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Nuclear and cpDNA sequences combined provide strong inference of higher phylogenetic relationships in the phlox family (Polemoniaceae)

Leigh A. Johnson [a,b,*], Lauren M. Chan [a], Terri L. Weese [c], Lisa D. Busby [a], Samuel McMurry [a]

[a] Department of Biology, Brigham Young University, Provo, UT 84602, USA
[b] M.L.B. Life Science Museum, Brigham Young University, Provo, UT 84602, USA
[c] Biology Department, Grand Valley State University, Allendale, MI 49401, USA

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ABSTRACT

Members of the phlox family (Polemoniaceae) serve as useful models for studying various evolutionary and biological processes. Despite its biological importance, no family-wide phylogenetic estimate based on multiple DNA regions with complete generic sampling is available. Here, we analyze one nuclear and five chloroplast DNA sequence regions (nuclear ITS, chloroplast *matK*, *trnL* intron plus *trnL–trnF* intergeneric spacer, and the *trnS–trnG*, *trnD–trnT*, and *psbM–trnD* intergenic spacers) using parsimony and Bayesian methods, as well as assessments of congruence and long branch attraction, to explore phylogenetic relationships among 84 ingroup species representing all currently recognized Polemoniaceae genera. Relationships inferred from the ITS and concatenated chloroplast regions are similar overall. A combined analysis provides strong support for the monophyly of Polemoniaceae and subfamilies Acanthogilioideae, Cobaeoideae, and Polemonioideae. Relationships among subfamilies, and thus for the precise root of Polemoniaceae, remain poorly supported. Within the largest subfamily, Polemonioideae, four clades corresponding to tribes Polemonieae, Phlocideae, Gilieae, and Loeselieae receive strong support. The monogeneric Polemonieae appears sister to Phlocideae. Relationships within Polemonieae, Phlocideae, and Gilieae are mostly consistent between analyses and data permutations. Many relationships within Loeselieae remain uncertain. Overall, inferred phylogenetic relationships support a higher-level classification for Polemoniaceae proposed in 2000.

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1. Introduction

At a variety of taxonomic levels, Polemoniaceae (the phlox family) serve as model systems for evolutionary investigations including speciation, fitness, plant response to herbivory, breeding system evolution, and pollination biology (e.g., Grant, 1981; Galen, 2000; Juenger and Bergelson, 2000; Galen and Cuba, 2001; Goodwillie et al., 2004; Campbell and Waser, 2007). Single-gene comparative DNA sequencing studies provide insights into patterns of relationships in this family of ca. 400 species in 26 genera (Steele and Vilgalys, 1994; Baldwin et al., 1995; Johnson and Soltis, 1995; Johnson et al., 1996; Porter, 1996; Prather et al., 2000). However, overall congruence, the degree of clade support, and instances of apparent discordance are difficult to assess because these studies differ greatly in sampling, with from 20 to 59 ingroup species representing 16–25 genera (sensu Porter and Johnson, 2000). Furthermore, the breadth and phylogenetic distance of outgroup species varies substantially. As a result, differences among analyses attributable to taxon sampling versus biological processes such as hybridization, lineage sorting, or heterogeneity in nucleotide substitution rate are difficult to distinguish. At present, the only published exploration of levels and sources of incongruence between chloroplast and nuclear genes across multiple genera in the phlox family is limited in sampling to a single chloroplast and single nuclear gene, and just 26 species representing subfamily Polemonioideae (Johnson and Soltis, 1998). The robustness of inferred relationships to different tree building techniques remains unexplored.

In spite of the variability in taxon sampling that exists in prior DNA-based analyses, notable consistencies between studies are apparent. Strong support exists for the monophyly of Polemoniaceae (Johnson et al., 1996; Porter, 1996; Prather et al., 2000), as well as for a close relationship between *Allophyllum*, *Collomia*, and *Navarretia* within tribe Gilieae (Steele and Vilgalys, 1994; Baldwin et al., 1995; Johnson and Soltis, 1995; Johnson et al., 1996; Porter, 1996; Prather et al., 2000). The recognition of *Ipomopsis* separate from *Gilia* (Grant, 1956, 1959) is well supported (Steele and Vilgalys, 1994; Johnson and Soltis, 1995; Johnson et al., 1996; Prather et al., 2000), and the paraphyly of a broad circumscription (Grant, 1959, 1998a, 2004) of the taxonomically complex

* Corresponding author. Address: Department of Biology, Brigham Young University, Provo, UT 84602, USA. Fax: +1 801 422 0090.
E-mail address: leigh_johnson@byu.edu (L.A. Johnson).

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genus *Gilia* is also evident (Johnson and Soltis, 1995; Johnson et al., 1996; Porter, 1996; Johnson, 2007). However, other phylogenetic issues remain unresolved by these studies, including relationships among subfamilies and tribes, some relationships among genera within tribes, and the placement of the root of Polemoniaceae (Steele and Vilgalys, 1994; Johnson et al., 1996; Porter, 1996; Prather et al., 2000).

Stimulated by renewed interest in the phylogeny of Polemoniaceae, Grant (1998b) updated his earlier classification of this family (Grant, 1959) and elaborated his philosophical and methodological views (see also Grant, 2001, 2003a,b). Porter and Johnson (2000) proposed an alternative classification based on inferences from the early DNA studies cited above, unpublished *trnL–trnL–trnF* sequence comparisons, and insights based on their observations of morphology. Subsequent works such as Wilken (2004) and Simpson (2005) followed Porter and Johnson (2000) in part, but not entirely; others, such as Heywood et al. (2007), followed Grant's most recent revisions (2003b, 2004). Though similarities between the classifications of Grant (2003b, 2004) and Porter and Johnson (2000) exist, there are also substantial differences (Table 1). Importantly, these differences extend beyond the arrangement of taxa to fundamental differences in philosophy and methodology that challenge Grant's assertions that morphology and molecules in the phlox family often disagree (e.g., Grant, 1998b, 2003b). Whereas Porter and Johnson constructed their classification to reflect phylogenetic patterns inferred from explicit analyses and emphasized the recognition of monophyletic (i.e., holophyletic) groups, Grant first constructed his classification and then derived phylogenetic hypotheses from it (phyletic patterns are outlined in Grant, 1998b but not in Grant, 2003b, 2004); his view of monophyly follows older parlance that includes recognition of paraphyletic groups. As such, though it is tempting to compare, through empirical tests, the alternative hypotheses proposed by Grant (2003b, 2004) and Porter and Johnson (2000), it is incongruous to use cladistic methods, as we do here, to assess the robustness of a classification system (i.e., Grant, 2003b, 2004) derived from a methodology that is non-cladistic, accepts paraphyly, emphasizes "reliable" characters designated as such without analytical scrutiny, prioritizes identification and assumed phenetic similarity over phylogeny and explicit analysis, and presents a hierarchical list of taxa but does not outline the phylogenetic relationships between such taxa.

Table 1
Comparison of taxonomic classifications for Polemoniaceae proposed by Porter and Johnson (2000) and Grant (2003b, 2004)

Porter and Johnson (2000)	Grant (2003a,b, 2004)
Acanthogilioideae	Acanthogilioideae
Acanthogilieae	Acanthogilieae
Acanthogilia	*Acanthogilia*
Cobaeoideae†	Gilieae
Bonplandieae	*Gilia*
Bonplandia	Subgen. *Gilia*
Cantueae	Sect. *Gilia* (*Gilia*)
Cantua	Sect. *Arachnion* (*Gilia*)
Cobaeeae	Sect. *Saltugilia* (*Saltugilia*, *Gilia*)
Cobaea	Subgen. *Greeneophila*
Polemonioideae†	Sect. *Giliastrum* (*Giliastrum*, *Bryantiella*, *Dayia*)
Polemonieae	Sect. *Giliandra* (*Aliciella*)
Polemonium	Sect. *Gilmania* (*Aliciella*)
Phlocideae†	Sect. *Campanulastrum* (*Linanthus*)
Linanthus	*Ipomopsis* (*Ipomopsis*, *Microgilia*, *Loeseliastrum*)
Leptosiphon	*Eriastrum*
Gymnosteris	*Langloisia* (*Langloisia*, *Loeseliastrum*)
Microsteris	Cobaeoideae
Phlox	Bonplandieae
Gilieae†	*Bonplandia*
Saltugilia	Cantueae
Gilia	*Cantua*
Lathrocasis	Cobaeeae
Allophyllum	*Cobaea*
Collomia	Loeselieae
Navarretia	Loeselia
Loeselieae†	Polemonioideae
Aliciella	Polemonieae
Giliastrum	*Allophyllum* (*Allophyllum*, *Navarretia*)
Bryantiella	*Collomia*
Dayia	*Navarretia*
Loeselia	*Polemonium*
Ipomopsis	Phlocideae
Eriastrum	*Phlox*
Microgilia	*Gymnosteris*
Langloisia	*Microsteris*
Loeseliastrum	Leptodactyloneae
	Leptodactylon (*Linanthus*)
	Linanthus (*Linanthus*, *Leptosiphon*)
	Maculigilia (*Linanthus*)

Following the taxa recognized by Grant, the genus, or genera where the species in those taxa are placed by Porter and Johnson (2000) are indicated in parentheses, when the circumscription differs between the two systems.
† Indicate taxa to which monophyly tests were applied.

Rather than contrast the fit of these alternative classifications to each other or to new sequence data point for point, our aims here are to provide a well-sampled framework of DNA-based relationships that enables congruence between chloroplast and nuclear ITS sequences in Polemoniaceae to be adequately assessed; to establish a base-line reference for further comparative study; to identify areas of higher-level relationship in Polemoniaceae that yet need focused investigation; and to evaluate the strength of support for the two multigeneric subfamilies and three multigeneric tribes circumscribed by Porter and Johnson (2000; Table 1).

To achieve these aims, first, we employ separate and combined analyses of nuclear (ITS) and concatenated chloroplast DNA (cpDNA) regions (partial *matK*, *trnL–trnL–trnF* intra- and intergenic spacer, and the *trnS–trnG*, *trnD–trnT*, and *psbM–trnD* intergenic spacers) sampled from taxonomically equivalent datasets to assess relationships among all Polemoniaceae genera presently recognized (Porter and Johnson, 2000; see also Grant (2003b, 2004). Second, we conduct phylogenetic analyses using parsimony and Bayesian approaches to assess the robustness of these phylogenetic inferences to different methods of tree reconstruction. Third, we employ Templeton tests (Templeton, 1983) and other measures of incongruence to assess the significance of differences between the shortest parsimony trees, the Bayesian Majority rule consensus trees, and to test the monophyly of the multigeneric taxa recognized by Porter and Johnson (2000; Table 1). To assess the potential for some relationships to be misleading due to long branch attraction, we also identify which branches are most likely to be affected by this problem.

2. Materials and methods

2.1. Taxon sampling

We sampled 84 of the approximately 370 recognized species of Polemoniaceae (Appendix A). This sampling includes the 26 genera recognized by Porter and Johnson (2000) as well as two additional genera recognized by Grant (2003b, 2004), with a minimum of two species from each non-monotypic genus. For 22 of the 26 genera sensu Porter and Johnson (2000), a sample considered conspecific with the type species was included. For larger genera, we included breadth of diversity as recognized in either subgeneric classifications or other molecular studies (e.g., Spencer and Porter, 1997; Porter, 1998), although we did not necessarily sample all sections or lineages. *Fouquieria splendens* and *Fouquieria columnaris* were included as outgroups based on the growing consensus for a sister relationship between Polemoniaceae and Fouquieriaceae (e.g.,

Downie and Palmer, 1992; Johnson et al., 1999; Schönenberger et al., 2005).

