS), 6689366, 54021625,–, **GQ983690,**–, **GQ983868, GQ983938,**–, 125632303,–,–,–; *Tragopogon porrifolius* L., *Fay 625* (K),–,–, 847675,–, 295289,–,–, **GQ984037,**–, **GQ984091,**–,–; *Villarsia calthifolia* F. Muell., N/A,–,–, 6424715,–, 343653,–,–, **–,**–, **–,**–,–; *Villarsia capitata* Nees, N/A, 15425595,–,–,–,–,–,–, **–,**–, **–,**–,–; *Villarsia sp.* J. F. Gmel., *Fay s. n.* (K),–, **GQ983655,**–, **GQ983781,**–, **GQ983873, GQ983906, GQ984032, GQ984045, GQ984133,**–,–; *Wittsteinia panderi, McKee 4852* (K),–, **GQ983647,**–,–,–, **GQ983846, GQ983892,**–, **GQ984054, GQ984074,**–,–; *Wittsteinia vacciniacea* F. Muell., N/A, 15425599,–, 7414843,–, 1304372,–,–,–,–,–,–,–

BRUNIALES: ***Berzelia lanuginosa* (L.) Brongn.,** *Price s. n.* (WTU), 6686964, 45548852, 6686966, **GQ983769,** 348066, **GQ983824, GQ983939, GQ984001, GQ984070,**–, 1777638,–; ***Brunia albiflora* Phillips,** N/A,–, 22795856, 3764068,–, 1922258,–,–, 22796012, 22795977, 22795924,–,–; ***Brunia laevis* Thunb.,** *Chase 5810* (K), **GQ983608,**–,–, **GQ983740,**–, **GQ983861, GQ983912,**–,–,–,–,–; ***Columellia oblonga* Ruiz & Pav.,** *van der Werff et al. 14912* (MO), 18075914, 22795858, 3764070, **GQ983784,** 1922260,–, **GQ983940,** 22796013, 22795978, 22795926, **GQ983564,**–; ***Desfontainia spinosa* Ruiz & Pav.,** *Maldonado 3238* (MO), 18075916, 22795860, 3808131, **GQ983765,** 496561, **GQ983860, GQ983890,** 22796014, 22795979, 22795928, **GQ983565, GQ983582**

