Phylogenetic Systematics of *Ipomopsis* (Polemoniaceae): Relationships and Divergence Times Estimated from Chloroplast and Nuclear DNA sequences

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Abstract—The genus *Ipomopsis* (Polemoniaceae) encompasses about 29 species and 24 subspecies generally divided into three sections: sect. *Ipomopsis*, sect. *Microgilia*, and sect. *Phloganthea*. We employed maximum likelihood and Bayesian inference of DNA sequences from the nuclear ribosomal ITS region (ITS1, 5.8S ribosomal subunit, ITS2) and the chloroplast *trnL–F* region (*trnL* intron + *trnL–trnF* intergenic spacer) to estimate phylogenetic relationships within this genus and its placement among other genera of Polemoniaceae. The chloroplast and combined sequences provide support for the monophyly of *Ipomopsis*, but only if four species previously included in the genus are removed: *Ipomopsis havardii*, *I. sonorae*, *Microgilia minutiflora* (= *I. minutiflora*), and *Loeseliastrum depressum* (= *I. depressa*). Of the three sections, two are conditionally supported as being monophyletic. Section *Microgilia* (with 11 species and 11 infra-specific taxa) is supported as monophyletic if *I. polycladon*, *I. sonorae*, *I. depressa*, and *I. minutiflora* (the type of the section) are removed. This clade is treated here as section **Elaphocera**. Section *Ipomopsis* is inferred to be monophyletic with the inclusion of several members of sect. *Phloganthea* (*I. multiflora*, *I. pinnata*, and *I. polyantha*). There is no support for monophyly or paraphyly of sect. *Phloganthea*. The *Giliopsis* group (*I. effusa*, *I. guttata*, and *I. tenuifolia*) is supported as monophyletic by both data sets, and the cp sequences place it as sister to the remainder of *Ipomopsis*. This clade is treated as a new section, **Giliopsis**. Nuclear data place *Giliopsis* in a clade with *Ipomopsis havardii*, *I. sonorae*, *Microgilia minutiflora*, *Loeseliastrum depressum*, *Eriastrum* spp., *Langloisia*, and *Dayia grantii*. Using the Eocene fossil *Gilisenium hueberii* to calibrate the most recent common ancestor of tribe Gilieae, we estimate that *Ipomopsis* has its origin 28.2 ± 0. 40 to 39.0 ± 1.14 MYA (*trnL–F* and ITS, respectively). Using this same relaxed clock, the node (or coalescent event) that defines the *I. aggregata* complex is dated at 16.2 ± 0.38 and 27.1 ± 0.83 MYA (*trnL–F* and ITS, respectively).

The deep divergence of the *I. aggregata* complex suggests that reticulation, rather than lineage sorting, is the source of conflict among phylogenetic markers used to infer the placement of *I. macrosiphon*.

Keywords—cpDNA, *Ipomopsis*, maximum likelihood, nrITS, nonparametric rate smoothing, Polemoniaceae.

The genus *Ipomopsis* Michx. has its origin in the works of Michaux (1803), Nuttall (1818; under the name *Ipomeria*), and Wherry (1936). The current and most enduring synthesis, contributed by Grant (1956), was formed by uniting the *Gilia aggregata* (Pursh.) Spreng. group (Wherry 1946), the *Gilia multiflora* Nutt. group (Kearney and Peebles 1943), the *Gilia congesta* Hook. group (Constance and Rollins 1936), *Loeselia* L. sect. *Giliopsis* (Gray 1876), and miscellaneous species formerly treated in *Gilia* Ruiz & Pav. As circumscribed by Grant (see also contributions by Moran 1977; Day 1980; Wilken and Allard 1986; Grant and Wilken 1986; Henrickson 1987; Wilken and Fletcher 1988; Wilken and Hartman 1991; Porter and Johnson 2000; Wilken 2001), *Ipomopsis* encompasses about 29 species and 24 subspecies. With the exception of one South American species, these taxa are distributed in North America with a center of diversity in western North America. The base chromosome number in examined species of *Ipomopsis*, $x = 7$ (Grant 1959; Taylor and Taylor 1977; Ward 1983a,b, 1984; Wilken 1986; Freeman and Brooks 1988) is consistently different from the $x = 9$ of *Gilia* (Grant 1959; Ward and Spellenberg 1986; Wilken 1986). Grant (1956, 1998a,b) suggested that *Ipomopsis* is derived from *Gilia* as part of an aneuploid reduction in tribe Gilieae. Morphological traits that distinguish these genera are cryptic. Rather than any single feature, trends and different combinations of traits distinguish species groups of *Ipomopsis* from other genera. Consequently, the segregation of *Ipomopsis* from *Gilia* was ignored or rejected by some authors (Cronquist 1984; Welsh et al. 1993, but not Welsh et al. 2003), in favor of historical circumscriptions (e.g. Gray 1870). Today, *Ipomopsis* is widely accepted.

Ipomopsis are annual or perennial herbs that are sometimes woody and branch from the base. Plants usually produce an over-wintering rosette of leaves, and leaves are alternate, entire, or once- to twice–pinnatifid, with cuspidate or mucronate tips. Inflorescences are cymose, and

either thyrsoid or in terminal heads. The corolla tube varies from salverform to campanulate, and the five lobes are radially or bilaterally symmetric (Fig. 1). The five stamens are either equally or unequally inserted in the distal corolla tube or in the sinuses of the corolla lobes, and the filaments are unequal or equal in length. As with other species of Loeselieae, styles are usually persistant after the corolla is shed (Johnson et al. 2008). All species examined have zonocolporate pollen with a striate, striate–reticulate or reticulate exine (Stuchlik 1967; Porter, pers. obs.). All but two species are diploid, with a gametic chromosome number of $n = 7$, as noted above. The two exceptions, *I. roseata* (Rydb.) V. E. Grant and certain populations of *Ipomopsis multiflora* (Nutt.) V. E. Grant, are tetraploids, with gametic chromosome numbers of $n = 14$.

Grant (1956) partitioned *Ipomopsis* into three sections: sect. *Ipomopsis*, sect. *Microgilia* (Benth.) V. E. Grant, and sect. *Phloganthea* (Gray) V. E. Grant (Table 1). These "working groups" (Grant 1956) were organized based on shared features and interpretation of the "primitive" or "advanced" nature of these features relative to "primitive" Dicotyledons (Grant 1959). Grant (1959, 1998a,b) suggested that both *Ipomopsis* and *Eriastrum* originated from *Gilia* sect. *Giliastrum*, which, in turn, was derived from *Loeselia* (Fig. 2). The more derived sections, sect. *Ipomopsis* and sect. *Microgilia*, were suggested to have their origin in the woody species of sect. *Phloganthea* (Grant 1959).

Ipomopsis has served as an important group for scientific and evolutionary inquiry. This is attributable, in part, to wide variation in pollination mechanisms and life histories, coupled with a high degree of interfertility among species (Grant 1959; Grant and Grant 1967). Most research has focused on *Ipomopsis aggregata* and its near relatives, although the lines of inquiry have been diverse (see bibliography deposited as online supplemental data). Few studies have

investigated other species (but see Freeman and Wilken 1987; Juenger and Bergelson 2002; Wood and Nakazato 2009).

Since Grant's (1956) synopsis, *Ipomopsis* has not been treated synthetically. The few existing studies have generally focused on more tightly defined species complexes. The *Ipomopsis aggregata* complex has been examined in varied scope (Grant and Wilken 1986; Wilken and Allard 1986; Grant and Wilken 1988a). The *Ipomopsis spicata* complex was revised by Wilken and Hartman (1991). Moran (1977) and Wood and Nakazato (2009) contributed a review of the "*Giliopsis* group" (*I. effusa*, *I. gutatta*, and *I. tenuifolia*). Descriptions and notes on several of the Chihuahuan Desert representatives of *Ipomopsis* were given by Henrickson (1987). In addition, several contributions are largely nomenclatural, the focus ranging from small groups, such as the *Ipomopsis congesta* group (Day 1980), to the entire genus (Porter and Johnson 2000).