2.2. DNA extraction, amplification, and sequencing

We isolated genomic DNA from fresh or silica dried leaf tissue, or from herbarium specimens using a modified CTAB protocol (Doyle and Doyle, 1987; Cullings, 1992). Standard PCR reactions used a 52 °C annealing temperature and gene-specific primers as follows. ITS: ITS4 and ITS5 for PCR, ITS4i and ITS5i for sequencing (White et al., 1990; Porter, 1996). *matK*: up131F and 1920R for PCR and sequencing, occasionally 541R and 480F for either PCR or sequencing (Johnson and Johnson, 2006). *trnL–trnL–trnF*: "c" and "f" for PCR and sequencing, occasionally "d" and "e" for sequencing (Taberlet et al., 1991). *trnS–trnG*: trnS^{GCU} and trnG^{UCC} for PCR and sequencing (Hamilton, 1999). *trnD–trnT*: trnD^{GUC}F and trnT^{GGU} for PCR and sequencing (DeMesure et al., 1995). *psbM–trnD*: psbMF and trnD^{GUC}R for PCR and sequencing (Shaw et al., 2005). Sequencing reactions used Big-Dye3 (Applied Biosystems, Foster City, CA) at either 1/4 or 1/8 the standard reaction volume and products were run at the DNASC core facility at BYU. Sequences were obtained for each of the 86 taxa for ITS and five chloroplast regions with the exception of five taxa: *Bryantiella palmeri* is missing the *psbM–trnD* and *trnD–trnT* regions, *Phlox glaberrima* and *P. hoodii* are missing the *trnD–trnT* region, and *Polemonium caeruleum* and *P. viscosum* are missing a 3′ portion of the *psbM–trnD* region. These missing data, all from samples acquired from herbarium specimens, likely stem from degraded template quality. We generated the majority of sequences reported here anew, though many of the *matK* sequences and a few sequences from other regions come from previously published work (Johnson and Soltis, 1995; Johnson et al., 1996; Johnson and Weese, 2000; Weese and Johnson, 2005; Johnson, 2007; Appendix A).

2.3. Sequence alignment and indel treatment

We aligned sequences using Se-Al (Rambaut, 1996) with attention given to mechanisms such as inversions that may contribute to alignment ambiguities (Kelchner, 2000). ITS sequences approach 30% divergence within Polemoniaceae (52% across only parsimony informative characters), yet we achieved a reasonable alignment by including additional sequences from species-rich groups that we removed after alignment to preserve taxon parity between datasets. The two *Fouquieria* outgroup species were not included in the final ITS matrix because unambiguous alignment of these sequences with Polemoniaceae across much of the region could not be achieved, a difficulty compounded by the high sequence divergence observed within Polemoniaceae alone for this region. Among chloroplast regions, the *matK* sequences (the only protein-coding region we surveyed) were the most straightforward to align. The *trnS–trnG* intergenic spacer was the most difficult, but even this region had large segments of unambiguous alignment. We discovered a few inversions in the alignment process, including an 11-base inversion in the *trnL–trnF* spacer that apparently occurred independently in *Bonplandia* and Phlocideae. Failure to account for this event as an inversion in alignments resulted in a spurious relationship between these groups in exploratory analyses of *trnL–trnL–trnF*, whereas accounting for the inversion by aligning the inverted bases apart from the non-inverted nucleotides in the remaining taxa recovered *trnL–trnL–trnF*-based trees with relationships of *Bonplandia* and Phlocideae congruent with those recovered in each of the other genes (independent cpDNA region analyses are not, however, detailed here). The cpDNA regions were concatenated into a single matrix and 22 segments (436 total aligned bp) were excluded such as poly-N strings of variable length, or less often, ambiguous alignment where hypervariability of length and nucleotides led to low confidence in homology assignments. In all matrices, we treated gap positions as missing rather than as a fifth state. We subsequently coded indels using simple-indel coding (Simmons and Ochoterena, 2000; Simmons et al., 2007) as implemented in SeqState (Müller, 2005), and analyzed matrices both with and without the coded indels. In total, we assembled six primary matrices for this study: ITS with indels, ITS without indels (both excluding outgroups), cpDNA with indels, cpDNA without indels, ITS + cpDNA with indels, and ITS + cpDNA without indels. The later four matrices were also modified to exclude the two outgroup species to assess the impact of phyletically distant outgroups on ingroup relationships given that the same studies that show Fouquieriaceae as sister to Polemoniaceae reveal relatively long branches leading to Polemoniaceae, Fouquieriaceae, and many other ericalean families (e.g., Johnson et al., 1996, 1999; Schönenberger et al., 2005).

2.4. Sequence analysis—parsimony methods

Parsimony analyses were performed on each data matrix using PAUP* 4b10 (Swofford, 2002). Treating multistate characters as polymorphic, rather than uncertain, was explored for the ITS region, resulting in identical topologies with different tree and branch lengths. To facilitate comparisons between datasets and the combination of data, we subsequently treated multistate characters as uncertain in all analyses. We applied equal weights to all characters in analyses implementing TBR branch swapping with 1000 random addition replicates, and evaluated branch support via fast bootstrapping with 10,000 random addition replicates. Base substitutions were reconstructed using ACCTRAN optimization, and topological differences among trees recovered from analyses (both parsimony and Bayesian) of the different matrices were assessed visually and using the partition metric (PM; implemented as the symmetrical difference metric in PAUP*). PM values were converted into percent similarity (abbreviated S_T here) values using the transformation $S_T = 1 - (PM/(2(N-3)))$ where N is the number of taxa (Johnson and Soltis, 1998). We also examined bootstrap values at conflicting nodes identified between the ITS and cpDNA trees to assess "soft" versus "hard" incongruence (Seelanan et al., 1997; Johnson and Soltis, 1998) using values $\geqslant$90% as indication of hard incongruence (Hillis and Bull, 1993; Wiens, 1998).

2.5. Sequence analysis—Bayesian methods

We partitioned data initially by gene region (ITS, *mat*K, *trnL–trnL–trnF*, *trnS–trnG*, *trnD–trnT*, and *psbM–trnD*) with coded indels from all gene regions concatenated as a single partition. For each gene region, we estimated a general model of sequence evolution for the ingroup taxa using the Akaike information criterion as employed in MrModeltest (Nylander, 2004). We conducted Bayesian analyses in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) of each matrix using gene-specific models of sequence evolution for each data partition and allowed rates to vary independently across partitions. After exploration of data parameter space including varying the number of chains and the temperatures of heated chains, we analyzed all datasets using two replicate runs each initiated from a random tree. The ITS + cpDNA + indels dataset with outgroups excluded employed six Markov chains whereas all other datasets included four chains; each run consisted of sampling every 1000 generations for 20,000,000 generations. We used Tracer 1.3 (Rambaut and Drummond, 2005) to examine the trends in model parameters, likelihood values, and clade posterior probabilities within and between runs to assess convergence. We summarized results with a majority rule consensus tree after discarding the first 2001 samples as burnin (Huelsenbeck and Imennov, 2002; Huelsenbeck et al., 2002), and inspected convergence be-

tween independent runs visually by plotting the probability of all clades identified in the first Bayesian run against the probabilities of identifying those same clades in the second run (using the comparetree command in MrBayes 3.1.2).

We assessed incongruence between the ITS and chloroplast analyses in a manner similar to that used with parsimony; nodes were considered strongly conflicting if posterior probability values $\geqslant$95% in separate data partitions supported different relationships. This percentage (95%) is higher than the 90% bs used in our parsimony comparisons given that Bayesian pp values are inflated relative to bootstrap and jackknife values (Suzuki et al., 2002; Simmons et al., 2004).

2.6. Long branch assessment

We assessed the potential for spurious reconstruction of relationships due to long branch attraction by identifying branches with high affinity for random sequences (Wheeler, 1990; Bergsten, 2005). We first generated 1000 random sequences in MacClade for the ITS with indels and cpDNA with indels datasets (both with outgroups excluded; G, A, T, and C were randomly chosen at each nucleotide position in equal probability, while 0 and 1 were randomly chosen in equal probability in the coded indel portion of the data matrix). We added these random sequences individually to the ITS and cpDNA matrices and then performed heuristic searches with random addition, TBR branch swapping, and Multrees off. From the resulting trees, we tallied the taxon or clade resolved as sister to each random sequence. Taxa showing high affinities for random sequences in this first round or rounds of analyses were removed and the analysis repeated to identify additional branches with high affinity for random sequences that were not identified in the first rounds (Cobaeoideae + *Acanthogilia* were removed from the ITS matrix and *Bonplandia* was removed from the cpDNA matrix). This exercise does not imply that relationships involving branches most attractive to random sequences are, in fact, spurious, but it does highlight branches that may be problematic.

2.7. Tree–tree comparisons and tests of monophyly

We selected trees determined to be topologically most similar (using the PM) from among the best trees recovered from each analysis to assess the significance of topological differences across the entire tree with Templeton's test (1983; implemented as the Wilcoxon Signed Rank test in PAUP*). Bonferroni corrections for multiple comparisons resulted in $a = 0.5$ levels of significance corresponding to $p = 0.0065$ for each comparison. We also used Templeton's test to assess the significance of support for monophyly for the multigeneric taxa recognized by Porter and Johnson (Table 1). For the combined ITS + cpDNA dataset with indels coded, constraint trees with a single branch resolving Cobaeoideae, Polemonioideae, Gilieae, Loeselieae, and Phlocideae as monophyletic were used in parsimony searches to find the shortest trees that failed to support the constraint. An arbitrarily selected, shortest unconstrained tree was then compared with an arbitrarily selected tree from the constrained search using an $a = 0.05$ level of significance corresponding to a Bonferroni corrected $p = 0.01$.

3. Results

3.1. Sequence and matrix metrics

All sequenced regions showed length heterogeneity. The greatest disparity in sequence length occurred in the *psbM–trnD* spacer where all members of tribe Phlocideae possess sequences ca. 350–800 bp shorter than those observed in other Polemoniaceae. The three *Eriastrum* species have ca. 150 bp at the 5′ end of the *trnS–trnG* region that we could not align with the first ca. 50 bp of other Polemoniaceae. Because these 5′ nucleotides were autapomorphic for *Eriastrum*, they were truncated and the *Eriastrum* sequences for this region consequently began with a gap. Overall, length variation in the different regions was as follows (reported lengths for chloroplast introns do not necessarily span the entire length between primers; rather, consistent yet arbitrary starting and ending motifs were selected for uniformity in alignment): ITS, 618–638 bp; *matK*, 1065–1086 bp (5′ two-thirds of gene); *trnL–trnL–trnF*, 775–1030 bp; *trnS–trnG*, 646–805 bp; *trnD–trnT*, 661–1125 bp; *psbM–trnD*, 320–1123 bp. Aligned matrix lengths and numbers of potentially parsimony informative characters are given in Table 2.