DIPSACALES: *Abelia x grandiflora* (Rovelli ex André) Rehder, no voucher (Donoghue lab DNA collection, Yale University),–, 20530892, 20805313, **GQ983776,** 17863842, **GQ983814, GQ983898, GQ984029,** 17980593, **GQ984109,**–,–; *Abelia triflora* R. Br., N/A, 6686863,–,–,–,–,–,–,–,–,–,–,–; *Acanthocalyx alba* (Hand.-Mazz.) M. J. Cannon, *Boufford 28401* (GH),–, 20530896, 20805321, **GQ983697,** 17863850, **GQ983819, GQ983952, GQ984026,** 17980597, **GQ984119,**–,–; ***Adoxa moschatellina* L.,** *Boufford et al.* 28906 (A), **GQ983610,** 20530883, 20805296, **GQ983718,** 7239765, **GQ983872, GQ983901, GQ983993,** 28172965, **GQ984103, GQ983560, GQ983578**; *Centranthus ruber* DC., Royal Botanic Garden Edinburgh 19880314, **GQ983626,** 20530909, 20805347, **GQ983743,** 18873584,–, **GQ983965, GQ983985,** 17980610, **GQ984106,**–,–; *Cryptothladia chinensis* (Pai) M. J. Cannon, *Boufford 27846* (GH),–, 20530897, 20805323,–, 17863852,–, **GQ983926, GQ984019,** 17980598,–,–,–; ***Diervilla rivularis* Gatt.,** N/A,–, 22795880,–,–,–,–,–, 22796024, 22795990, 22795946,–,–; ***Diervilla sessilifolia* Buckley,** Cult. Arnold Arboretum; *Donoghue & Winkworth 29* (YU, A), **GQ983617,**–, 12232566, **GQ983692,** 496563, **GQ983799, GQ983968,**–,–,–, **GQ983566, GQ983583**; ***Dipelta yunnanensis* Franch.,** no voucher (Donoghue lab DNA collection, Yale University), **GQ983629,** 20530893, 20805315, **GQ983768,** 17863844, **GQ983832, GQ983885, GQ984013,** 17980594, **GQ984136, GQ983567, GQ983584**; ***Dipsacus laciniatus* L.,** Cult. Royal Botanic Garden Edinburgh 19822247,–,–,–, **GQ983732,**–, **GQ983854, GQ983888,**–,–,–,–,–; ***Dipsacus sativus* [Honck.],** N/A, 14718031, 22795882, 6424725,–, 290071,–,–, 22796025, 22795991, 22795948,–, 19919672; ***Dipsacus sp.* L.,** N/A,–,–,–,–,–,–,–,–,–,–, 1777740,–; *Fedia cornucopiae* (L.) Gaertn., Cult. Royal Botanic Garden Edinburgh 20011423-32,–, 20530906, 20805341, **GQ983753,** 17863870, **GQ983825,**–, **GQ983983,** 17980607,–,–,–; *Heptacodium miconioides* Rehder, Cult. Arnold Arboretum 1549-80A; *Donoghue & Winkworth 7* (YU, A), **GQ983604,** 20530889, 28172969, **GQ983724,** 18873574, **GQ983878, GQ983917, GQ984004,** 28172947, **GQ984127,**–,–; *Kolkwitzia amabilis* Graebn., Cult. Arnold Arboretum 20447-B; *Elsiket al. 844* (A), **GQ983600,** 20530895, 20805319, **GQ983746,** 17863848, **GQ983865, GQ983959, GQ983989,** 17980596, **GQ984116,**–,–; *Leycesteria formosa* Wall., *Boufford 29867* (GH), **GQ983636,** 20530885, 20805300, **GQ983767,** 18873572, **GQ983827, GQ983881, GQ984000,** 17980586, **GQ984114,**–,–; *Linnaea borealis* L., no voucher (Donoghue lab DNA collection, Yale University), **GQ983619,** 22796590, 8954304, **GQ983766,** 22796544, **GQ983804, GQ983907,** 22796680, 22796660, 22796634,–,–; ***Lonicera japonica* Thunb.,** *Smith s. n.* (YU), **GQ983602,**–, 6424729, **GQ983785,**–, **GQ983875, GQ983915,**–,–,–,–, **GQ983587**; ***Lonicera maackii* (Rupr.) Herder,** N/A,–,–,–,–,–,–,–,–,–,–, 1857127,–; ***Lonicera orientalis* M. Bieb.,** N/A,–, 22796641,–,–, 1304321,–,–, 22796679, 22796659, 22796632,–,–; ***Morina coulteriana* Royle,** N/A,–,–,–,–, 2065206,–,–,–,–,–,–,–; ***Morina longifolia* Wall. ex DC.,** Cult. Bergius Bot. Gard., Sweden; *Eriksson s. n., 2 Nov. 1999* (SBT), **GQ983601,** 20530898, 22796550, **GQ983745,**–, **GQ983798, GQ983953,** 22796681, 22796661, 22796636, **GQ983569,**–; *Nardostachys jatamansi* DC., *Boufford 29364* (GH), **GQ983614,** 20530903, 20805335, **GQ983749,** 17863864,–,–, **GQ984007,** 17980604,–,–,–; ***Patrinia triloba* Miq.,** Cult. Royal Botanic Garden Edinburgh 19980546, **GQ983625,** 20530904, 12232582, **GQ983708,** 17863866, **GQ983845, GQ983921, GQ984014,** 17980605, **GQ984108, GQ983572,**–; *Pterocephalodes hookeri* (C. B. Clarke) V. Mayer & Ehrend., *Boufford 27845* (GH), **GQ983623,** 20530899, 20805327, **GQ983712,** 17863856, **GQ983802, GQ983923, GQ984025,** 17980600, **GQ984098,**–,–; *Sambucus caerulea* Raf., no voucher (Donoghue lab DNA collection, Yale University), **GQ983634,**–,–, **GQ983704,**–, **GQ983796, GQ983967, GQ984038,**–, **GQ984092,**–,–; *Sambucus racemosa* L., N/A,–, 20530881, 20805292,–, 17863820,–,–,–,–,–,–,–; *Sambucus williamsii* Hance, N/A,–,–,–,–,–,–,–,–, 28172967,–,–,–; *Scabiosa columbaria* L., Cult. Royal Botanic Garden Edinburgh 10001354, **GQ983595,** 20530901, 20805331, **GQ983763,** 17863860,–,–, **GQ984021,** 17980602, **GQ984081,**–,–; *Sinadoxa corydalifolia* C. Y. Wu, Z. L. Wu & R. F. Huang, *Boufford et al. 26555* (A), **GQ983638,** 20530882, 20805294, **GQ983779,** 18873560, **GQ983877,**–,–, 28172964,–,–,–; *Symphoricarpos orbiculatus* Moench, N/A,–, 20530887, 20805303,–, 17863832,–,–,–, 28172945,–,–,–; *Symphoricarpos sp.* Duhamel, Cult. Arnold Arboretum; *Donoghue & Winkworth 28* (YU, A), **GQ983633,**–,–, **GQ983709,**–, **GQ983787, GQ983961, GQ984017,**–,–,–,–; *Tetradoxa omeiensis* (Hara) C. Y. Wu, *Donoghue et al.* 4000 (A), **GQ983607,** 20530884, 20805298, **GQ983711,** 17863826, **GQ983847, GQ983947, GQ984033,** 17980585, **GQ984131,**–,–; *Triosteum perfoliatum* L., no voucher (Donoghue lab DNA collection, Yale University), **GQ983597,** 20530888, 20805305, **GQ983682,** 18873570, **GQ983793, GQ983942, GQ983994,** 17980589, **GQ984101,**–,–; ***Triplostegia glandulifera* Wall. ex DC.,** *Boufford 30108* (GH), **GQ983612,** 20530902, 20805333, **GQ983698,** 17863862, **GQ983833, GQ983920, GQ984027,** 28172959, **GQ984079, GQ983577, GQ983593**; ***Valeriana hirtella* Kunth,** N/A,–,–,–,–,–,–,–, 22797863,–, 22797837,–,–; ***Valeriana officinalis* L.,** Cult. Royal Botanic Garden Edinburgh 19721065, 8452795, 38231958, 40068099, **GQ983774,** 40068129, **GQ983864, GQ983977,**–, 27529350,–, 6689554, 19919640; ***Valerianella locusta* (L.) Laterr.,** *Patterson 2001* (SFSU), **GQ983622,** 20530905, 20805343, **GQ983713,** 17863872,–, **GQ983911,**–, 17980608, **GQ984123,**–,–; ***Viburnum acerifolium* L.,** N/A,–,–,–,–,–,–,–,–,–,–, 6689555,–; ***Viburnum opulus* L.,** no voucher (Donoghue lab DNA collection, Yale University), 8452798,–,–, **GQ983679,**–, **GQ983843, GQ983930,**–,–,–,–, **GQ983594**; ***Viburnum rhytidophyllum* Hemsl. ex Forb. & Hemsl.,** N/A,–, 22797815, 2605738,–, 1304368,–,–, 22797862, 22797852, 22797835,–,–; *Weigela hortensis* C. A. Mey., Cult. Arnold Arboretum 1897-77-A; *Kelly and Buckland 28* (A), **GQ983609,** 20530891, 20805311, **GQ983755,** 18873576, **GQ983855, GQ983918, GQ984018,** 17980592, **GQ984082,**–,–; *Zabelia corymbosa* (Regel & Schmalh.) Makino, no voucher (Donoghue lab DNA collection, Yale University),–,–,–, **GQ983760,**–,–,–,–,–,–,–,–; *Zabelia taihyoni* (Nakai) Hisauti & Hara, no voucher (Donoghue lab DNA collection, Yale University), **GQ983627, GQ983641, GQ983675,**–, **GQ983786, GQ983788,**–,–, **GQ984046, GQ984135,**–,–