Cladistic analyses of *Ipomopsis* also vary in their scope and inclusion. Porter (1993) included four species of *Ipomopsis* (*I. gunnisonii*, *I. longiflora*, *I. multiflora*, and *I. tenuifolia*), representing samples from all of Grant's (1956) sections, in a family-wide survey of nuclear ribosomal internal transcribed spacers (nrITS) DNA sequence variation. Three of the *Ipomopsis* species formed a clade, but *I. tenuifolia* was sister to members of *Eriastrum*, *Langloisia*, and *Loeseliastrum*. These relationships, however, lacked significant support. Importantly, representatives of *Ipomopsis* have not been inferred to be closely related to *Gilia*. Wolf et al. (1993) evaluated chloroplast DNA restriction site variation of 16 taxa in the *I. aggregata* complex, concluding that the pattern of intra- and interspecific variation was consistent with recent divergence followed by frequent hybridization. In a family wide survey of chloroplast *matK* DNA sequences, Steele and Vilgalys (1994) included only *I. aggregata*; though the monophyly of *Ipomopsis* could not be addressed, *Ipomopsis* was again shown to be distant from

Gilia. Using broader sampling and a different portion of *matK*, Johnson and Soltis (1995) recovered *I. aggregata*, *I. congesta*, and *I. polycladon* as a clade. Johnson et al. (1996), again using *matK*, added *I. minutiflora* to their analysis that placed *I. minutiflora* sister to *Eriastrum*, *Langloisia*, and *Loeseliastrum* rather that part of a monophyletic *Ipomopsis*. Porter (1996), in a reanalysis of nrITS data, used an expanded data set and included *I. sonorae*, in addition to the four *Ipomopsis* species included in the 1993 study, and obtained similar results, as did Johnson et al. (2008) in their family wide study of nrITS and several cpDNA regions that included eight species of Grant's (1959) *Ipomosis*. The only cladistic analysis using morphological data was that of Wilken and Hartman (1991), examining variation in the *I. spicata* complex.

The phylogenetic results described above are difficult to interpret broadly because taxon sampling is sparse and differs among analyses. Several molecular studies provide evidence that questions the monophyly of *Ipomopsis*, sensu Grant (1956). Had a greater diversity of *Ipomopsis* been included, it is conceivable that species could well be distributed across many of the major lineages of Polemoniaceae, as was shown to be the case with *Gilia* (Johnson et al. 1996; Porter 1996; Prather et al. 2000; Johnson et al. 2008). This possibility is heightened given that Porter (1996) demonstrated the strong influence that taxon sampling has on phylogenetic estimates using nrITS sequences in Polemoniaceae, an observation likely extendable to chloroplast regions in this family when sampling is meager. Thus, previous phylogenetic analyses involving *Ipomopsis* lack sufficient sampling to adequately address species relationships, generic and subgeneric (sectional) circumscription, and monophyly.

We present a comparative study of DNA sequence variation from the ITS region (ITS1 + 5.8S ribosomal subunit + ITS2) and chloroplast *trnL–F* region *(trnL* intron + *trnL–trnF* intergenic spacer) in *Ipomopsis*, with more complete taxon sampling than previous studies to

examine phylogenetic relationships within *Ipomopsis*, evaluate the integrity of sectional and generic delimitaiton, and further explore the placement of the genus within Polemoniaceae. Grant did not propose that *Ipomopsis*, or its sections as he delimited them, were monophyletic in the cladistic sense. Rather, Grant uses the term "monophyly" to refer to "any group descended from a close common ancestor" (Grant 2004: 533). Such a definition can result in the application of the term "monophyletic" to mono-, para-, and polyphyletic groups (as the terms are defined by cladists; see Grant 1998b). We find no advantage to this broader definition, as it does not lead to an objective criterion for defining groups. However, because classifications are frequently used as proxies for phylogenies, classifications are often interpreted in the context of the strict interpretation of monophyly. It is therefore important to determine the extent to which classifications deviate from this expectation. Secondarily, we use these data to estimate the age of divergence of *Ipomopsis* as a whole, and the *Ipomopsis aggregata* complex, to provide a preliminary test of the hypotheses concerning age of the complex and possible timing of hybridization in this complex, which has been well documented (Wilken and Allard 1986; Grant and Wilken 1987, 1988a; Melendez-Ackerman and Campbell 1998; Wolf et al. 1997).

MATERIALS AND METHODS

Plant Samples—Nomenclature below follows Porter and Johnson (2000). Samples of leaf material from 109 collections of Polemoniaceae were obtained from air-dried herbarium collections. This sampling (Appendix 1) included 52 populations of *Ipomopsis* sensu Grant, representing 31 species. *Gilia polyantha* Rydb. var. *whitingii* Kearney & Peebles is included in this number because the material is recognized as belonging to *Ipomopsis* (it is generally considered synonymous with *I. multiflora*); however, the varietal epithet "*whitingii*" has never

been transferered to *Ipomopsis* even though the specific epithet "*polyantha*" has. Representatives of all genera of Polemoniaceae sensu Porter and Johnson (2000) were included, as was one representative from Fouquieriaceae, the sister-family to Polemoniaceae, as an outgroup. This broad sampling ensures adequate representation of family-wide diversity in case some members of *Ipomopsis* should be related to rather distant genera, as is the case for the traditional circumscription of *Gilia* (see Johnson et al. 1996; Porter 1996; Prather et al. 2000; Johnson et al. 2004, 2008). Equally important, the broad sampling is necessary to apply an external calibration point for estimation of the age of *Ipomopsis* (see below).

DNA Isolation and Sequencing—DNA was isolated using a modified 2X CTAB buffer (Porter 1996) or with the DNEasy plant DNA extraction kits (Qiagen Inc., Valencia, California). Approximately ten milligrams of dried leaf material was used for each extraction.

Double stranded DNAs of the ITS region (ITS1, 5.8S ribosomal subunit, and ITS2; White et al. 1990; Baldwin et al. 1995) were amplified directly by polymerase chain reaction (PCR), as previously described by Porter (1996) and Baldwin (1992), using a 1:1 ratio of primers "ITS5" and "ITS4". Polymerase chain-reaction (PCR) amplifications included 40 cycles of denaturation at 97°C for 10 sec, primer annealing at 48°C for 30 sec, and primer extension at 72°C, for 20 sec (with an increase of 4 sec per successive cycle). The final extension time was increased to 7 min at 72°C.

Templates of the cpDNA *trnL* intron and *trnL–trnF* intergenic spacer (Taberlet et al. 1991) were prepared using a 1:1 ratio of primers “trnLc” and “trnLf”. PCR consisted of 35 cycles of denaturation at 95°C for 60 sec, primer annealing at 55°C for 60 sec, and primer extension at 72°C, for 60 sec. The final extension time was increased to 7 min at 72°C.

PCR products were separated in 0.8% agarose with 0.5× TBE (pH 8. 3) buffer. The gel was subsequently stained with ethidium bromide to confirm a single product. The PCR products were precipitated with 20% polyethylene glycol 8000 (PEG) in 2.5M NaCl, using a 1:1 volume ratio with the PCR reaction mixture. Following an incubation period at 37°C for 15 min, the DNA pellet was recovered by centrigugation for 15 min in a desktop centrifuge at maximum speed and washed using ice-cold 80% and 95% EtOH.

Sequencing was performed using an Applied Biosystems Model 373A DNA Sequencing System (Foster City, California) and run on 6% polyacrylamide gels (Sequagel-6, National Diagnostics, Atlanta, Georgia). Direct cycle-sequencing of purified template DNAs followed manufacturer's specifications but at 1/2 the reaction volume, using the PRISM™ DyeDeoxy™ Terminator Kit (Perkin Elmer, Waltham, Massachussetts). Sequencing of the *trnL-trnF* region made use of primers "trnLci" (5'-TCG GTA GAC GCT ACG GAC TT-3'), trnLf, "trnLd", and "trnLe". Similarly, sequencing of the ITS region (amplified using primers ITS5 and ITS4, see above) employed the following four primers: "ITS5I" (5'-AGG TGA CCT GCG GAA GGA TCA TT-3'), "ITS2", "ITS3" (White et al. 1990), and "ITS4I" (5'-GGT AGT CCC GCC TGA CCT GG-3').