3.2. Data analyses

Representative trees recovered from parsimony analyses of the ITS data (Fig. 1), cpDNA data (Fig. 2), and combined ITS + cpDNA data (Fig. 3), all including coded indels, are presented here (see also

Table 2
Matrix characteristics, parsimony shortest tree lengths, and goodness of fit indices for each of the primary datasets (in columns)

Comparison	Dataset (matrix)					
	ITS	ITS + indels	cpDNA	cpDNA + indels	ITS + cpDNA	ITS + cpDNA + indels
Total number of characters	711	847	6383	7121	7094	7832
Number of potentially informative characters	320	405	1401	1803	1721	2123
Number of most parsimonious trees	120	3168	2	1	2	4
MPT length for matrix	**1700**	**1906**	**4247**	**5215**	**6001**	**7174**
CI (autapomorphies retained)	0.39	0.42	0.69	0.70	0.60	0.62
RI	0.69	0.72	0.87	0.88	0.83	0.83
ITS tree lengths	**0**	3–6	285–365*	338–429*	230–311*	290–382*
ITS + indel tree lengths	1–8	**0**	178–364*	201–434*	130–310*	159–381*
cpDNA tree lengths	72–74*	70–73*	**0**	8–9	18–20	26–28
cpDNA + indel tree length	69*	67*	4	**0**	19	14
ITS + cpDNA tree lengths	35–36*	33–34*	18–19*	23–24*	**0**	4
ITS + cpDNA + indel tree lengths	36–39*	34–36*	20–22*	17–19*	4–5	**0**
Bayes ITS + indel tree length	15	9	329*	397*	289*	353*
Bayes cpDNA + indel tree length	69*	67*	10	9*	25*	23*
Bayes ITS + cpDNA + indel tree length	59*	57*	11	6	16	10

Lower rows (tree lengths) show the additional steps required when data from a given matrix (columns) is measured on the shortest trees from each matrix (rows) relative to the shortest trees for each matrix (indicated by bold values). For cpDNA and ITS + cpDNA datasets (with and without indels), additional steps for the ITS and ITS + indels trees are based on length differences after pruning the outgroups from the cpDNA containing matrices. Significant differences ($a = 0.5$) in tree topologies as measured with the Wilcoxon signed-rank test (Templeton, 1983) with Bonferroni corrections ($p = 0.00625$) are indicated with "*".

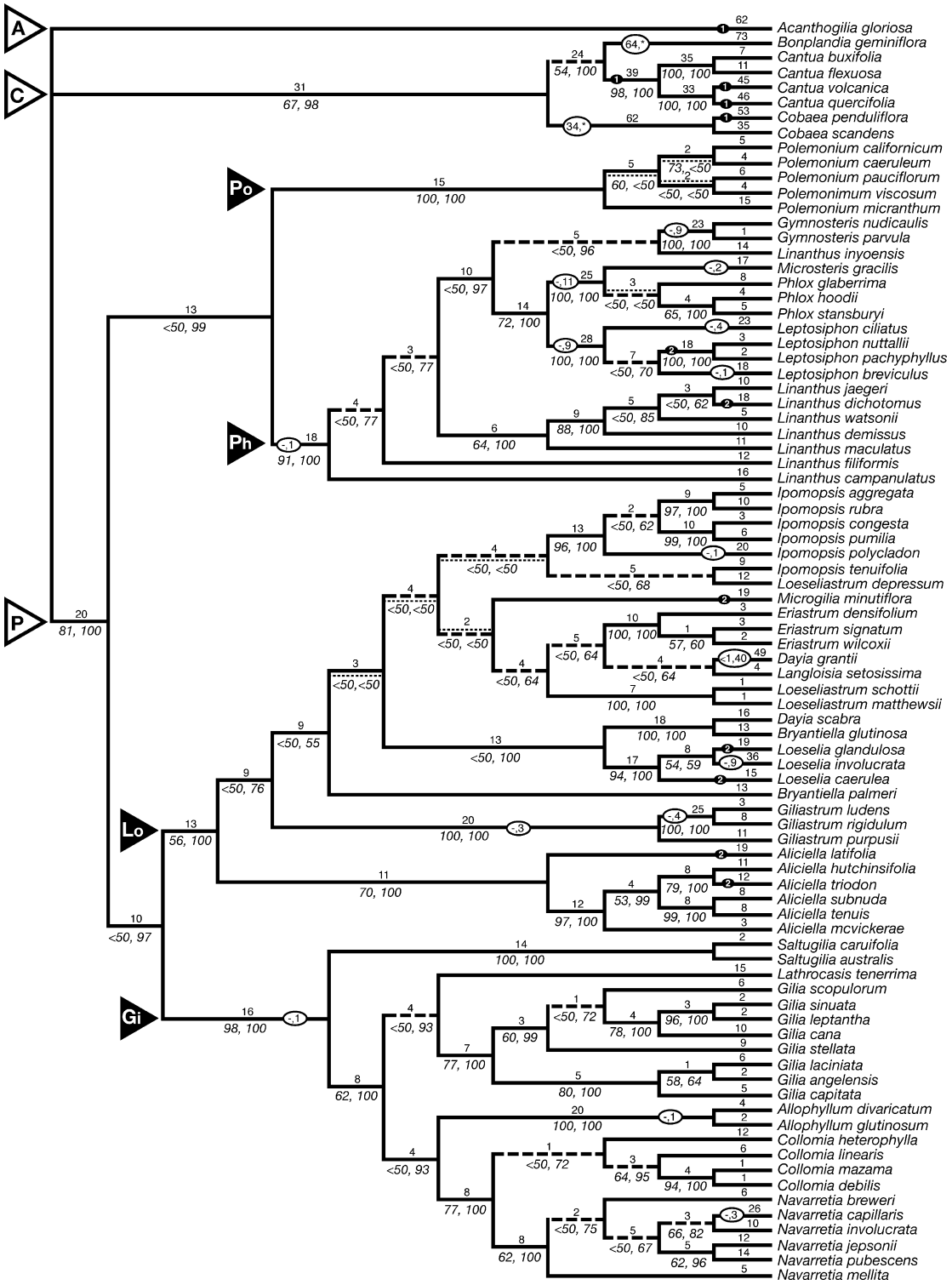

Fig. 1. One of three most parsimonious trees (out of 6336) recovered from analyses of ITS data with indels coded that are topologically most similar to the consensus tree derived from Bayesian analyses of the same data (symmetrical difference = 12). Thick dashed lines indicate branches not present in the strict consensus of the most parsimonious trees. Thin dashed lines indicate branches not present in the Bayesian tree. Numbers above branches are base substitutions as reconstructed using parsimony (ACCTRAN optimization); numbers below branches are parsimony bootstrap and Bayesian posterior probabilities, respectively. Numbers in white ovals indicate percentage of random sequences that attach to that branch in each of two iterations of adding random sequences (first iteration, second iteration); numbers in black ovals indicate branches where at least three, but less than 10 (<1%) random sequences attached in the first (1) or second (2) iteration of random sequence addition. White triangles indicate subfamilies following Porter and Johnson (2000; A = Acanthogilioideae, C = Cobaeoideae, P = Polemonioideae). Black triangles represent tribes within Polemonioideae following Porter and Johnson (2000; Gi = Gilieae, Lo = Loeselieae, Ph = Phlocideae, Po = Polemonieae).

Table 2). Summary trees from parsimony analyses with outgroups excluded, coded indels excluded, and all Bayesian analyses are available as electronic supplementary files (Appendix B).

3.2.1. Effect of outgroup on ingroup relationships

For both parsimony and Bayesian analyses of the concatenated cpDNA data (with and without indels coded), topologically identi-

cal trees among ingroup taxa were recovered when outgroups were included versus excluded. Furthermore, nodal support as measured by bootstrapping (parsimony) and posterior probabilities (Bayesian) did not differ substantially between analyses.

In the Bayesian analyses only, excluding outgroups had a minor impact on ingroup relationships with the ITS + cpDNA + indels matrix. The three *Loeselia* species formed a polytomy when outgroups were excluded, whereas *Loeselia involucrata* was resolved, but weakly supported, as sister to *Loeselia caerulea* when outgroups were included. When indels were excluded, the three *Loeselia* species form a polytomy regardless of outgroup inclusion or exclusion (Appendix B).

3.2.2. Impact of indel coding on topologies and nodal support

Coded indels had little effect on the topology of trees recovered by parsimony analyses. The Wilcoxon sign-rank test (Templeton, 1983) indicated that the parsimony topologies recovered with indels coded were not significantly different from topologies recovered without indels coded (Table 2). Topologies between Bayesian analyses with or without coded indels were also very similar although some rearrangements among major clades generally with lower posterior probabilities were apparent (Appendix B).

Coded indels did affect nodal support: in general, bootstrap values from parsimony analyses varied modestly (<5%) or increased substantially with indels coded (Table 3). In general, bootstrap values from parsimony analyses were unaffected (varied less than 5%) or substantially increased by coding indels. For example, the branch uniting Cobaeoideae increased from below 50% to 67%, the branch uniting Polemonioideae increased from 53% to 81%, and the branch uniting Gilieae increased from 89% to 98% in analyses of ITS, whereas only support for the branch uniting *Langloisa* with *Eriastrum* decreased substantially (by 15%; Table 3). Patterns were similar with the cpDNA and ITS + cpDNA matrices (Table 2), as well as in the posterior probability scores recovered through Bayesian analyses of these same matrices (Table 3; Appendix B).

3.2.3. Parsimony analyses

Because recovered trees were similar between analyses with or without indels included (Table 2), our discussion focuses on analyses with indels coded. The ITS matrices did not contain outgroups so trees based on these data (e.g., Fig. 1) are rooted with a basal polytomy consistent (yet unresolved) with the root identified in the cpDNA (Fig. 2) and ITS + cpDNA (Fig. 3) analyses. Clades comprising Cobeaoideae, Polemonioideae, Polemonieae, Phlocideae, Loeselieae, and Gilieae were consistently recovered in all most parsimonious ITS trees. Furthermore, Polemonieae was weakly but consistently recovered as sister to Phlocideae (<50% bs), and Loeselieae was weakly yet consistently resolved as sister to Gilieae (<50% bs). Within each of these major clades, homoplasy gives rise to a lack of resolution among some relationships on the strict consensus of the most parsimonious trees (Fig. 1).

The most parsimonious trees for cpDNA (Fig. 2) and ITS + cpDNA (Fig. 3) reveal the same major clades and sister relationships among them as does the ITS data. In these analyses, the *Fouquieria* species indicate a root for Polemoniaceae that places Cobaeoideae as sister to remaining Polemoniaceae, and Acanthogiliodeae as sister to Polemonioideae with weak support (54% bs). The cpDNA data show less homoplasy than the ITS data (Table 3), resulting in more fully resolved strict consensus trees and generally greater bootstrap values for various nodes for the cpDNA and ITS + cpDNA analyses.

Conflict between the cpDNA and ITS trees is evident at the topological level, but the severity of such topological discordance is insubstantial based on character support for conflicting relationships. Many instances of topological discordance are either only weakly to marginally supported by both datasets (<90% bs) or strongly supported by only one dataset. For example, neither ITS nor cpDNA provide strong support for their differing resolution of relationships among the three sampled *Loeselia* species: ITS sequences unite *L. glandulosa* and *L. involucrata* (54% bs) while chloroplast data unite *L. involucrata* with *L. caerulea* (69%). Similarly, only weak support exists for the discordant placement of *Aliciella mcvickerae*. Strongly-supported incongruence in both datasets (bootstrap values both >90%) is limited to just two instances: *Leptosiphon pachyphyllus* as sister to *L. nutallii* (ITS, 100% bs) versus sister to *L. breviculus* (cpDNA, 99% bs); and *Collomia mazama* as sister to *C. debilis* (ITS, 94% bs) versus sister to *C. linearis* (cpDNA, 100% bs).

3.2.4. Long branch assessment

Randomly generated sequences attached to several taxa in both the ITS with indels and cpDNA with indels datasets (Figs. 1 and 2). For the ITS data, *Bonplandia* was sister to random sequences in 64% of 1000 replications, *Cobaea* was sister in 34% of the replications, and other members of Cobaeoideae, individually or as clades, were sister in over 1.5% of the replications (Fig. 1). Although random sequences attached to *Acanthogilia* only twice in the first 1000 replicates, this taxon became the favored attachment point for random sequences after the removal of all Cobaeoideae. *Dayia grantii* was the only taxon/clade from Polemonioideae to unite with random sequences more than once in the first 1000 replicates (3 times); however, after removing Cobaeoideae and *Acanthogilia*, this species became the preferred attachment point for 40% of the random sequences, and several other branches in Polemonioideae became attractive to greater than 5% of the random sequences (Fig. 1).