ESCALLONIACEAE: *Anopterus macleayanus* F. Muell., *Forster 26573* (GH), 15422205, **GQ983639,** 9856890, **GQ983722,** 1922256, **GQ983874, GQ983946, GQ984023, GQ984061,**–,–,–; ***Eremosyne pectinata* Endl.,** *Soltis s. n.* (WS), 6687549, 22795862, 22795799, **GQ983716,** 1015304, **GQ983791, GQ983945,** 22796015, 22795980, 22795930, 1777710,–; ***Escallonia coquimbensis* J. Rémy,** no voucher (Soltis lab DNA collection, University of Florida),–,–,–, **GQ983719,**–, **GQ983838, GQ983943,**–,–,–, 1777678, **GQ983585**; ***Escallonia rubra* Pers.,** N/A, 15425575, 22795864, 7572953,–, 18075930,–,–, 22796016, 22795981, 22795932,–,–; *Forgesia racemosa* J. F. Gmel., *J. F. 425* (MO), 18075918, **GQ983661,** 18075944, **GQ983780,** 39725408, **GQ983789, GQ983913, GQ984036, GQ984041, GQ984076,**–,–; ***Polyosma sp.* Blume**, *McPherson 18643* (MO),–,–,–, **GQ983695,**–, **GQ983815, GQ983899,**–,–,–, **GQ983575,**–; ***Polyosma cunninghamii* Benn.,** N/A,–, 22797811, 22797771,–, 9910001,–,–, 22797860, 22797850, 22797831,–,–; ***Tribeles australis* Phil.,** N/A, 18077602, 22797124, 22797094,–, 9909898,–,–, 22797183, 22797168, 22797148,–,–; *Valdivia gayana* J. Rémy, *Zapata 99* (MO), 18077606, **GQ983642,** 18077616, **GQ983751,** 39725410,–, **GQ983979, GQ984039, GQ984052, GQ984085,**–,–