Sequence Editing and Alignment—DNA sequence chromatograms were proofed, edited, and assembled into contigs using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences of the *trnL–F* region were truncated to include only the *trnL* group I intron, the 3' *trnL* exon, and the *trnL–trnF* intergenic spacer, based on comparisons of the *trnL–F* region of other taxa (e.g. Gielly and Taberlet 1994, 1996; Gielly et al. 1996). Sequences of the ITS region were also truncated to include only ITS1, 5.8S, and ITS2, based on comparisons of the ITS region from other members of Polemoniaceae (e.g. Porter 1996). Sequences were

initially aligned using Clustal W 1.4 (Thompson et al. 1994), a gap-cost:gap-extension cost ratio of 10:5. This preliminary alignment was followed by manual editing of the alignment, which considered canonical secondary structure estimates of the *trnL* (a group I) intron (Michel and Westhof 1990), as well as parsimony considerations. Even so, some regions were difficult for the assignment of positional homology and are considered ambiguously aligned. These regions were sequentially included and excluded from analyses. The aligned DNA sequence files have been deposited in TreeBASE (study number S2350; matrix number M4463).

***Phylogenetic Analyses*—**MAXIMUM LIKELIHOOD—Phylogenetic estimates were obtained using maximum likelihood (ML; Felsenstein 1981) as implemented in PAUP* 4.0b10 (Swofford 2001). ML analyses were performed on the ITS region, *trnL–F* region, and combined data sets. A model of nucleotide evolution was selected using the Akaike Information Criterion (AIC; Akaike 1974; Posada and Crandall 2001) from Modeltest version 3.7 (Posada and Crandall 1998). Twenty-five starting trees were randomly sampled from the posterior distribution of phylogenetic trees of Bayesian analyses (see below) for each data set. This approach was used to decrease search time, taking advantage of the exploration of the likelihood surface provided by Bayesian analysis. Bootstrap procedures (Felsenstein 1985) for the assessment of clade support were not used for two reasons. Although confusion over the interpretation and degree of confidence associated with particular bootstrap percentage values because of bias exists (Hillis and Bull 1993; Felsenstein and Kashino 1993; Sanderson and Wojciechowski 2000), this is not the nature of our objection. First and most problematical from a statistical perspective is the nature of our sampling, which is not IID, a requirement of bootstrapping (Sanderson 1995). Second, though perhaps less important, bootstrap analysis, as applied to maximum likelihood estimation in standard software (e.g. PAUP*), fails to 1) hold all parameters constant across

pseudoreplicates (i.e. branch length parameters vary, but model parameters do not), or 2) allows all parameters to vary among pseudoreplicates. Technically appropriate methods should either 1) fix all parameters, drawing branch lengths from a predetermined set of parameters (i.e. a branch length spectrum) or 2) estimate all parameters, including the model of nucleotide evolution, for all pseudoreplicates. As a result, currently, different models are being compared among the different pseudoreplicates. This is inappropriate in bootstrap analysis and its consequence has never been explored.

BAYESIAN INFERENCE—Bayesian inference (Mau and Newton 1997; Mau et al. 1999) was implemented in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). General models of nucleotide substitution were selected for each data matrix using the Akaike information criterion (Akaike 1974; Posada and Crandall 2001), as implemented in Modeltest 3.7 (Posada and Crandall 1998). The same general model was selected for both data sets, requiring six substitution parameters, a gamma distribution of the rate parameter, and a specified frequency of invariant sites (GTR + Ģ + I; lset nst = 6 rates = invgamma). Each data set was analyzed running four Monte Carlo Markov chains, a random tree as a starting point, sampling every 1000 generations, and continuing for 5,000,000 generations. The posterior distribution was determined as described by Ronquist et al. (2005). We allowed each run to continue at least 1,000,000 generations after the average standard deviation of split frequencies reached a value of 0.01. Ten replicate Bayesian runs were conducted for each data matrix to insure that the posterior distribution was both stable and identical. Convergence among independent Bayesian runs was verified by direct comparison of posterior probabilities of nodes across the 10 runs.

***Hypothesis Testing*—**We examine two a priori hypotheses of relationship regarding the monophyly of *Ipomopsis.* One, based on Grant (1956; Table 1) and one based on Porter and Johnson (2000). The likelihood-based Shimodaira-Hasegawa (S-H) test (Shimodaira and Hasegawa 1999; see also Goldman et al. 2000) was employed, with a topology constrained to match each hypothesis. Differences in optimal tree scores with differing constraints imposed were analyzed using PAUP*. Bonferroni corrections for multiple pairwise tests were applied to S-H test (Rice 1989).

***Estimation of Divergence Dates for Ipomopsis and the Ipomopsis aggregata Complex*—**We used non-parametric rate smoothing to estimate divergence times (Sanderson 1997), a method that relaxes the molecular clock assumption. Simulation studies have shown relaxed clock methods to be superior to clock-based methods in divergence date estimation (Ho et al. 2005; Lepage et al. 2007). Penalized likelihood estimates provide an improvement over non-parametric rate smoothing in branch length rescaling (Sanderson 2002), but at a substantial time cost. Given that our estimates employ a single calibration point and we use a sample of trees with alternative branch lengths (see below), the improvement that penalized likelihood might provide on a per tree basis will likely be overwhelmed by the variance in branch lengths among trees. Equally important, the improvement in estimation precision provided by penalized likelihood is moot if the accuracy of the estimate is poor (Pulquério and Nichols 2007).

Rather than focusing only on a single tree for date estimation, we used a random sample from the posterior tree distribution from runs of MrBayes 3.1. The order of posterior distribution of trees (the set of trees remaining after the "burn-in" trees were discarded) was randomized in Microsoft Excel and imported into TreeEdit 1.0a10 (Rambaut and Charleston 2001). The first 100 trees were sampled. All trees were rooted using midpoint rooting. Branch lengths produced

by MrBayes were transformed using the nonparametric rate smoothing (Sanderson 1997) function in TreeEdit. Each tree was calibrated with estimated age of the fossil *Gilisenium hueberii* (Lott et al. 1998), using the Scale tree option. *Gilisenium* was discovered in the Green River Formation (45 MYA) and is hypothesized to be a member of tribe Gilieae. Although the fossil has been compared to members of *Gilia* section *Gilia* (Lott et al. 1998), we place the calibration node as the common ancestor of tribe Gilieae. Placement of the calibration point at more derived nodes results in unrealistically ancient divergence estimates. For example, given ITS DNA sequences, if the node representing the common ancestor of *Gilia* section *Gilia* is used to calibrate a relaxed-clock, then the origin of Polemoniaceae averages 220.95 MYA. This seems unreasonably old. Six nodes were recorded for the *trnL–F*, ITS and combined posterior probability distributions of trees. Clade A = common ancestor of *Ipomopsis*, excluding *Ipomopsis havardii* and *I. sonorae* (this clade was never recovered in analyses of nrITS sequences alone, therefore for nrITS we report Clade A' = common ancestor of *Ipomopsis*, excluding *Ipomopsis havardii*, *I. sonorae*, and *Ipomopsis* subgenus *Giliopsis*, for comparative purposes); Clade B = common ancestor of all subspecies of *Ipomopsis aggregata*; Clade C = common ancestor of all subspecies of *Ipomopsis aggregata*, treating, subsp. *bridgesii* as if it were not a member of *I. aggregata*; Clade D = common ancestor of *Ipomopsis* subgenus *Giliopsis*; Clade E = common ancestor of *Ipomopsis* sect. *Elaphocera*. Summary statistics of divergence times were generated in SPSS11 (SPSS Inc., Chicago, Illinois) for Mac OSX.

This process was repeated on the trees resulting from the ML analysis of the combined *trnL–F* and nrITS sequences to serve as a general illustration of the date estimates.

RESULTS

***Sequence Matrices*—**The *trnL–F* region varied in length within *Ipomopsis* from 954 nucleotides (nt) in *Ipomopsis gutatta* to 980 nt in *I. congesta* subsp. *congesta*. The length of the *trnL–F* region displayed greater variation in the entire family, ranging from 917 nt (*Cobaea scandens*) to 994 nt (*Microsteris gracilis*). Approximately 21 indels were imposed, producing a matrix of 1308 characters, with 289 phylogenetically informative characters (22.1%). Sequences are deposited in GenBank (Appendix 1).