Similar to the ITS matrix, random sequences added to the cpDNA matrix formed a sister relationship to *Bonplandia* more than any other species (70% of replications). Other Cobaeoideae were not particularly singled out, although *Cobaea* became sister in 20% of the replications after removing *Bonplandia* and repeating the analyses (Fig. 2). In Polemonioideae, *Lathrocasis*, *Gymnosteris*, and *Linanthus inyoensis* were identified as the longest branches both before and after removing *Bonplandia* (Fig. 2).

3.2.5. Tests of monophyly

Parsimony searches of the combined ITS + cpDNA dataset (including indels) for trees that failed to recover each of the two multigeneric subfamilies and three mutligeneric tribes proposed by Porter and Johnson, (2000; Table 1) all resulted in trees significantly longer than the most parsimonious trees as measured with Templeton's test: disrupting Cobaeoideae required an increase of 34 steps ($p = 0.0003$), Polemonioideae required 37 steps ($p = 0.0001$), Phlocideae required 64 steps ($p < 0.0001$), Loeselieae required 33 steps ($p < 0.0001$), and Gilieae required 47 steps ($p < 0.0001$).

3.2.6. Bayesian analyses

MrModeltest selected the GTR + G model of nucleotide substitution for the *trnL–trnL–trnF*, *trnS–trnG*, *trnD–trnT*, and *psbM–trnD* regions, and the GTR + I + G model for ITS and *matK* regions. The two independent Bayesian runs for each dataset implementing the appropriate model converged on similar parameter estimates, probabilities, and topologies within datasets; indicating that global optima, rather than local, were achieved by the Markov chains.

Majority rule consensus trees formed from Bayesian searches are highly similar to their parsimony counterparts (Figs. 1–3; Appendix B) with strong support for the monophyly of subfamilies and tribes. The cpDNA data reveal a polytomy between the three subfamilies (Acanthogilioideae, Cobaeoideae, and Polemonioideae). Although the ITS data lack outgroup sequences, combining ITS with the cpDNA resolves *Acanthogilia* as sister to all other Polemoniaceae, but with little support (63% pp). The ITS consensus

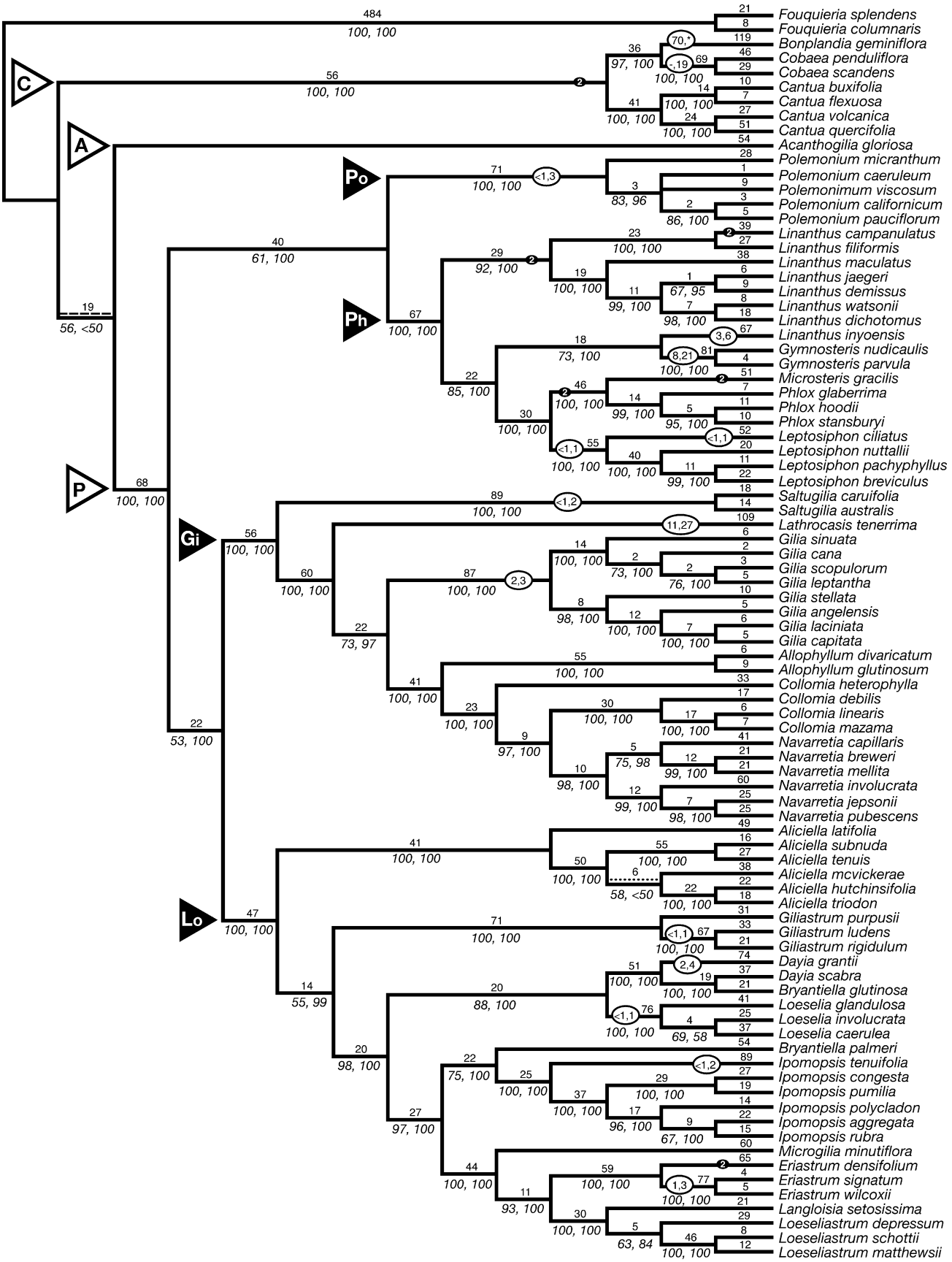

Fig. 2. Single most parsimonious tree recovered from analyses of the concatenated cpDNA regions with indels coded. Symbology as described in Fig. 1.

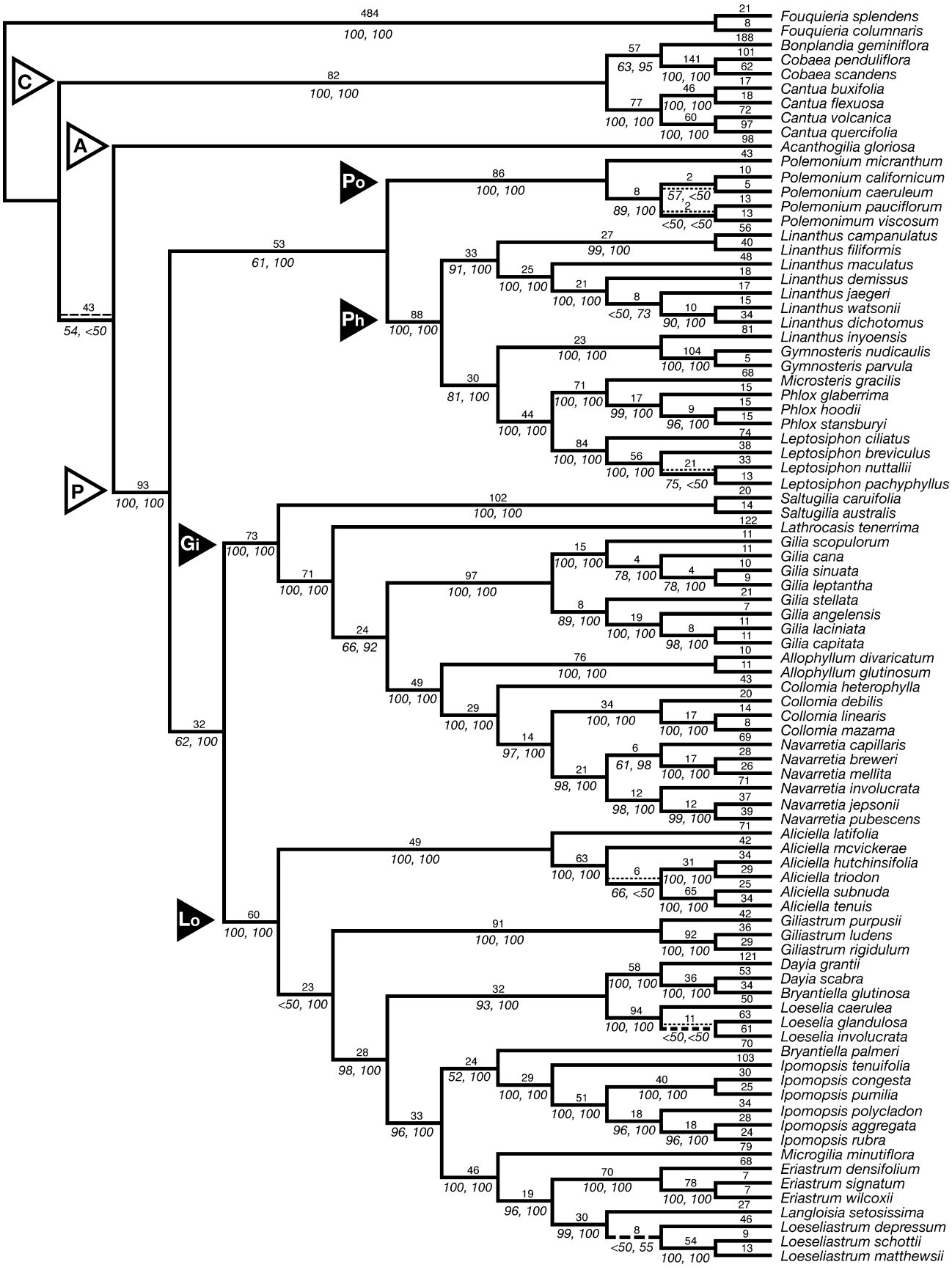

Fig. 3. One of four most parsimonious trees recovered from analyses of the combined ITS + cpDNA regions with indels coded. Symbology as described in Fig. 1 except that the attachment of random sequences was not assessed with the combined data.