PARACRYPHIACEAE: ***Paracryphia alticola* (Schltr.) Steenis,** *Pintaud 561* (K), 18077600, 22797122, 22797092, **GQ983689,** 9909880, **GQ983794, GQ983900,** 22797182, 22797158, 22797146, **GQ983571, GQ983589; *Quintinia quatrefagesii* F. Muell.,** *Lundberg 31* (UPS),–,–,–, **GQ983727,**–, **GQ983805, GQ983922,**–,–,–, **GQ983576, GQ983590; *Quintinia verdonii* F. Muell.,** N/A, 18077604, 22797480, 7414459,–, 1304335,–,–, 22797626, 22797594, 22797544,–,–; *Sphenostemon lobosporus* (F. Muell.) L. S. Sm., *Jensen 280* (QRS), **GQ983631, GQ983660, GQ983668,**–, 9909967, **GQ983826, GQ983919, GQ983981, GQ984044, GQ984086,**–,–.

Appendix 2. Amplification and sequencing primers (5′ to 3′) for the ten chloroplast and two nuclear ribosomal DNA regions used in this study. References indicate the original source of primer sequences; for the *rpoC2* and *rps4* sequences, numbers in brackets indicate the 5′ most base for that gene region in the *Panax ginseng* complete chloroplast genome sequence (GI: 52220789).

***ndhF* gene - Forward:** ndhF_5′-PCR: ATGGAACAGACATATCAATA TGSGTGG (Olmstead and Reeves 1995), ndhF_274: CTTACTTCTATTAT GTCAATATTAAT, ndhF_536: TTGTAACTAATCGTGTAGGGGA, ndhF_ 972: GTCTCAATTGGGTTATATGATG, ndhF_1318: GGATTAACYGCA TTTTATATGTTTCG, ndhF_1603: CCTYATGAATCGGACAATACTATGC, **Reverse:** ndhF_3′-PCR: CCYASATATTTGATACCTTCKCC (Olmstead

and Reeves 1995), ndhF_274R: ATTAATATTGACATAATAGAAGTAAG, ndhF_536R: TCCCCTACACGATTAGTTACAA, ndhF_972R: CATCATAT AACCCAATTGAGAC, ndhF_1318R: CGAAACATATAAAATGCRGTT AATCC, ndhF_1603R: GCATAGTATTGTCCGATTCATRAGG (except where otherwise noted, from Olmstead and Sweere 1994).