The nrITS region varied in length within *Ipomopsis* from 617 nt in *Gilia polyantha* var. *whitingii* to 627 nt in *I. effusa* and *I. guttata*. The modal length was 625 nt, found in 33 *Ipomopsis* samples. ITS length variation elsewhere in the family showed a nearly identical span, ranging from 619 nt (*Eriastrum densifolium* subsp. *mojavensis*, *Gymnosteris parvula*, and *Loeselia pumila*) to 638 nt (*Cantua quercifolia*). Approximately 36 indels were imposed for the alignment of the ITS region. The aligned sequences produced a matrix of 708 characters, with 310 phylogenetically informative characters (43.8%). Sequences are deposited in GenBank (Appendix 1).

One or more polymorphic nucleotide positions from direct sequencing of the ITS region were present in thirteen of the *Ipomopsis* sequences. The pattern of polymorphisms did not appear to be the additive combination of two other *Ipomopsis* sequences within the data set. In *Ipomopsis sancti-spiritus*, direct sequencing of PCR products resulted in highly polymorphic and uninterpretable chromatograms. For this sample, PCR products were cloned and sequenced, which resulted in the recovery of two ITS types. In the aligned ITS data matrix and in the figures, these two ITS types are identified as "*Ipomopsis sanctispiritus*" and "*Ipomopsis sanctispiritus* b. "

Bayesian Inference from trnL–F Data—All of the replicate Bayesian analyses of *trnL–F* sequences converged on similar parameter estimates, clade posterior probabilities, and topologies, suggesting that the Markov chains were not trapped at local optima. The majority rule consensus of the posterior distribution of phylogenetic trees (not shown) possessed branching order nearly identical to the ML analysis. Fifty clades had posterior probabilities at or above 0.95, representing 23.5% of the possible branches in a tree of 108 OTUs (note that only 81.7% of possible branches were present, the remainder were of length zero).

Likelihood Analyses of the trnL–F Region—ML analysis of the *trnL–F* data recovered two trees with $-\ln(L) = 7265.75514$. The two trees differed in branch order only in the placement of the three representatives of *Aliciella*. In one tree, the *Aliciella* clade was sister to remaining members of large clade here referred to as Loeselieae, a clade corresponding to tribe Loeselieae sensu Porter and Johnson (2000). The alternative tree placed the *Aliciella* clade and a clade composed of three representatives of *Giliastrum* unresolved in the sister group position (Fig. 3). All relationships involving members of *Ipomopsis* were identical in the two ML trees.

As noted above, all samples of *Ipomopsis* display coalescence within the Loeselieae clade, corresponding to tribe Loeselieae. This clade, composed of *Ipomopsis*, *Bryantiella*, *Langloisia*, *Loeseliastrum*, *Eriastrum*, *Loeselia*, *Dayia*, *Giliastrum*, and *Aliciella*, had significant support ($P = 1.00$) from Bayesian posterior probabilities. There was a similarly high degree of support ($P = 0.972$) for the sister group relationship between *Bryantiella palmeri* and a clade incorporating nearly all species of *Ipomopsis*.

The *trnL–F* data provided significant support ($P = 1.00$) for a clade corresponding to *Ipomopsis* (Fig. 3) excluding *Ipomopsis havardii* and *I. sonorae*, which were in a clade with *Dayia* and *Bryantiella glutinosa* (Fig. 3); *Loeseliastrum depressum* and *Microgilia minutiflora*

(both formerly included in *Ipomopsis*) were associated with a clade including *Eriastrum*, *Loeseliastrum,* and *Langloisia*. The placement of the above taxa with genera other than *Ipomopsis* (all with posterior probabilities, $P = 1.00$), yielded *Ipomopsis* sensu Grant (1956) and sensu Porter and Johnson (2000) non-monophyletic.

Within *Ipomopsis*, species relationships were largely unresolved in the ML trees, but several major lineages that had significant posterior probabilities are identified. The *Giliopsis* group had significant support for monophyly, and for sharing ancestry with the remainder of *Ipomopsis* (Fig. 3), but lacked significant support for sister group relationship. The branch order among the species of the *Giliopsis* group was not evident. There were no nucleotide substitutions that supported the monophyly of the members of Grant's *Ipomopsis* section *Microgilia* (exclusive of *Microgilia minutiflora* and *Loeseliastrum depressum*); however, three indels did support its monophyly (deletion of GA at alignment position 180, insertion of TA at position 345, and insertion of GAAA at position 937). There was also support for a clade that included members of sect. *Ipomopsis* and the remaining species from sect. *Phloganthea* (Fig. 3).

Bayesian Inference from ITS Data—All of the replicate Bayesian analyses of the ITS data converged on similar parameter estimates, clade posterior probabilities, and topologies, suggesting union at a single optimum posterior distribution. The majority rule consensus of the posterior distribution of phylogenetic estimates (not shown) possessed branching order nearly identical to the ML analysis. Forty-three clades had posterior probabilities at or above 0.95, representing 20.2% of the possible branches in a tree of 108 OTUs (note that 88.7% of possible branches were present). Of the 23 clades with this level of support in tribe Loeselieae, about 35% conflicted with clades possessing equal support from *trnL–F* data. Many of these involve species of *Ipomopsis*.

Likelihood Analyses of the ITS Region—ML analysis of the ITS data recovered two trees with –ln(*L*) = 9604.35904. The two trees differed only in the placement of *Ipomopsis aggregata* subsp. *candida*. In one tree, *I. aggregata* subsp. *candida* was in a clade with *I. aggregata* subsp. *candida*1, *I. aggregata* subsp. *formosissima*, and *I. tenuituba* subsp. *latiloba* (Fig. 4). In the alternative tree *I. aggregata* subsp. *candida* was unresolved with two other terminals (*I. arizonica*, and *I. macrosiphon*) in the sister position of the clade with *I. aggregata* subsp. *candida*, *I. aggregata* subsp. *formosissima*, and *I. tenuituba* subsp. *latiloba*. Branch order of the ML trees from analysis of ITS sequences, shared much in common with that of the *trnL–F* estimates, but were not identical.

All samples of *Ipomopsis* coalesce within the Loeselieae clade (tribe Loeselieae sensu Porter and Johnson 2000). This clade lacked support from Bayesian analyses. The *Giliopsis* group was represented as sister group to *Loeseliastrum depressum* rather than to *Ipomopsis*; however, this relationship lacked posterior probability support. Similarly, relationships among *Ipomopsis*, *Bryantiella*, *Langloisia*, *Loeseliastrum*, *Eriastrum*, *Loeselia*, *Dayia*, *Giliastrum*, and *Aliciella* lacked posterior probability support.

The ITS sequences provided strong support (*P* = 1.00) for the monophyly of a clade that included all of the *Ipomopsis* samples, except *Ipomopsis havardii*, *I. sonorae*, *I. effusa*, *I. gutatta*, and *I. tenuifolia* (Fig. 4). There was significant support for monophyly of *I. havardii* and *I. sonorae* (*P* = 1.00); however, relationships among this clade and other genera of Loeselieae (e.g. *Bryantiella*, *Dayia*, *Eriastrum*, *Ipomopsis*, *Langloisia*, *Loeselia*, and *Loeseliastrum*) remain uncertain. Likewise, the *Giliopsis* group (Fig. 4) had significant support for monophyly (*P* = 1.00), but its placement among the same genera was unresolved. The phylogenetic relationships

of *Microgilia minutiflora* and *Loeseliatrum depressum* were similarly unclear, with respect to *Ipomopsis* clade.

Monophyly of two major lineages within *Ipomopsis* were supported by ITS data; however, the relationship of *I. polycladon*, a species formerly included in sect. *Microgilia*, was ambiguous. Significant support ($P = 1.00$) was found for the monophyly of the *Elaphocera* clade, incorporating all species of Grant's (1959) sect. *Microgilia*, with the exception of *I. polycladon*, *I. sonorae*, *Microgilia minutiflora*, and *Loeseliastrum depressum*. Monophyly of the remainder of *Ipomopsis* was also supported ($P = 1.00$). While we refer to this clade as "sect. *Ipomopsis,*" it is important to note that this clade included species formerly included in sect. *Phloganthea*. Common ancestry of the *Elaphocera* Clade, sect. *Ipomopsis* and *I. polycladon* was strongly supported ($P = 1.00$), providing support for the monophyly of much of Grant's (1956) circumscription of *Ipomopsis*.