Table 3
Summary of topological and dataset incongruence between different permutations and comparisons of the various data matrices

Comparison (matrix)	PM	S_T (%)	B+	B−	*I*
Parsimony					–
Within (ITS)	1–23	86–99	–	–	–
Within (ITS + indels)	2–40	75–99	–	–	–
Between (ITS) & (ITS + indels)	22–50	70–87	9 (6–28)	1 (15)	–
Within (cpDNA)	1–3	98–99	–	–	–
Within (cpDNA + indels)	1	99	–	–	–
Between (cpDNA) & (cpDNA + indels)	7–9	95–96	6 (5–13)	4 (10–13)	–
Within (ITS + cpDNA)	2	99	–	–	–
Within (ITS + cpDNA + indels)	2–4	98–99	–	–	–
Between (ITS + cpDNA) & (ITS + cpDNA + indels)	4–8	95–98	6 (6–26)	2 (6–7)	–
Between (ITS + indels) & (cpDNA + indels)	49–65	60–70	–	–	2
Bayesian					
Between (ITS) & (ITS + indels)	14	91	6 (6–15)	1 (9)	–
Between (cpDNA) & (cpDNA + indels)	9	95	2 (5–20)	0	–
Between (ITS + cpDNA) & (ITS + cpDNA + indels)	5	97	2 (24–39)	0	–
Between (ITS + indels) & (cpDNA + indels)	58	64	–	–	4
Between Parsimony and Bayesian					
(ITS + indels)	11–42	74–93	–	–	–
(cpDNA + indels)	2–3	98–99	–	–	–
(ITS + cpDNA + indels)	11–13	92–93	–	–	–

PM = partition metric values between pairwise comparisons of sets of trees. S_T = percent similarity index calculated from PM values as described in the materials and methods. B+ = number of branches mutually resolved where parsimony bootstrap or Bayesian posteriori values increased more than 5% when coded indels were included in analyses compared to analyses without coded indels; the range of increased values is given in parentheses. B− = number of branches mutually resolved where parsimony bootstrap or Bayesian posteriori values decreased more than 5% when coded indels were included in analyses compared to analyses without coded indels; the range of decreased values is given in parentheses. *I* = number of significant incongruent branches as determined by parsimony bootstrap or Bayesian posteriori values ⩾95% in both ITS + indels and cpDNA + indels analyses.

tree shows a polytomy among many genera of Loeselieae, within *Polemonium*, and within *Phlox*; this tree is 11 PM units from three of the most parsimonious ITS trees (e.g., Fig. 1). The Bayesian majority rule consensus tree for the cpDNA and ITS + cpDNA data are 2–3 and 11–13 PM units, respectively, from their parsimony counterparts. Topologically, the ITS and cpDNA Bayesian consensus trees are 58 PM units apart. Four conflicting relationships are strongly supported by both datasets (i.e., pp > 95%): *Bonplandia* as sister to *Cantua* (ITS, 100% pp) versus sister to *Cobaea* (cpDNA, 100% pp); *Leptosiphon pachypyllus* as sister to *L. nutallii* (ITS, 100% pp) versus sister to *L. breviculus* (cpDNA, 100% pp); *Collomia mazama* as sister to *C. debilis* (ITS, 100% pp) versus sister to *C. linearis* (cpDNA, 100% pp), and *Loesliastrum depressum* in a clade with *Microgilia minutifolora* and *Ipomopsis tenuifolia* (ITS, 97% pp) versus in a clade with *Langloisia setosissima* and other *Loeseliastrum* species (cpDNA, 100% pp).

4. Discussion

4.1. Taxon and character sampling

Relationships of Polemoniaceae at the family level have been the subject of previous molecular phylogenetic analyses (Steele and Vilgalys, 1994; Baldwin et al., 1995; Johnson and Soltis, 1995; Johnson et al., 1996; Porter, 1996; Prather et al., 2000) as well as inferred from an "evolutionary systematics" approach (Grant, 1998b). The sequence-based studies provide hypotheses of relationships based on single nucleotide sequence regions, but all lack sufficient taxonomic sampling to confirm the monophyly of some taxonomic groups. For example, Steele and Vilgalys (1994) recovered a monophyletic *Gilia*, but they did not sample any of the species subsequently excluded from *Gilia* by Porter and Johnson (2000). Similarly, Prather et al. (2000) included only half of the genera recognized in Gilieae today. No study has yet included all of the genera recognized by Porter and Johnson (2000). Here, we included 84 ingroup species (~22% of Polemoniaceae diversity) representing every genus with a particular effort to include species that may break up long branches observed in some earlier analyses (e.g., "*Gilia scabra*"; Baldwin et al., 1995; Johnson et al., 1996; Porter, 1996). On average, ~52% of species within genera were represented; sampling density was higher in smaller or more morphologically complex genera (e.g., *Ipomopsis* with six of 29 species sampled), and lower in larger, or well-defined genera (e.g., *Phlox* with 3 of ca. 69 species sampled).

While efforts to extend beyond ITS with low copy nuclear genes are promising (e.g., Weese and Johnson, 2005; Johnson and Johnson, 2006), the extensive cloning required has precluded the timely compilation of such data for all of the taxa surveyed here. Instead, we included both coding genes (*matK*) and rapidly evolving intra- and intergenic spacers from the cpDNA genome that together provided a concatenated alignment of 6383 nucleotide characters. Analyses of these data resulted in better resolution and much stronger support of higher-level taxonomic relationships relative to previous studies while providing a taxonomically equivalent framework for assessing congruence between cpDNA- and nuclear ITS-based phylogenetic hypotheses (Figs. 1–3).

4.2. Hard incongruence between ITS and cpDNA matrices

An implicit assumption of phylogenetic analyses is that sampled characters share a common phylogenetic history (de Queiroz et al., 1995). Although the ITS and cpDNA trees presented here are not completely congruent topologically, the weak character support for most instances of topological incongruence indicate that, overall, ITS and cpDNA sequences provide estimates of phylogenetic relationships at higher taxonomic levels in Polemoniaceae that are not strongly conflicting. The few instances of hard incongruence are isolated to one or a few taxa generally near the tips of the trees (compare Figs. 1 and 2). These discordances may well reflect different phylogenetic histories between the nuclear and chloroplast genomes that merit additional study with both additional character data and more thorough taxon sampling. For example, both ITS sequences and morphology support a relationship between the perennial species *Collomia mazama* and *C. debilis*

(Fig. 1; 94% bs, 100% pp). The cpDNA data (Fig. 2), in contrast, strongly support a close relationship between *C. mazama* and an annual species, *C. linearis* (100% bs, 100% pp). Broader taxon sampling further confirms this pattern of discordant cpDNA relationship versus the ITS + morphology inference, but shows that *C. mazama* is sister to a clade of species that includes *C. linearis* and not just *C. linearis alone* (unpublished data). A working hypothesis is that *C. mazama*, early in its evolutionary history, captured a chloroplast through introgression with a species ancestral to several annual species. The hard incongruence for *C. mazama* revealed in this study is thus supported, but the details that can be inferred by the data presented here alone provide an incomplete view of the taxa involved in the putative introgression event. Similar caution should be exercised in inferring sister relationships among species within any of the incompletely sampled genera in this study.

The discordant placement of *Leptosiphon pachyphyllus* as sister to *L. nuttallii* (ITS, 100% bs, 100% pp) versus as sister to *L. breviculus* (cpDNA, 99% bs, 100% pp) is the only additional incongruence between ITS and cpDNA data that is strongly revealed by both parsimony and Bayesian analyses. A greater taxonomic sampling within *Leptosiphon* did not recover this same discordance, but that study only used 568 bp of *matK* to represent the chloroplast genome and recovered generally unresolved or poorly supported relationships (Bell and Patterson, 2000). With our sampling including only four of 31 *Leptosiphon* species, greater taxonomic and character sampling is clearly needed to better understand the biological significance of this conflict between ITS and cpDNA trees.

Bayesian, but not parsimony, analyses indicate three additional instances of hard incongruence. The first of these places *Bonplandia* as sister to *Cantua* (ITS, 54% bs, 100 pp) versus as sister to *Cobaea* (cpDNA, 97% bs, 100% pp). All three genera are morphologically distinct, placed in monogeneric tribes (Grant, 1959; Porter and Johnson, 2000), and alignment issues within ITS may be a contributing factor to the discordance (compare Fig. 1 with the ITS topologies of Baldwin et al., 1995 and Porter, 1996). The preference of random sequences for attaching to *Bonplandia* in both ITS and cpDNA matrices and other members of Cobaeoideae, particularly in the ITS matrix, indicate "long branches" that may be susceptible to arbitrary resolution among these three genera (Figs. 1 and 2).

The second incongruence involves the reversed position of *Gilia scopulorum* and *G. sinuata*. ITS trees resolve *G. scopulorum* as sister to a clade containing species of *Gilia* section *Arachnion*, a topology consistent with *matK* studies alone (Johnson and Soltis, 1995; Johnson et al., 1996). In contrast, our concatenated cpDNA data place *G. scopulorum* embedded in this clade as sister to just *G. leptantha*. With very few nucleotide differences observed among these species in the over 6000 bp of cpDNA data (Fig. 2) we sampled, and with hybridization potential well documented within section *Arachnion* (Grant and Grant, 1960; Grant, 1964), this discordance is, again, an area where both more data and more taxa are needed to elucidate the evolutionary dynamics of these species.

The third incongruence involves the placement of *Dayia grantii* clearly as sister to *D. scabra* + *Bryantiella glutinosa* by cpDNA data (Fig. 2; 100% bs, 100% pp) versus the 100% pp exclusion of *D. grantii* from a larger clade containing *D. scabra*, *Bryantiella glutinosa,* and *Loeselia* in the ITS analyses (Fig. 1). Given the long autapomorphic branch *for D. grantii* in the ITS trees (Fig. 1), the high preference for random sequences to attach to this taxon following the removal of Cobaeoideae and *Acanthogilia* (40%; Fig. 1), and the different placement of this taxon in early ITS analyses (as "*Gilia scabra*"; Baldwin et al., 1995; Porter, 1996), long branch attraction and alignment ambiguities may confound the ability of ITS alone to place this species unambiguously. We are confident, however, that among known Polemoniaceae, the taxa most likely to stabilize this species' position have been included in our analysis.

4.3. Phylogenetic inference—subfamily circumscription and the root of Polemoniaceae

Previous analyses in Polemoniaceae delimited the major clades in this family with sufficient support from ancillary evidence to give rise to both informal groupings (Johnson et al., 1996; Porter, 1996) and a formal classification (Porter and Johnson, 2000). Porter and Johnson (2000) recognized three subfamilies with uncertain sister relationships among them: the monotypic Acanthogilioideae, the mid-latitude Cobaeoideae, and the higher latitude Polemonioideae. All trees recovered in this study, regardless of analytical method or data permutation, recovered each of these subfamilies as monophyletic. For the combined ITS + cpDNA with indels data, an additional 34 steps are required to disrupt the monophyly of Cobaeoideae ($p = 0.0003$) and 37 steps to disrupt the monophyly of Polemonioideae ($p = 0.0001$). The question that remains involves the location of the root of Polemoniaceae—that is, whether Acanthogilioideae is (a) sister to all other Polemoniaceae; (b) sister to just Cobaeoideae; or (c) sister to just Polemonioideae. Prather et al. (2000) discussed the position of *Acanthogilia* in earlier analyses, consistently recovered a clade with *Acanthogilia* as sister to Cobaeoideae (52% bs) using cpDNA *ndhF* sequences, and suggested including the former in the latter. Here, a sister relationship between *Acanthogilia* and Polemonioideae is consistently recovered from parsimony analyses of cpDNA and combined ITS + cpDNA data, but still with only weak support (54–56% bs). Bayesian analyses place *Acanthogilia* as either sister to all Polemoniaceae (ITS + cpDNA, 63% pp) or as part of a trichotomy including all three subfamilies (cpDNA). The data presented here thus support the recognition of three groups, but reassert the uncertainty in the relationships among them. It is unclear that additional taxonomic sampling alone within Cobaeoideae or Polemonioideae will clarify the root, or that already surveyed morphology (e.g., Day and Moran, 1986) alone will answer this question. Instead, new comparative studies and additional sequence data, preferably from the nuclear genome and encompassing a greater number of outgroup species, is needed.

4.4. Phylogenetic inference—tribal circumscription and relationships

Similar to its placement in the monotypic Acanthogilioideae, Porter and Johnson (2000) erected the monotypic Acanthogilieae for *Acanthogilia*, using a phylogenetic definition that holds open the possibility of including yet undiscovered taxa, extant or extinct, that show clear affinities to *Acanthogilia gloriosa*, a species endemic to Baja California, Mexico. These higher ranks were erected for parity with the rest of the family under the frequently used "principle of exhaustive subsidiary taxa"—a nomenclatural convention not mandated by the ICBN then (Greuter et al., 2000) or now (McNeill et al., 2006). While Acanthogilieae can accommodate additional taxa, it presently circumscribes a single species and is effectively redundant with the name *Acanthogilia* (Acanthogilioideae is similarly redundant). Our preference is to recognize *Acanthogilia* as the only superspecific taxon for *Acanthogilia gloriosa* with the understanding that it represents a lineage cladogenically comparable to Cobaeoideae and Polemonioideae. Treating *Acanthogilia* in this fashion is compatible with the data presented here and the classification of Porter and Johnson (2000), but avoids the use of names that are presently redundant.