***atpB* gene - Forward:** S1494R: TCAGTACACAAAGATTTAAGGTCAT, S20: CTTCTGATCCTGGGGTTTCCACACT, S335: ACGTGCTTGGGG AGCCTGTTGATAA, S611: AACGTACTCGTGAAGGAAATGATCT, S1022; CGACATTTGCACATTTAGATGCTAC, S1277: AAATTGAGCG TTTCTTATCACAACC, **Reverse:** S2: TATGAGAATCAATCCTACTACT TCT, S2R: AGAAGTAGTAGGATTGATTCTCATA, S352R: TATCTACA GGACCTAAATTATCAAC, S385R: GCGCAGATCTATGAATAGGAGAC GT, S766R: TAACATCTCGGAAATATTCCGCCAT, S1186R: TGTCCTGA AGTTCTTTGTAACGTTG (all from Hoot et al. 1995).

***matK* gene - Forward:** matK_1F: ACTGTATCGCACTATGTATCA (Sang et al. 1997), matK_2F: GTTCACTAATTGTGAAACGT (Sang et al. 1997), matK_3F: AAGATGCCTCTTCTTTGCAT (Sang et al. 1997), matK_4F: CTT CGCTAYTGGGTAAAAGATGC (Bremer et al. 2002), **Reverse:** matK_4R: GC ATCTTTTACCCARTAGCGAAG (Bremer et al. 2002), matK_6R: TTCTAG MATTTGACTCCGTACC (Bremer et al. 2002), matK_3R: GATCCGCTGT GATAATGAGA (Sang et al. 1997).

***rpoC2* gene - Forward:** rpoC2_1F: ATGGAGGTACTTATGGCAGAACG [1], rpoC2_1.1F: AACGGGCCAATCTGGTCTTTC [20], rpoC2_2F: ATTC AAAGCAATTTACGCGAAGG [505], rpoC2_3F: TACTGCAGAACATGT GCGAGC [1053], rpoC2_4F: GCTTATTTCGATGATCCTCGATACAG [1836], rpoC2_5F: GTCACATATGAAATAACGGACGG [2337], rpoC2_6F: TAACTAACTATTTGCAACTTGACAATT [3015], rpoC2_7F: CAGGTGT TAGAAGTGCGTTCG [3447], **Reverse:** rpoC2_1R: TGTGGTGGAACAA AATATCTCTC [4083], rpoC2_1.1R: TGGTGGAACAAAATATCTCTCAT [4081], rpoC2_7R: CGAACGCACTTCTAACACCTG [3467], rpoC2_6R: AATTGTCAAGTTGCAAATAGTTAGTTAC [3042], rpoC2_5R: CCGTCC GTTATTTCATATGTGAC [2359], rpoC2_4R: CTGTATCGAGGATCAT CGAAATAAGC [1861], rpoC2_3R: GCTCGCACATGTTCTGCAGTA [1073], rpoC2_2R: CCTTCGCGTAAATTGCTTTGAAT [527]. ***rps4* gene - Forward:** rps4_1F: GTTACCGAGGACCTCGTTTC [8], **Reverse:** rps4_1R: GCAATTCATTTATTTTCAAACCGACC [573].