Relationships among most of the species of *Ipomopsis* were not resolved; however, there was slightly more resolution in the strict consensus than observed from chloroplast *trnL–F* sequences. For example, the relationships among *I. thurberi*, *I. pringlei*, *I. pinnata*, *I. macombii*, and *I. wendtii* had support ($P = 1.00$). Likewise, the *I. polyantha* and *I. sancti-spiritus* clade also had support ($P = 1.00$). Note, however, that the second ITS copy detected in *I. sancti-spiritus* was inferred to be related to *I. aggregata*, *I. tenuituba*, *I. macrosiphon*, *I. multiflora*, and *I. rubra*.

***Bayesian Inference of Combined Data*—**All of the replicate Bayesian analyses of combined *trnL–F* and ITS data converged on similar parameter estimates, clade posterior probabilities, and topologies, suggesting union at a single optimum posterior distribution. The majority rule consensus of the posterior distribution of phylogenetic estimates (not shown) possessed branching order nearly identical to the ML analysis of combined data. 74 clades had

posterior probabilities at or above 0.95, representing 34.7% of the possible branches in a tree of 108 OTUs (note that 95.3% of possible branches were present).

Likelihood Analyses of the Combined trnL–F and ITS Regions—ML analysis of combined *trnL–F* and nrITS DNA sequences recovered a single tree with $-\ln(L) = 17862.76502$ (Fig. 5). In terms of branching order, this tree most resembled the ML tree from *trnL–F*; however, there was a 41.9% increase in the number of clades with posterior probability support > 0.95 relative to the *trnL–F* analysis.

The combined data provided support for most intergeneric relationships among members of Loeselieae. In particular, there was a high degree of support ($P = 0.963$) for the sister group relationship between *Bryantiella palmeri* and a clade incorporating all species of *Ipomopsis*, except *I. havardii*, *I. sonorae*, *Loeseliastrum depressum*, and *Microgilia minutiflora*. The placement of these taxa with genera other than *Ipomopsis* (all with posterior probabilities, $P = 1.00$), yielded *Ipomopsis* sensu Grant and sensu Porter and Johnson non-monophyletic.

Within *Ipomopsis*, species relationships were largely unresolved in the ML tree from combined data, but the primary lineages had significant posterior probabilities ($P = 1.00$). The *Giliopsis* group had significant support for monophyly (Fig. 5), including significant support for a sister group relationship with the remainder of *Ipomopsis*. Similarly, the monophyly of sect. *Elaphocera* and sect. *Ipomopsis*, as well as their sister group relationship had significant posterior probabilities ($P = 1.00$). The species formerly included in sect. *Phloganthea* (Fig. 5) were unquestionably non-monophyletic.

Hypothesis Testing—Application of Shimodaira-Hasegawa tests of a priori hypotheses of the monophyly to *trnL–F* and nrITS DNA sequences provided different inferences (Table 2). Chloroplast *trnL–F* sequences displayed a significant improvement in the likelihood when

contrasting the Porter and Johnson (2000) hypothesis versus that of Grant (1959). By contrast, nrITS DNA sequences could not distinguish the two hypotheses.

Combined cp*trnL–F* and nrITS sequences showed a significant improvement in the likelihood with the Porter and Johnson (2000) hypothesis, relative to that of Grant (1956). At the same time, it is important to recognize that the Bayesian posterior probabilities of both of these hypotheses were $P = 0.000$.

***Divergence Dates for Ipomopsis and the Ipomopsis aggregata Complex*—**Likelihood ratio tests revealed that branch length estimates of *trnL–F*, ITS, and combined data differed significantly from expectations under the molecular clock model ($P < 0.001$, in all three cases). This provided justification for employing a relaxed-molecular clock method. Nonparametric rate smoothing estimates of divergence times for the target clades (Fig. 6) showed similarity among the data sets, given the different genome sources of the genes (Table 3). However, most mean divergence values differed significantly among the *trnL–F*, ITS, and combined sequences estimates (note confidence limits in Table 3). The exceptions to this general pattern included the mean age of the most recent common ancestor (coalescence) for the *Giliopsis* group. In addition, the divergence date estimates using *trnL–F* and the combined data for *Ipomopsis aggregata*, excluding subsp. *bridgesii*, fell within the contrasting confidence limits.

The mean divergence of *Ipomopsis* was estimated by the Bayesian posterior distribution of trees at 31.2 and 36.6 MYBP, for *trnL–F* and combined *trnL–F* + ITS, respectively. The age of coalescence for *Ipomopsis aggregata* was estimated at between 15.4 and 27.0 MYBP, if subspecies *bridgesii* was included, and between 15.4 and 20.3 MYBP if this subspecies was excluded.

DISCUSSION

These analyses demonstrate both the degrees to which *trnL–F* and ITS DNA sequences contribute to our understanding of the diversification of *Ipomopsis* and the uncertainty (areas of conflict and discrepancies in divergence dates) of inferences from these regions. Both *trnL–F* and ITS provide significant evidence for some higher level relationships (e.g. subgeneric or sectional). At the same time, these regions may be of limited utility for inferring phylogenetic relationships among species at lower levels. This is not because the DNA sequences are identical in all, or even many, of the samples. For example, samples of *I. aggregata* differ at between 0 and 8 nucleotide sites (mean = 3.0) and two indels, with respect to *trnL–F* sequences. Variability in ITS sequences is even greater for the same group, differing at between 0 and 10 nucleotide sites (mean = 5.25). It appears, in the case of *trnL–F*, that the signal from these mutations is not greatly hierarchical for samples of *I. aggregata*. This may be the result of a rapid process of diversification, during which no or very few mutations were fixed early in the radiation. If *I. aggregata* were both widespread and maintained large populations early in its diversification, then this pattern is a reasonable outcome. By contrast, the ITS sequence data are hierarchical, but are also ambiguous for the same samples. This may be due in part to polymorphic coding at some sites. Cloning the different ITS variants may improve the apparent ambiguity, however, this was beyond the scope and resources of our study.

Regardless of the issues at lower taxonomic levels, there are consistent, or at least non-contradictory patterns of coalescence between chloroplast and nuclear DNA regions. These include the monophyly of the *Ipomopsis* clade (i.e. all species previously considered part of *Ipomopsis*, exclusive of the *Giliopsis* group, *I. havardii*, *I. sonorae*, *Microgilia minutiflora*, and *Loeseliastrum depressum*). A second group supported by all analyses is the *Giliopsis* group. The

monophyly of the *Elaphocera* clade has significant support from ITS sequences, and three indels from the *trnL–F* data. Although the indels were not included as additional binary characters in the analyses, the nucleotide substitutions provide no contradictory evidence. Both nuclear and chloroplast sequences also display significant support for the sister-group relationship of *I. havardii* and *I. sonorae,* and their relationship to species outside of *Ipomopsis*.

Patterns of agreement are matched by patterns of disagreement, with the two regions providing significant statistical support for conflicting inferences of relationship. As noted above, of the clades involving taxa from *Ipomopsis* with statistical support, nearly one-third are in conflict. Given evidence for hybridization and introgression within *Ipomopsis* (e.g. Campbell and Waser 2001; Campbell et al. 1998, 2002; Grant 1992b; Grant and Wilken 1986, 1987, 1988a; Wilken and Allard 1986; Wolf and Soltis 1992; Wolf et al. 2001), this degree of conflict is not unexpected. At the same time, other phenomena may play a role in the conflict in gene coalescences, including lineage sorting, gene duplication, or gene conversion (in ITS).

Phylogenetic Inferences Concerning Ipomopsis—Grant (1959, 1998a,b; Fig. 2) provided a concise hypothesis describing phylogenetic relationships and the origin of *Ipomopsis*. Grant suggested that the origin of *Ipomopsis* ultimately must be sought within the genus *Loeselia*, which he treats as a monogeneric, tropical tribe (Grant 1997). It is from *Loeselia* that *Gilia* (in the broad sense, e.g. Grant 1998a) has its origin, and more specifically, *Gilia* sect. *Giliastrum*, with several woody-based, perennial representatives. Grant proposed that from *Gilia* sect. *Giliastrum,* the remainder of *Gilia* has its origin; furthermore, from this section another lineage gives rise to *Ipomopsis*, *Eriastrum*, and *Langloisia*. It should be clear from this description that Grant indirectly infers that *Gilia* and *Loeselia* are paraphyletic groups or grades,

rather than monophyletic groups in the sense we employ the term here. However, he adamantly states that *Gilia* is not polyphyletic (Grant 1998a,b, 2004).