The three genera comprising Cobaeoideae are morphologically distinct and traditionally circumscribed in individual tribes (Grant, 1959). Porter and Johnson (2000) also treated these genera in separate tribes for rank parity throughout their classification. Whether tribes are recognized for each genus or not, resolving relationships among the genera remains an outstanding issue. As noted above, the sister relationship between *Bonplandia* and *Cobaea* is strongly

supported by cpDNA and ITS + cpDNA, but ITS sequences alone place *Cantua* as sister to *Cobaea* (Figs. 1–3). Recent studies have investigated the relative lability of floral characters in *Cobaea* (Prather, 1999), relationships among Cobaea species (Prather and Jansen, 1998), and demonstrated uniformity in pollen morphology in *Cantua* (Monfils and Prather, 2004).

Porter and Johnson (2000) recognized four tribes within Polemonioideae. All analyses with indels coded (Figs. 1–3) resolve Phlocideae + Polemonieae as sister to Gilieae + Loeselieae with high posterior probabilities (>97%), but weak bootstrap support (<62%). Analyses excluding indels resolve these relationships to varying degrees but fail to resolve Loeselieae as monophyletic with ITS sequences alone (Appendix B). Our best estimate from the combined data (Fig. 3), therefore, provides a hypothesis of relationships among these four tribes but with tenuous support. Given the lack of morphological synapomorphies that unambiguously unite these tribes as sisters, we see a need for additional study of both morphological and gene sequence data to more substantially assess relationships along the backbone of Polemonioideae.

Considerable in-depth discussion of morphological features and historical background for the individual tribes and genera within Polemonioideae is available (Johnson et al., 1996; Porter, 1996; Porter and Johnson, 2000). We therefore elaborate these details minimally and focus our remaining discussion on relationships and examples of current research within these taxa.

4.4.1. Tribe Polemonieae

Polemonieae circumscribes the single genus *Polemonium* (Porter and Johnson, 2000). This genus of about 28 species includes mostly perennial herbs with alternate, compound pinnate leaves, herbaceous and accressent calyces, and dark, shiny seeds. Some taxonomists recognize the single annual species, *P. micranthum*, as the segregate genus *Polemoniella* (e.g., Wherry, 1944). Known as "Jacob's ladder" in horticultural circles, *Polemonium* includes both shade loving mesic species and alpine tufted plants. *Polemonium viscosum* has been a model organism in studying plant reproductive biology (e.g., Galen, 2000; Galen and Cuba, 2001; Galen and Geib, 2007).

The position of *Polemonium* within Polemoniaceae varied in previous analyses. Chloroplast *matK* and mitochondrial *nad1B* sequences placed it as sister to all other Polemonioideae while cpDNA *ndhF* sequences placed it as sister to Gilieae + Phlocideae, and ITS as sister to just Phlocideae (Johnson et al., 1996; Porter, 1996; Porter and Johnson, 1998; Prather et al., 2000). Here, parsimony analyses of cpDNA without indels recover *Polemonium* as sister to Gilieae alone, but all other analyses recover *Polemonium* as sister to Phlocideae ($\leqslant$61% bs, $\geqslant$99% pp).

4.4.2. Tribe Phlocideae

Strong support exists for the monophyly of Phlocideae (Figs. 1–3). An additional 64 steps are required to disrupt the monophyly of this tribe ($p = 0.0001$). This tribe includes approximately 127 species in five genera (*Gymnosteris*, *Leptosiphon*, *Linanthus*, *Microsteris*, and *Phlox*) with a tendency toward opposite leaves and salverform corollas (though exceptions exist). Relationships among the genera do not vary substantially among analyses (compare Figs. 1–3), with most disagreements the result of conflicting information regarding relationships within the ITS data alone. *Microsteris*, a monotypic genus that has been circumscribed in *Phlox* previously (Mason, 1941; Patterson and Wilken, 1993), is here resolved as sister to *Phlox* in agreement with previous focused analyses of these genera with greater sampling (Ferguson et al., 1999; Ferguson and Jansen, 2002). *Leptosiphon* is sister to *Phlox* + *Microsteris* and a surprising clade of *Linanthus inyoensis* + *Gymnosteris* is sister to these groups combined, with remaining *Linanthus* forming a monophyletic clade sister to these other genera.

Porter and Johnson (2000) discussed the anomalous placement of *Linanthus inyoensis* as sister to *Gymnosteris* in ITS (Porter, 1996) and unpublished *trnL–trnF–trnF* datasets. As they noted, *L. inyoensis* appears morphologically close to *L. campanulatus*, and Jepson (1957) considered the two conspecific. *Gymnosteris* shows a propensity for attracting random sequences in both ITS and cpDNA matrices, as does *Linanthus inyoensis* with cpDNA data. Excluding *L. inyoensis* or both *Gymnosteris* species prior to analysis results in no difference in the relative placement of the other taxon in the recovered trees. Given stark differences in macromorphology between *Gymnosteris* and *L. inyoensis*, a comparative survey of micromorphological characters including seed coats, pollen exine sculpting, and trichomes would be illuminating, as would additional molecular data from the nuclear genome.

As circumscribed by Porter and Johnson (2000), *Linanthus* includes species formerly treated in *Leptodactylon* and four species previously circumscribed in *Gilia*: *Linanthus campanulatus*, *L. inyoensis*, *L. filiformis*, and *L. maculatus*. Chloroplast data strongly support the monophyly of *Linanthus* as thus circumscribed (Fig. 2, excepting the placement of *L. inyoensis*). Grant and Day (1998), in contrast, created *Maculigilia* to house *L. maculatus* and resurrected *Tintinabulum* to house *L. campanulatus*, *L. inyoensis*, and *L. filiformis*. To emphasize a putative relationship between *Tintinabulum* and *Gilia*, Grant (2004) returned these latter three species to *Gilia* (in tribe Gilieae) while retaining *Maculigilia* with *Linanthus* and *Leptodactylon* in his tribe Leptodactylonae. This alternative treatment of these species is difficult to reconcile with the data presented here and the similarities in seed coats between these former *Gilia* species and *Linanthus* as traditionally circumscribed (Johnson et al., 2004).

Strong support exists for the segregation of *Leptosiphon* from *Linanthus* by these data, by focused studies of *Linanthus* (Bell et al., 1999; Bell and Patterson, 2000), and by several early molecular studies in this family (Johnson et al., 1996; Porter, 1996). Porter and Johnson (2000) noted historical precedence for the segregation of *Leptosiphon* from *Linanthus*. In constrast, the recognition of *Linanthastrum* on the basis of its perennial habit (e.g., Cronquist, 1984; Welsh, 2003), is not supported. Instead, "*Linanthastrum*" species (represented in this study by *Leptosiphon pachyphyllus* and *L. nutallii*) are shown to be derived within *Leptosiphon* with putative past introgression with annual *Leptosiphon* as evidenced by the strongly discordant relationships among *L. pachyphyllus*, *L. breviculus*, and *L. nutallii* in the ITS and cpDNA trees (compare Figs. 1 and 2).

Against a background of historical use as a model for investigations of hybridization and population biology (e.g., Levin and Clay, 1984; Levin and Bulinska Radomska, 1988; Strakosh and Ferguson, 2005; Ruane and Donohue, 2007), the horticultural value of *Phlox* has extended its role as a model system (Hammond et al., 2006; Mackay and Sankha, 2006; Madeiras et al., 2007). *Linanthus* and *Leptosiphon* are also important models for evolutionary biology (e.g., Goodwillie, 1997, 1999; Goodwillie et al., 2004; Schemske and Bierzychudek, 2007).

4.4.3. Gilieae

Gilieae sensu Porter and Johnson (2000) circumscribes about 100 species in six genera (*Allophyllum*, *Collomia*, *Gilia*, *Lathrocasis*, *Navarretia*, and *Saltugilia*). Strong support for this tribe exists in each of our analyses, and disrupting the monophyly in the combined ITS + cpDNA matrix requires 47 additional steps ($p = 0.0001$). Though not a synapormorphy for the entire tribe, *Gilia*, *Allophyllum*, *Navarretia*, and most *Collomia* share a seed coat morphology not observed elsewhere in Polemonioideae (Johnson et al., 2004).

Relationships among genera in Gilieae are mostly well resolved and confirm patterns revealed by earlier studies. *Saltugilia* is sister

to the remaining genera, *Lathrocasis* is sister to either to *Gilia* alone or *Gilia* + *Allophyllum* + *Collomia* + *Navarretia*. The latter three genera form a strongly supported clade with *Allophyllum* as sister to *Collomia* + *Navarretia*.

Saltugilia, once more broadly defined and recognized as a section of *Gilia*, resemble many *Gilia* in possessing a well-developed basal rosettes of leaves, reduced upper leaves, and a well-branched scapiform stem. It differs in seed coat morphology (Johnson et al., 2004) and in its combination of chain-like hairs on the lower leaves with subsessile capitate glands on the pedicels and sometimes calyx. DNA sequence data, including partial sequences of the nuclear *idh* gene (Weese and Johnson, 2005; Johnson, 2007) support a narrow circumscription of this taxon apart from *Gilia*.

An easily overlooked tetraploid species with diminutive flowers, *Lathrocasis tenerrima* represents the sole member of this lineage isolated early in the diversification of tribe Gilieae. A combination of characters, including seed coat and trichome features, distinguishes this genus from *Gilia* (Johnson and Weese, 2000; Johnson et al., 2004). The two positions *Lathrocasis* occupies in the analyses presented here is one fewer than previous results that also placed *Lathrocasis* weakly as sister to just *Allophyllum* + *Collomia* + *Navarretia* (Johnson and Weese, 2000).

Historically, *Gilia* has been taxonomically complex and defined by the absence of distinguishing features that could readily place taxa into other genera (Wherry, 1940; Mason and Grant, 1948; Johnson and Soltis, 1995). The narrow circumscription of *Gilia* outlined by Porter and Johnson (2000) is morphologically cohesive and recovered as monophyletic here. These species vary not only in DNA sequence from all species segregated from *Gilia* by Porter and Johnson (2000), but also in seed coat morphology and combinations of characters including habit, trichomes, and life history. We have already discussed the placement of *G. scopulorum* within the cobwebby gilias in our analyses of cpDNA (Fig. 2); Johnson (2007) advocated leaving *G. scopulorum* without sectional affiliation within *Gilia* until greater evidence bearing on its relationships is put forth.

Gilia has been an important model for understanding plant speciation (e.g., Day, 1965; Grant, 1981). Morrell and Rieseberg (1998) examined relationships in *Gilia* section *Gilia* while the cobwebby gilias (*Gilia* section *Arachnion*, represented in our analyses by *G. cana*, *G. leptantha*, and *G. sinuata*) appear to have diversified via a rapid radiation that has made many species both morphologially and molecularly difficult to differentiate. Hypervariable markers such as microsatellites and a population-level sampling approach will likely provide the best opportunity to test evolutionary hypotheses and the relationships in this group (e.g., Grant and Grant, 1960; Grant, 1964; Day, 1965).

Allophyllum, *Collomia* and *Navarretia* form a strongly supported clade although the monophyly of *Collomia* and *Navarretia* with respect to each other is ambiguous in our analyses. Though cpDNA does not recover *Collomia* as monophyletic, monophyly of the entire genus cannot be rejected statistically (Johnson et al., unpublished data). Morphologically and chromosomally, *Collomia* and *Navarretia* are well-differentiated though they share a common seed coat with *Allophyllum* and accrescent calyces with each other. Represented by *N. capillaris* in our analyses, the placement of "*Gilia* section *Kelloggia*" within *Navarretia* as suggested by Porter and Johnson (2000), rather than *Allophyllum* as suggested by Grant and Day (1998), is well supported by these data (see also Spencer and Porter, 1997).