***psbBTNH* gene - Forward:** psbBTNH_60F: ATGGGTTTGCCTTGGTAT CGTGTTCATAC, psbBTNH_B60F: CATACAGCTCTAGTTKCTGGTTGG, psbBTNH_B61F: CGGGTMTTTGGAGTTAYGARGG, psbBTNH_63F: GGATTRCGTATGGGMAATATTGAAAC, psbBTNH_65F: TGCCTACT TTTTTTGAAACATTTCC, psbBTNH_67F: GAGATGTTTTTGCTGGTA TTGA, psbBTNH_69F: TCGCTATCTTYTTYCGAGAACCRC, **Reverse:** psbBTNH_61R: TCCCAATAYACCCAATGCCAGATAG, psbBTNH_B64R: CTTGCTGRAAGTATCCYTGATCCC, psbBTNH_B66R: CCCCTTGGAC TRCTACGAAAAACACC, psbBTNH_66R: CCAAAAGTRAACCAACC CCTTGGAC, psbBTNH_B68R: GTAGTTGGATCTCCAAGTTTTTGG, psbBTNH_68R: AAYGTATAAACCAATGCTTCCAT, psbBTNH_70R: TA TCTGGTTTACTTGTAAGYTTTACTGG, psbBTNH_B71R: CCAGGAGC TACTTTACCATATTC, psbBTNH_71R: CCCATMAAAGGAGTAGTYCC CC (all from Graham and Olmstead 2000).

***rps16* intron - Forward:** rpsF: GTGGTAGAAAGCAACGTGCGACTT, **Reverse:** rpsR2: TCGGGATCGAACATCAATTGCAAC (all from Oxelman et al. 1997).

***trnL/trnF* region - Forward:** trnL/F_C: CGAAATCGGTAGACGCTACG, trnL/F_e: GGTTCAAGTCCCTCTATCCC, **Reverse:** trnL/F_f: ATTTGA ACTGGTGACACGAG, trnL/F_d: GGGGATAGAGGGACTTGAAC (all from Taberlet et al. 1991).

***atpE/trnV* IGS - Forward:** atpe/trnV_1F: TAGGGCTATACGGAG TCGAACCG, atpE/trnV_3F: GTGTAAACGAGTTGCTCTACC, atpE/ trnV_5F: CATACGGCGGGAGTCATTGGTTC, **Reverse:** atpE/trnV_2R: GGTAGAGCAACTCGTTTACAC, atpE/trnV_4R: GAACCAATGACTC CCGCCGTATG, atpE/trnV_6R: GGAGAGCAATTTGAAGAAATG (all from Bremer et al. 2002).

18S nrDNA gene - Forward: N-NS1: GTAGTCATATGCTTGTCTC, N-18E: CTGCCCTATCAACTTTCGATGGTA, N-18G: AGGGCAAGT CTGGTGCCA, N-18H: GCTGAAACTTAAAGGAATTGACGGAAGGGC, N-18J: CAATAACAGGTCTGTGATGCCCTTAGA, **Reverse:** C-18L: GA AACCTTGTTACGACTT, C-18E: TACCATCGAAAGTTGATAGGGCAG, C-18G: TGGCACCAGACTTGCGCT, C-18H: GCCCTTCCGTCAATTCC TTTAAGTTTCAGC, C-18J: TCTAAGGGCATCACAGACCTGTTATTG (all from Bult et al. 1992).

26S nrDNA gene - Forward: N-nc26S1: CGACCCCAGGTCAGGCG, N-nc26S3: AGGGAAGCGGATGGGGGC, N-nc26S5: CGTGCAAATC GTTCGTCT, N-nc26S7: GATGAGTAGGAGGGCGCG, N-nc26S9: AAT GTAGGCAAGGGAAGT, N-ncs6S11: AATCAGCGGGGAAAGAAG, N-nc26S13: CCTATCATTGTGAAGCAG, N-nc26S14: TTATGACTGAAC GCCTCT, **Reverse:** 268rev: GCATTCCCAAACAACCCGAC, 641rev: TT GGTCCGTGTTTCAAGACG, 950rev: GCTATCCTGAGGGAAACTTC, 1499rev: ACCCATGTGCAAGTGCCGTT, 1839rev: TTCACCTTGGAGAC CTGATG, 2426rev: MCTACACCTCTCAAGTCAT, 2782rev: GGTAACT TTTCTGACACCTC, 3331rev: ATCTCAGTGGATCGTGGCAG (all from Kuzoff et al. 1998).