The more recent reclassification of Polemoniaceae by Porter and Johnson (2000), incorporating both traditional comparative data and molecular phylogenetic studies, provides a contrasting but similar suggestion regarding the origin of *Ipomopsis*. In their classification, only those groups for which there was evidence of monophyly were accorded taxonomic recognition. *Ipomopsis* is classified as a member of tribe Loeselieae, which includes, among other genera, *Giliastrum* (included by Grant 1959, 1998a, 2004 within *Gilia*), *Eriastrum*, *Langloisia*, *Loeseliastrum*, and *Loeselia*. Although these are the same genera (or groups) specified by Grant as involved in the origin of *Ipomopsis*, these genera are all supported as monophyletic by Porter and Johnson (i.e. *Giliastrum* and *Loeselia*). Further, *Gilia* sensu stricto (see Porter and Johnson 2000) is not included as part of this tribe. Porter and Johnson (2000) do not specify any details regarding how *Giliastrum*, *Eriastrum*, *Langloisia*, *Loeseliastrum*, and *Loeselia* are related to *Ipomopsis*, merely that these genera all share a common ancestor. Our sampling outside *Ipomopsis* incorporates representatives from all genera of Polemoniaceae. This should allow a reasonable assessment of Grant's (1959, 1989b) hypothesis concerning the origin and phylogenetic relationships of *Ipomopsis*, and also of Porter and Johnson's (2000) assertions of monophyly, with respect to the chloroplast and nuclear genes we have examined. Rather than considering these hypotheses only in total (i.e. they are either supported or unsupported), we also deconstruct the hypotheses, examining support for the elements that compose them. In doing so, some aspects of both Grant's and the Porter and Johnson hypotheses are supported by phylogenetic inferences from *trnL–F* and ITS sequence data, but others are not.

The close phylogenetic relationship among *Ipomopsis*, *Eriastrum*, *Langloisia* and *Loeseliastrum*, groups sharing the uncommon chromosome number of $2n = 14$, find support from our data (Fig. 5), as has been found in previous studies of ITS (Porter 1993, 1996), *matK* (Johnson et al. 1996), *ndhF* (Prather et al. 2000), and nrITS and five chloroplast DNA sequence regions (Johnson et al. 2008). Whether this close phylogenetic relationship is a sister group relationship and whether other taxa are involved differs depending on the gene used for inference. For example, *trnL–F* and combined *trnL–F* + nrITS sequences (Figs. 3, 5) support a sister group relationship between *Ipomopsis* (including the *Giliopsis* group) and *Bryantiella palmeri*; this group is sister to a clade composed of *Microgilia* (included by Grant 1959 in *Ipomopsis*), *Loeseliastrum*, *Eriastrum* and *Langloisia*. By contrast, ITS sequence data support common ancestry of *Ipomopsis* with members of tribe Loeselieae (Fig. 4; see also Porter and Johnson 2000), which includes *Langloisia*, *Loeseliastrum*, and *Eriastrum*, but the branch order is ambiguous (i.e. clades lack significant posterior probabilities).

The origin of *Ipomopsis* (as well as *Eriastrum* and *Langloisia*) from *Loeselia*, through *Gilia* sect. *Giliastrum* is not supported by these data. Indeed, the origin of the remainder of *Gilia* sensu Grant (1959, 1998a, 2004) through *Giliastrum* is unsupported by both *trnL–F*, ITS and combined sequences. Indeed, *Gilia* sensu Grant is unquestionably inferred polyphyletic from both nuclear and chloroplast genes (e.g. Fig. 6). While both *Loeselia* and *Giliastrum* are members of Loeselieae sensu Porter and Johnson (2000), and thus related to *Ipomopsis*, they do not form grades associated with *Ipomopsis*. That is, *Loeselia* is inferred to be a monophyletic group and sister to either a clade including *Dayia*, *Bryantiella glutinosa*, *Ipomopsis havardii*, and *I. sonorae* (*trnL–F* and combined sequences), or sister to a clade composed of *Dayia scabra* and *B. glutinosa* (ITS). In either case, *Loeselia* is not implicated as the putative ancestor of

Giliastrum nor *Ipomopsis*. Indeed, *trnL–F* and combined sequences infer a closer relationship between *Ipomopsis* and *Loeselia* than between *Ipomopsis* and *Giliastrum* s.s. *Bryantiella palmeri*, a member of Grant's *Gilia* sect. *Giliastrum*, is inferred to be sister to *Ipomopsis* (*trnL–F* and combined sequences). This might be interpreted as support for Grant's hypothesis (e.g. origin of *Ipomopsis* through sect. *Giliastrum*); however, Grant suggested that *Giliastrum rigidulum* or a similar ancestor provided the link between *Giliastrum* and *Ipomopsis*, rather than *B*. *palmeri*. In fact, the greatest shortcoming of Grant's hypothesis for the origin of *Ipomopsis* appears to lie in the use of extant groups (e.g. *Loeselia* and *Giliastrum*) as ancestors. *Loeselia* and *Giliastrum* are apparently clades that are contemporary with *Ipomopsis*, diversifying largely at the same time. These groups share ancestry, but are not themselves ancestors.

Monophyly of Ipomopsis—*Ipomopsis*, as taxonomically accepted today, dates only from the mid-1950s, even though the genus was first proposed in the early 1800s and the names of some species date to the works of Linnaeus, Lamarck, and Cavanilles. Grant's benchmark classification of *Ipomopsis* (1956, 1959) asserted that it was a natural lineage, though the term monophyly was not used. At the same time, Grant's discussions concerning the origin and diversification of Polemoniaceae (1959, 1998b) leads one to the conclusion that *Ipomopsis* is monophyletic. Porter and Johnson (2000) were very explicit in hypothesizing that their circumscription of *Ipomopsis* (i.e. excluding *Microgilia minutiflora* and *Loeseliastrum depressum*) was a monophyletic group. The chloroplast DNA sequences presented in this study reject the monophyly of Grant's (1956, 1959) circumscription of *Ipomopsis*, based on the Shimodaira-Hasegawa test. The monophyly of the more contemporary circumscription of *Ipomopsis* (Porter and Johnson 2000) cannot be rejected by this test. Nuclear ITS sequences can reject neither Grant's nor the Porter and Johnson circumscriptions of *Ipomopsis*, indicating that

the ITS data cannot distinguish between these two hypotheses. Regardless of the tests of monophyly, there is little question that the Grant and the Porter and Johnson circumscriptions are very similar and both are largely consistent with the data presented here. Of the 29 species and 24 subspecies that have been placed in *Ipomopsis*, four species are inferred by these data to be more closely related to genera other than to *Ipomopsis*. A fifth species, previously transferred to *Acanthogilia*, was not considered as part of the *Ipomopsis* ingroup, because previous work strongly supports its placement outside of Loeselieae (Day and Moran 1986; Johnson et al. 1996; Porter 1996; Porter and Johnson 2000). The remainder and majority of *Ipomopsis* are arguably monophyletic.

Although ITS and *trnL–F* sequences infer a monophyletic *Ipomopsis*, with exceptional taxa discussed below, these data sets individually disagree on the relationships between the *Giliopsis* group and the remainder of *Ipomopsis*. Chloroplast *trnL–F* and combined sequences unambiguously provide significant evidence that *Giliopsis* is the sister group to the remainder of *Ipomopsis* (Figs. 3, 5). On the other hand, ITS sequences weakly infer that the *Giliopsis* group is related to *Microgilia minutiflora* and *Loeseliastrum* (Fig. 4). Although the inference concerning the placement of *Giliopsis* lacks posterior probability support by ITS, it is difficult to say that the phylogenetic estimate is due only to error. While it is true that there is a higher degree of homoplasy within the ITS data (CI excluding uninformative characters 0.39) than found with *trnL–F* data (CI excluding uninformative characters 0.61), alternative explanations such as differential gene conversion, lineage sorting, and hybridization should not be ruled out. The sister group relationship between the *Giliopsis* group and the remainder of *Ipomopsis* remains the better-supported hypothesis from our data as evidenced by the combined analysis (Fig. 5); however, relationships involving *Giliopsis* are evidently more complex and require further study.