4.4.4. Loeselieae

Of the four Polemonioideae subfamilies, the monophyly of the ca. 100 species of Loeselieae and relationships among its 10 genera have been the least well supported in single-gene analyses (Porter, 1996; Johnson et al., 1996; Prather et al., 2000). Coding of gaps was important in the recovery of a monophyletic Loeselieae by our ITS sequence data (Fig. 1) compared to the analyses without coded gaps (Appendix B), but the cpDNA and ITS + cpDNA data both strongly support the recognition of this tribe (100% bs; 100% pp). An additional 33 steps are required to disrupt the monophyly of Loeselieae in the ITS + cpDNA matrix ($p = 0.0001$). The cpDNA data (Fig. 2) also provide strong support for relationships among genera at many nodes, revealing topological relationships that are both congruent and discordant with those recovered by the ITS data (Fig. 1); however, most of the discordant relationships are only weakly supported by ITS sequences.

The combined ITS + cpDNA data recover *Aliciella* as sister to the remainder of the tribe, followed by *Giliastrum*. *Dayia* + *Bryantiella glutinosa* + *Loeselia* form a well supported clade that is sister to a *Bryantiella palmeri* + *Ipomopsis* clade and a *Microgilia* + *Eriastrum* + *Langloisia* + *Loeseliastrum* clade. Of these genera, *Aliciella*, *Giliastrum*, *Dayia*, and *Bryantiella* were commonly treated within *Gilia* prior to Porter and Johnson (2000).

Many *Aliciella* share the scapiform habit with a well-developed basal rosette found commonly in *Gilia*, but they all differ in trichome characteristics, leaf shape, and seed morphology. The basal rosette itself is actually common throughout Polemonioideae. Many *Navarretia*, for example, form a basal rosette before bolting that ultimately withers with age. The scapiform inflorescence bearing reduced leaves is less common, yet occurs in Gilieae, Loeselieae, and Phlocideae particularly among the basal members of each tribe, suggesting this condition is a plesiomorphy for much of the temperate radiation. Porter (1998) examined relationships within *Aliciella* with ITS sequence data. One species, *Aliciella heterostyla*, is the only known heterostylous species of Polemoniaceae (Cochrane and Day, 1994). Like *Aliciella*, *Giliastrum* tend to retain their styles after the corolla senesces, which is a common characteristic in Loeselieae. *Giliastrum* are perennial and differ considerably from *Gilia* in morphology of corollas and seed surfaces (Day and Moran, 1986; Johnson et al., 2004).

Porter (in Porter and Johnson, 2000) erected *Dayia* and *Bryantiella* to house species commonly included in *Gilia* but without clear phylogenetic affinities to any of the other genera segregated from *Gilia*. *Dayia grantii* was included in earlier analysis as "*Gilia scabra*" (Johnson et al., 1996; Porter, 1996) or as "*Gilia* sp. nov." (Prather et al., 2000); the true "*Gilia scabra*" is included here as *Dayia scabra*. All of our analyses strongly support *Bryantiella glutinosa* as sister to *D. scabra* (100% bs, 100% pp), confirming the unpublished results of J.M. Porter (Rancho Santa Ana Botanic Garden) suggesting *B. glutinosa* should be circumscribed in *Dayia* (Johnson et al., 2004). Seed coat morphology is similar between both *Bryantiella* species and *D. scabra*, but not *D. grantii* (Johnson et al., 2004). The latter species clusters with *D. scabra* and *B. glutinosa* in the cpDNA and ITS + cpDNA analyses, but not with ITS alone. However, *D. grantii* shows many autapomorphies in that dataset (Fig. 1) and strongly attracts randomly generated sequences; this suggests both anagenic change and sequence alignment may contribute to its poorly supported (and variable) placement in the set of ITS most parsimonious trees.

Upon reclassification of *B. glutinosa*, *B. palmeri* will represent the only species of *Bryantiella*. This species was weakly supported (52%) by parsimony analyses of cpDNA sequences as sister to *Ipomopsis* (but with 100% pp), and weakly (<50% bs, 55% pp) as sister to all Loeselieae except *Aliciella* and *Giliastrum* by ITS sequences. As noted by Johnson et al. (2004), *Dayia* and *Bryantiella* are among the least studied species formerly treated in *Gilia*; our analyses clearly show their segregation from that genus, but additional study is needed.

The sister relationship between *Dayia* and *Loeselia* was consistently recovered but weakly to well-supported (<50–93% bs, 100% pp). Early molecular work clearly established the affinities

of *Loeslia* with the other genera of this tribe, and our work confirms the derived position of *Loeselia* within Loeselieae, rather than as ancestral to these other taxa (Grant, 1998a,b). The arrangement of the three sampled *Loeselia* species differs between the ITS and cpDNA analyses, but with only weak support for the differing topologies.

Excluding from discussion the weak support by cpDNA (52% bs) for a sister relationship between *Ipomopsis* and *Bryantiella palmeri*, the genera *Ipomopsis*, *Microgilia*, *Eriastrum*, *Langloisia*, and *Loeseliastrum* formed a well-supported clade in both cpDNA and combined ITS + cpDNA analyses and share a chromosome number of $x = 7$. Relationships among these genera vary and are less well supported by ITS sequences alone (Fig. 1). *Ipomopsis* are recovered as monophyletic by the cpDNA and combined data, but not by analyses of the ITS data alone.

A more striking lack of monophyly in our analyses is the discordant placement of *Loeseliastrum depressum* between the ITS (Fig. 1) and cpDNA (Fig. 2) trees. Porter and Johnson (2000) discussed this discordance with the concomitant removal of *L. depressum* from *Ipomopsis* to *Loeseliastrum*. cpDNA data and some morphological features support the placement of *L. depressum* with *Loeseliastrum*, but morphology does not support this relationship unequivocally. *Loesliastrum depressum* may represent an evolutionary lineage isolated following ancient hybridization between taxa presently recognized as separate genera. The remaining two species of *Loeseliastrum* share a large number of cpDNA synapomorphies exclusive of *L. depressum*; *L. depressum* itself has a number of autapomorphies in its cpDNA. Without strong evidence tying this species to other genera through the nuclear genome, a classification placing *L. depressum* in a genus of its own may better reflect its evolutionary origins (putatively intergeneric hybridization) and phylogenetic relationships. Low copy nuclear sequence data and thorough comparative morphology should bear on this hypothesis.

As first suggested by analyses of chloroplast *matK* data (Johnson et al., 1996), our analyses corroborate the removal of *Microgilia minutiflora* from *Ipomopsis*. Both ITS and cpDNA data recover trees with *Microgilia* placed as sister to *Eriastrum*, *Langloisa*, and *Loeseliastrum*. ITS sequences, however, place this monotypic genus variously with weak support.

While all molecular datasets have been consistent with morphology in showing a close, yet distinct, relationship between *Loeseliastrum* and the monotypic *Langloisia*, the ITS data do not consistently recover these genera as sister in all trees (Fig. 1). Prather et al. (2000) favor including *Loeseliastrum* in *Langloisia* rather than proliferating small genera. Given clear morphological differences between the two genera (Timbrook, 1986) and great molecular divergence between *Langloisia setosissima* relative *to Loeseliastrum matthewsii* and *L. schotti*, we favor the recognition by Timbrook (1986), followed by Porter and Johnson (2000), of two distinct genera.

4.5. Summary

General patterns of phylogenetic relationships recovered in our separate and combined analyses of ITS and cpDNA matrices corroborate and extend earlier, single-gene analyses of Polemoniaceae. These relationships largely confirm the classification of Porter and Johnson (2000) in so far as these data serve as a proxy for underlying patterns of organismal relationships. The similarity between trees recovered by parsimony and Bayesian methods provides increased confidence in these patterns with respect to different assumptions incorporated into these phylogenetic reconstruction methods. With parity in sampling between ITS and cpDNA data and representation of all recognized genera, the data presented here improve upon earlier single-gene analyses by providing a stronger comparative framework for assessing agreement between the ITS region and the cpDNA genome, and the identification of some instances of probable chloroplast capture. Combined, these DNA regions provide a useful framework for subsampling within Polemoniaceae, empirical data that supports the major taxa postulated by Porter and Johnson (2000), testable hypotheses for comparative studies of morphology or new molecular data, and identification of outstanding problems where additional data are yet needed to resolve, substantiate, or refute relationships among genera, tribes, and subfamilies in Polemoniaceae. Indeed, despite sequencing both nuclear ITS and over 6000 aligned nucleotides of cpDNA including regions from tiers 1, 2, and 3 (Shaw et al., 2005), our data still do not unequivocally resolve the root of Polemoniaceae, relationships among tribes in Polemonioideae, or relationships among all genera. Thus, while these data provide the most comprehensively sampled and best-resolved estimates of relationships within Polemoniaceae to date, some questions identified at the outset of our study remain unanswered.

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Appendix A

Summary of taxa, vouchers, and GenBank accession numbers, provided in the following order: Taxon, collector, collection number, (herbarium where voucher is deposited), GenBank numbers [ITS, *matK, trnL–trnL–trnF, trnS–trnG, trnD–trnT, psbM–trnD*]. "—" indicates region is missing from final matrices for a particular taxon.

Outgroup taxa: *Fouquieria columnaris* (Kellogg) Kellogg ex Curan, *Johnson 97-008* (Cult., RSA), —, EU628508, EU628440, EU628160, EU628368, EU628295. *F. splendens* Engelm., *Johnson 96-010* (Cult., RSA), —, EU628509, EU628441, EU628159, EU628369, EU628294.

Ingroup taxa: *Acanthogilia gloriosa* A.G. Day and Moran, *Johnson and Mort 95-070* (WS), AY997920, L48562, AY997960, EU628161, EU628370, EU628296. *Aliciella hutchinsifolia* (Rydb.) J.M. Porter, *Johnson 93-069* (WS), AF208193, L34186, AF208164, EU628217, EU628423, EU628351. *A. latifolia* (S. Watson) J.M. Porter, *Johnson 93-018* (WS), AF208194, L48578, AY997961, EU628216, EU628422, EU628350. *A. mcvickerae* (M.E. Jones) J.M. Porter, *Johnson 94-044* (WS), EU628286, EU628537, EU628495, EU628221, EU628427, EU628355. *A. subnuda* (A. Gray) J.M. Porter, *Johnson 94-045* (WS), EU628284, L48582, EU628493, EU628219, EU628425, EU628353. *A. tenuis* (F.J. Sm. and Neese) J.M. Porter, *Johnson 03-065* (BRY), EU628285, EU628536, EU628494, EU628220, EU628426, EU628354. *A. triodon* (Eastw.) Brand, *Johnson 92-102* (BRY), EU628283, EU628535, EU628492, EU628218, EU628424, EU628352. *Allophyllum divaricatum* (Nutt.) A.D. Grant and V.E. Grant, *Johnson 04-128* (BRY), EF199700, EU628542, EU628501, EU628233, EF199695, EF199673. *A. glutinosum* (Benth.)

A.D. Grant & V.E. Grant, *Johnson 93-032* (BRY), AF208198, L48564, AF208168, EU628234, EU628430, EU628358. *Bonplandia geminiflora* Cav., *R. Patterson s.n.* (WS), EU628245, L34179, EU628442, EU628162, EU628371, EU628297. *Bryantiella glutinosa* (Phil.) J.M. Porter, *Taylor et al. 10772* (MO), EU628271, EU628527, EU628480, EU628204, EU628411, EU628339. *B. palmeri* (S. Wats.) J.M. Porter, *Tenorio L.* 10949 (MO), EU628272, EU628528, EU628481, EU628205, —, —. *Cantua buxifolia* Juss. Ex Lam., *R. Patterson s.n.* (WS), EU628246, L48566, EU628443, EU628163, EU628372, EU628298. *C. flexuosa* (Ruiz & Pav.) Pers., *R. Patterson s.n.* (WS), EU628248, L34180, EU628445, EU628165, EU628374, EU628300. *C. quercifolia* Juss., *R. Patterson s.n.* (WS), EU628249, L48567, EU628446, EU628166, EU628375, EU628301. *C. volcanica* J.M. Porter & Prather, *Porter 12199* (RSA), EU628247, EU628510, EU628444, EU628164, EU628373, EU628299. *Cobaea penduliflora* (H. Karst.) Hook. f., *Prather 1580* (TEX), EU628250, EU628511, EU628447, EU628167, EU628376, EU628302. *C. scandens* Cav., *R. Patterson s.n.* (WS), EU628251, L48568, EU628448, EU628168, EU628377, EU628303. *Collomia debilis* (S. Watson) Greene, *Johnson 00-072* (BRY), EU628290, EU628544, EU628503, EU628238, EU628433, EU628361. *C. heterophylla* Dougl. ex Hook., *Johnson 94-076* (BRY), AY997922, L48570, AY997963, EU628235, EF199696, EF199674. *C. linearis Nutt., Johnson 92-045* (WS), AF208200, L34188, AF208170, EU628236, EU628431, EU628359. *C. mazama* Coville, *Johnson 97-130* (BRY), EU628289, EU628543, EU628502, EU628237, EU628432, EU628360. *Dayia grantii* J.M. Porter, *Porter & Heil 7991* (SJNM), EU628270, L48581, EU628479, EU628202, EU628409, EU628337. *D. scabra* (Brandegee) J.M. Porter, *Porter 15422* (RSA), AY997924, EU628526, AY997965, EU628203, EU628410, EU628338. *E. densifolium* (Benth.) H. Mason, *Johnson 92-090* (WS), EU628267, L34184, EU628476, EU628199, EU628406, EU628334. *E. signatum* Gowen, *Johnson 97-124* (BRY), EU628268, EU628525, EU628477, EU628200, EU628407, EU628335. *E. wilcoxii* (A. Nelson) H. Mason, *Johnson 94-003* (BRY), EU628269, L48575, EU628478, EU628201, EU628408, EU628336. *Gilia angelensis* V.E. Grant, *Johnson 93-029*, (BRY), AF208202, EU628541, EU628498, EU628229, EF199686, EF199664. *Gilia cana* (M. E. Jones) A.A. Heller, *Johnson 93-016* (WS) AF208204, L48577, AY997966, EU628232, EF199692, EF199670. *G. capitata* Sims, *Johnson 92-015* (WS), AF208206, L34182, AY997967, EU628228, EF199685, EF199663. *G. laciniata* Ruiz & Pav., *Morrell 403* (RSA), AF208208, EU628540, AF208175, EU628227, EF199688, EF199666. *G. leptantha* Parish, *Johnson 93-045* (CAS), EF199705, L34197, EU628500, EU628231, EF199690, EF199668. *G. scopulorum* M.E. Jones, *R. Johnson 304* (BRY), AF208209, L34190, AF208176, EU628225, EF199689, EF199667. *Gilia sinuata* Dougl. ex Benth., *Johnson 92-004* (WS) EF199707, L34198, EU628499, EU628230, EF199693, EF199671. *G. stellata* A. Heller, *Johnson 93-059* (BRY), AF208212, L34199, AF208179, EU628226, EF199683, EF199661. *Giliastrum ludens* (Shinners) J.M. Porter, *Prather 1560* (TEX), EU628280, L48579, EU628489, EU628213, EU628419, EU628347. *G. purpusii* (K. Brandegee) J.M. Porter ssp. *stewartii* (I.M. Johnst.) J.M. Porter, *Porter 10367* (RSA), EU628282, EU628534, EU628491, EU628215, EU628421, EU628349. *G. rigidulum* (Benth.) Rydb., *Porter et al. 8723* (AZ), EU628281, L48580, EU628490, EU628214, EU628420, EU628348. *Gymnosteris nudicaulis* (Hook. & Arn.) Greene, *Harrison 13041* (BRY), EU628258, EU628516, EU628457, EU628177, EU628384, EU628312. *G. parvula* (Rydb.) A. Heller, *Patterson s.n.* (WS), AF067551, L48583, EU628458, EU628178, EU628385, EU628313. *Ipomopsis aggregata* (Pursh) V.E. Grant, *Johnson 92-100* (WS), EU628261, L34193, EU628470, EU628192, EU628399, EU628327. *I. congesta* (Hook.) V.E. Grant, *R. Johnson 166* (WS), EU628262, L34200, EU628471, EU628194, EU628401, EU628329. *I. polycladon* (Torr.) V.E. Grant, *Johnson 93-068* (WS), AY997925, L34194, AY997969, EU628193, EU628400, EU628328. *I. pumila* (Nutt.) V.E. Grant, *Johnson 03-067* (BRY), EU628263, EU628522, EU628472, EU628195, EU628402, EU628330. *I. rubra* (L.) Wherry, *Warrick 2041* (BRY), EU628264, EU628523, EU628473, EU628196, EU628403, EU628331. *I. tenuifolia* (A. Gray) V. E. Grant, *Weese 01-025* (BRY), EU628265, EU628524, EU628474, EU628197, EU628404, EU628332. *Langloisia setosissima* (Torr. & A. Gray) Greene, *Johnson 93-074* (WS), EU628279, L34201, EU628488, EU628212, EU628418, EU628346. *Lathrocasis tenerrima* (A. Gray) L.A. Johnson, *Johnson 93-103* (WS), AF208213, L34192, AF208180, EU628224, EF199694, EF199672. *Leptosiphon breviculus* (A. Gray) J.M. Porter & L.A. Johnson, *Johnson 92-024* (WS), AF067545, L48588, EU628467, EU628187, EU628394, EU628322. *L. ciliatus* (Benth.) Jeps., *Johnson 95-056* (BRY), AF067546, L48589, EU628464, EU628184, EU628391, EU628319. *L. nuttallii* (A. Gray) J.M. Porter & L. A. Johnson, *K. Clapier s.n.* (no voucher), AF067549, EU628517, EU628465, EU628185, EU628392, EU628320. *L. pachyphyllus* (R. Pratt) J.M. Porter & L.A. Johnson, *Schultz 93-092* (WS), AF067550, L48593, EU628466, EU628186, EU628393, EU628321. *Linanthus campanulatus* (A. Gray) J.M. Porter & L.A. Johnson, *Tommerup 355* (RSA), AF208205, EU628518, AF208172, EU628188, EU628395, EU628323. *L. demissus* (A. Gray) Greene, *Johnson 93-063* (WS), AF067547, L48590, EU628462, EU628182, EU628389, EU628317. *L. dichotomus* Benth., *Schultz 93-017* (WS), AF067548, L48592, EU628463, EU628183, EU628390, EU628318. *L. filiformis* (C. Parry ex A. Gray) J.M. Porter & L.A. Johnson, *Johnson 03-016* (BRY), EU628259, EU628519, EU628468, EU628189, EU628396, EU628324. *L. inyoensis* (I.M. Johnst.) J.M. Porter & L.A. Johnson, *Porter 10842* (RSA), EU628260, EU628521, EU628469, EU628191, EU628398, EU628326. *L. jaegeri* (P. A. Munz) J. M. Porter & L. A. Johnson *M. Hamilton s.n.* (WS), AF067542, L48585, EU628460, EU628180, EU628387, EU628315. *L. maculatus* (Parish) Milliken, *Johnson 97-002* (BRY), AY997926, EU628520, AY997971, EU628190, EU628397, EU628325. *L. watsonii* (A. Gray) Wherry, *Needy 4435* (BRY), AF067544, L48587, EU628461, EU628181, EU628388, EU628316. *Loeselia caerulea* (Cav.) G. Don, *Steinman 730* (RSA), EU628275, EU628530, EU628484, EU628208, EU628414, EU628342. *L. glandulosa* (Cav.) G. Don, *Porter & Cambell 9231* (AZ), EU628273, L48594, EU628482, EU628206, EU628412, EU628340. *L. involucrata* G. Don, *Appleby 1104* (RSA), EU628274, EU628529, EU628483, EU628207, EU628413, EU628341. *Loeseliastrum depressum* (M. E. Jones ex A. Gray) J. M. Porter & L. A. Johnson, *R. Johnson 685* (BRY), EU628276, EU628531, EU628485, EU628209, EU628415, EU628343.

L. matthewsii (A. Gray) Timbrook, *Weese 02-003* (BRY), EU628278, EU628533, EU628487, EU628211, EU628417, EU628345. *L. schottii* (Torr.) Timbrook, *Johnson 03-051* (BRY), EU628277, EU628532, EU628486, EU628210, EU628416, EU628344. *Microgilia minutiflora* (Benth.) J.M. Porter & L.A. Johnson, *Ertter 2374* (NY), EU628266, L48584, EU628475, EU628198, EU628405, EU628333. *Microsteris gracilis* (Dougl. ex Hook.) Greene, *Johnson 92-046* (BRY), AF067553, L34203, EU628459, EU628179, EU628386, EU628314. *Navarretia breweri* (A. Gray) Greene, *Johnson 93-101* (WS), AF208222, L48599, AF208189, EU628239, EU628434, EU628362. *N. capillaris* (Kellogg) Kuntze, *Johnson 93-104* (WS), AF008200, L34181, EU628504, EU628241, EU628436, EU628364. *N. involucrata* Ruiz & Pav., *Johnson 06-032* (BRY), EU628293, EU628547, EU628507, EU628244, EU628439, EU628367. *N. jepsonii* V.L. Bailey ex. Jeps., *Johnson 04-063* (BRY), EU628291, EU628545, EU628505, EU628242, EU628437, EU628365. *N. mellita* Greene, *Johnson 94-072* (BRY), AY997929, L48597, AY997974, EU628240, EU628435, EU628363. *N. pubescens* (Benth.) Hook. & Arn., *Johnson 04-055* (BRY), EU628292, EU628546, EU628506, EU628243, EU628438, EU628366. *Phlox glaberrima* L., *Coile 2989* (NCSC), EU628257, EU628515, EU628454, EU628174, —, EU628309. *P. hoodii* Richardson, *Johnson 92-001* (WS), AF067554, L34205, EU628455, EU628175, —, EU628310. *P. stansburyi* (Torr.) A. Heller, *Johnson 93-012* (WS), AF067555, L48600, EU628456,

EU628176, EU628383, EU628311. *Polemonium caeruleum* L., *McNeal 3530* (BRY), EU628253, EU628512, EU628450, EU628170, EU628379, EU628305. *P. californicum* Eastw., *Johnson & Patterson 93-089* (WS), EU628252, L34204, EU628449, EU628169, EU628378, EU628304. *P. micranthum* Benth., *Johnson 95-066* (BRY), EU628254, EU628513, EU628451, EU628171, EU628380, EU628306. *P. pauciflorum* S. Watson, *Johnson 94-084* (WS), EU628255, L48602, EU628452, EU628172, EU628381, EU628307. *P. viscosum* Nutt., *Collins & Harper 597* (BRY), EU628256, EU628514, EU628453, EU628173, EU628382, EU628308. *Saltugilia australis* (H. Mason & A.D. Grant) L.A. Johnson, *Johnson 97-049* (BRY), EU628288, EU628539, EU628497, EU628223, EU628429, EU628357. *S. caruifolia* (Abrams) L.A. Johnson, *Johnson 93-093* (BRY), EU628287, EU628538, EU628496, EU628222, EU628428, EU628356.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.05.036.

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