Chloroplast and nuclear DNA sequences collectively support the exclusion of four species, previously included in *Ipomopsis*. Two species were removed from *Ipomopsis* by Porter and Johnson (2000), based on morphological and preliminary molecular data. *Microgilia minutiflora* (= *Ipomopsis minutiflora*) is inferred to be the sister lineage to *Eriastrum*, *Langloisia*, and *Loeseliastrum* (e.g. Figs. 3, 5) based on *trnL–F* and combined sequences, but ITS inferences conflict in the placement of *M. minutiflora* (see Fig. 4). *Loeseliastrum depressum* (= *I. depressa*) is inferred to be sister to *Loeseliastrum matthewsii*. ITS sequences alone do not support these relationships; rather, *L. depressum* is in a clade with *Giliopsis* and *M. minutiflora*. The two remaining species that are not part of a monophyletic *Ipomopsis* are *I. havardii* and *I. sonorae*. Both ITS and *trnL–F* data provide strong support that these are sister species and, significant support (*trnL–F* and combined sequences) that they are related to *Bryantiella glutinosa*, *Dayia*, and *Loeselia*. Similar to the inferences concerning *Giliopsis*, the hypothesis with the greatest overall support (i.e. both *trnL–F* and combined data) is the sister relationship to the *Dayia-Bryantiella glutinosa* clade. While it may be ambiguous to what genus *I. havardii* and *I. sonorae* are most closely related, all of the candidate genera are known to have a base chromosome number different than that of *Ipomopsis*. For example, *Dayia* has been shown to have a gametic number of $n = 9$ (Porter and Johnson 2000) and *Loeselia* has a similar gametic number of $n = 9$ (Grant 1959). The chromosome number of *I. havardii* and *I. sonorae* have not been recorded, but the phylogenetic inferences presented here would predict that they should have a gametic number of $n = 9$ (parsimony reconstruction not shown).

Divergence Time Estimates for Ipomopsis and the Ipomopsis aggregata Complex—Our ability to estimate divergence times in Polemoniaceae is limited by the fossil record of this family, and the reliability of current methods of inference. Few fossils have been discovered. The

recent unearthing of *Gilisenium hueberii* (Lott et al. 1998) may represent the most important Polemoniaceae fossil discovered so far, due to its completeness and antiquity. Using this fossil to calibrate the common ancestor of tribe Gilieae, the divergence time for *Ipomopsis* (sensu Porter and Johnson 2000) is 31.2 ± 0.33 (*trnL–F*) and 36.6 ± 0.31 (combined data) MYBP (Table 3; Fig. 6). ITS sequences fail to infer this clade; however, divergence of *Ipomopsis* excluding the *Giliopsis* clade is 38.8 ± 0.46 MYBP. This corresponds to a Paleogene (Tertiary) origin for *Ipomopsis*, from as recent as the early Oligocene to as early as the late Eocene. This unquestionably predates the estimates of previous authors. For example, Grant (1959) envisioned the common ancestor of *Cantua* and *Cobaea* (and thus the family Polemoniaceae) to be present during the Eocene or early Oligocene, the same time period we estimate the origin of *Ipomopsis*. Grant (1959, 1998) and Raven and Axelrod (1978), in discussing diversification and origin of Grant's Gilieae (including *Ipomopsis*), suggest that genera of this tribe have arisen or expanded during the late Oligocene and early Miocene or, in many cases, as recent as the Pleistocene. In fairness to these previous authors, most of the earlier estimates were made prior to the discovery of Tertiary-aged, *Gilia*-like fossil, and as such were largely educated conjectures based on apparent correlations between species distributions and habitat and the estimated origin of those habitats. We show that use of estimated ages of origin of the habitats in which extant species occur can produce vastly different divergence dates than those when fossils are included. We caution that both methods should be interpreted with skepticism.

The timing of diversification of the *Ipomopsis aggregata* complex and hybridization among the members of this complex (*I. aggregata*, *I. tenuituba*, *I. macrosiphon*, and *I. arizonica*) has been debated. Grant (1981, 1992b) argued that the *I. aggregata* complex is characterized by gradual divergence and speciation, followed by recent introgressive hybridization. It was

suggested that the primary divergence was the result of a shift between hummingbird and hawkmoth pollination systems (Grant and Grant 1965; Grant and Wilken 1988a). Wolf et al. (1993) used allozyme and chloroplast restriction site data to infer that the complex is of recent divergence, with subsequent hybridization. Wolf et al. concluded that flower color, and thus hummingbird and hawkmoth pollination systems each have evolved more than once (see also Grant 1992a).

The coalescent event for the *I. aggregata* complex is estimated to be between 15.4 ± 0. 33 and 27.0 ± 0.45 MYA (*trnL–F* and ITS respectively). These dates place the origin of the *Ipomopsis aggregata* complex somewhere between the late Oligocene and the mid-Miocene. This age is likely far older than either Grant (1992b) or Wolf et al. (1993, 1997) would have proposed. The large discrepancy between the *trnL–F* and ITS estimates are in part a function of the disparate placement of *I. aggregata* subsp. *bridgesii* (compare Figs. 3–5). The placement of *I. aggregata* subsp. *bridgesii* may be a function of processes other than divergent speciation (e.g. hybridization or lineage sorting). For ITS, the coalescence of members of the *I. aggregata* complex exclusive of *I. aggregata* subsp. *bridgesii* is estimated at 20.3 ± 0.40 MYA, in the early Miocene. While this estimate is closer to that of *trnL–F* and combined data estimates, it remains significantly older.

Whether divergence has been slow or rapid is less straightforward than is estimating the absolute age of the *Ipomopsis aggregata* complex. If the *I. aggregata* clades (inclusive or exclusive of subsp. *bridgesii*) are considered, greater diversity in floral form, pollination, and habit is reflected than the forms found in *I. aggregata* alone. In addition to *I. aggregata*, *I. tenuituba*, *I. macrosiphon*, *I. sanctispiritus*, and *Gilia polyantha* var. *whitingii* are within this clade and therefore linked to the diversification of *I. aggregata*. Similarly, other species that are

red-flowered and hummingbird pollinated are also part of this clade (i.e. *I. arizonica* and *I. rubra*). This indicates that diversification as a whole has been far more complex than implied by a divergence event involving a shift from hawkmoth to hummingbird pollination. Indeed, the branching order necessitates multiple transitions from hummingbird pollination to hawkmoth pollination, multiple origins of hummingbird from hawkmoth pollination systems, or a combination of the two. Arguments can be made favoring gradual divergence coupled with interspecific and introgressive hybridization, rapid speciation 10–20 MYA followed by interspecific and introgressive hybridization, or recent, rapid speciation of 1) a compilospecies that has been taking in genes from many formerly isolated species, or 2) a series of diploid hybrid (or recombinational) species. These data alone cannot distinguish among these, and other possible hypotheses. Further, chloroplast restriction site (Wolf et al. 1993, 1997), allozyme (Wolf et al. 1991), and biogeographic (Grant 1992b) data can also be argued as consistent with many of these differing hypotheses. Additional data are essential to understanding the rate and mode of diversification in *I. aggregata* complex. Although Wolf et al. (1997) are correct that greater sampling (additional genes and additional populations for genetic analyses) is needed, we also lack the needed crossing studies (i.e. investigating the degree of reproductive isolation) and comparative data on pollination and floral traits (including floral pigment types and distribution) from the relevant taxa, including species not traditionally part of the *I. aggregata* complex (e.g. *I. tenuituba*, *I. macrosiphon*, *G. polyantha* var. *whitingii*, *I. rubra*, and *I. sanctispiritus* among others).

Ipomopsis macrosiphon provides a good case study of the limits of the explanatory power of our data when coupled with available evidence. This taxon was originally described as a variety of "*Gilia aggregata*. " More recently, Grant and Wilken (1986) included the populations

currently treated as *I. macrosiphon* as conspecific with *I. tenuituba* and later segregated three races (Grant and Wilken 1988b): subspp. *tenuituba*, *latiloba*, and *macrosiphon*. These three races were presumed to share a common origin. Wolf et al. (1991, 1993) and Wolf and Soltis (1992) showed a closer genetic and phylogenetic relationship between *I. tenuituba* subsp. *macrosiphon* and *I. thurberi*, than among the races of *I. tenuituba*, providing a serious challenge to species status of *I. tenuituba* sensu lato. In response to Wolf's research, Grant elevated *I. macrosiphon* to species status, arguing that a separate allopatric speciation event gave rise to *I. macrosiphon* and *I. thurberi*, the morphological similarity between *I. macrosiphon* and *I. tenuituba* being due to convergence.

The chloroplast and nuclear DNA sequences provide no evidence that *Ipomopsis tenuituba* subsp. *tenuituba*, *I. tenuituba* subsp. *latiloba*, and *I. macrosiphon* share common ancestry to the exclusion of other recognized taxa. As Wolf et al. (1993) found, *trnL–F* sequences suggest that *I. macrosiphon*, *I. thurberi*, and *I. macombii* form a clade with high posterior probability (Fig. 3). By contrast and again with high posterior probability, ITS sequences place *I. macrosiphon* in a clade with *I. arizonica*, a clade including several subspecies of *I. aggregata* and *Gilia polyantha* var. *whitingii*, and a clade composed of *I. aggregata* subspp. *formosissima* and *candida*, and *I. tenuituba* subsp. *latiloba*. ITS sequences also recover a clade with *I. thurberi* together with *I. pinnata*, *I. pringlei*, *I. wendtii*, and *I. macombii*, with a posterior probability of 1. We conclude that relationships of *I. macrosiphon* are not consistent with a bifurcating phylogenetic tree. This reticulate pattern could be due to hybridization. An alternative explanation is lineage sorting of the nuclear ITS copy, however, this would require the maintenance of polymorphism for 16 to 26 MY, which seems unlikely unless very large populations were maintained. Present day hybridization is believed to be occurring, based upon

morphologically intermediate individuals on Mount Graham, Arizona (Wolf et al. 1992). The ITS region of *I. macrosiphon* differs from that of *I. arizonica* at only one nucleotide position (aligned site 662). These sites are identical and polymorphic in *I. arizonica* (A and G), whereas in *I. macrosiphon* the position has only A. The differences between *I. macrosiphon* and *I. aggregata* subsp. *formosissima* are only at site 662 (A vs. G, respectively) and site 691 (G vs. A, respectively). By contrast, *trnL–F* sequences of *I. macrosiphon* and *I. thurberi* are identical except for an additional nucleotide associated with a simple sequence repeat in *I. thurberi*. We believe that our data are consistent with an additional alternative hypothesis for the origin of *I. macrosiphon* of diploid hybrid speciation, involving *I. thurberi* and either *I. arizonica* or *I. aggregata* subsp. *formosissima*. This latter hypothesis is consistent with traditional lines of evidence (morphology and biogeography), the genetic evidence, and unpublished crossing studies.

Crosses between *I. aggregata* subsp. *formosissima* (Cerro Potosi, Nuevo Leon, Mexico) and *I. thurberi* (Santa Rita Mountains, Santa Cruz County, Arizona, U.S.A.) produce fully fertile F1s (Porter, unpubl. data). While *I. aggregata* subsp. *formosissima* has crimson flowers with white at the orifice (the anthocyanin, pelargonidin predominating but cyanidin present in small amounts), and *I. thurberi* has purple flowers (the anthocyanin, delphinidin predominating), the F1 has pink-magenta flowers (anthocyanins, cyaniding, and delphinidin in high proportions). The fact that crossing is demonstrably possible between *I. aggregata* subsp. *formosissima* and *I. thurberi*, and that the progeny of this cross takes on a floral morphology and color (including floral pigments) similar to *I. macrosiphon*, is consistent with a hybrid origin, but does not rule out possible alternative hypotheses. The collective inferences from *trnL–F* and ITS combined with previous evidence are indicative of one of three scenarios: 1) hybridization between *I.*

aggregata and *I. thurberi*, no earlier than between 4.4 ± 0.591 and 7.9 ± 0.529 MYBP (late Miocene to early Pliocene) or more recently, giving rise to a recombinational species, *I. macrosiphon* on Mount Lemmon, in southeastern Arizona (the Mount Graham population of *I. macrosiphon* may have the same or an independent origin, i.e. Wolf et al. 1992); 2) allopatric divergence between sister taxa, *I. macrosiphon* and *I. thurberi*, followed by more recent introgressive hybridization with *I. aggregata*; or 3) allopatric divergence between sister taxa, *I. macrosiphon* and *I. aggregata*, followed by more recent introgressive hybridization with *I. thurberi*. Although we cannot select among these alternative hypotheses with the existing data, it may be possible to design future studies that can reject specific hypotheses.

Sectional Classification of Ipomopsis—Grant (1956, 1959) proposed a sectional classification for *Ipomopsis* (Table 1; Figs. 3–5) recognizing three sections. *Phloganthea* is the purported ancestral section, largely characterized as perennials with glomerulate, thyrsoidal inflorescences, medium-sized corollas with exserted stamen, and bee pollination. As the most primitive section, Grant may have envisioned *Phloganthea* as a grade; however, he did not specify precise relationships (see Grant 1959). *Microgilia* was considered a more advanced section, including small-flowered annuals and perennials, with derived pollination systems, including beetle, solitary bee, and autogamy (Grant and Grant 1967). Section *Ipomopsis* was also considered advanced, including species with large salverform corollas, and hawkmoth or hummingbird pollination. Both *trnL–F* and ITS data display a significantly poorer fit if the monophyly of each section is imposed as a constraint. Of the three sections recognized by Grant, two clades largely correspond to sections *Ipomopsis* and *Microgilia*, respectively (Figs. 3–5). Only section *Phloganthea* is unsupported, its members being within section *Ipomopsis*, in the *Giliopsis* clade, or outside of the genus (i.e. *I. havardii*).

One significantly supported monophyletic group, not treated nomenclaturally since the works of Gray (1875) and Brand (1907), is the *Giliopsis* group. The *Giliopsis* group is found in the Peninsular Ranges of Baja California, Mexico, and adjacent San Diego County, California, USA. The inclusion of these three species in *Loeselia* by Asa Gray bears testament to their morphological distinctness with respect to other species of *Ipomopsis*, which Gray placed in *Gilia* section *Ipomopsis*. For example, the corollas are strongly bilaterally symmetric (less so in *I. tenuifolia*) with truncate but cuspidate lobes. Grant's inclusion of this group in *Ipomopsis* is supported by a number of morphological similarities, including zonocolporate pollen with striate-reticulate exine, glandular trichomes with a single-celled terminal gland, and chromosome number of $2n = 14$. Chloroplast *trnL–F* and combined data display significant support for the sister group relationship between *Giliopsis* and *Ipomopsis*. As noted above, ITS sequences do not infer that *Giliopsis* is most closely related to *Ipomopsis*. From this, it might be argued that the *Giliopsis* group should be recognized at the generic rank. We suggest that such a transfer may be premature, as there is no significant support for this portion of the ITS inference, as evidenced by the combined analysis. Retention of *Giliopsis* within *Ipomopsis* seems the most appropriate course, unless or until data can be found that refutes the morphological, *trnL–F,* and combined data inferences. At the same time, the proposed sister group relationship between *Giliopsis* and *Ipomopsis* argues strongly for some degree of nomenclatural and taxonomic recognition of *Giliopsis*. We therefore propose that the *Giliopsis* group should be treated at the sectional rank (Table 4), within *Ipomopsis*.

Grant's (1959) section *Microgilia* includes annual and perennial species with capitate inflorescences. Most species formerly placed in *Microgilia* are inferred to be monophyletic, based on ITS and combined sequences (Figs. 4, 5; *Elaphocera* clade), with the exceptions of

Microgilia minutiflora, *Loeseliastrum depressum*, *Ipomopsis polycladon*, and *I. sonorae*. Interestingly, of these excluded species, only *I. polycladon* possesses congested, somewhat capitate inflorescences. The removal of *M. minutiflora* is problematical only from a nomenclatural point of view. Because the type of *Ipomopsis* section *Microgilia* is *M. minutiflora*, the sectional name, *Microgilia,* can no longer be applied to this section if *M. minutiflora* is removed. However, Nuttall's section *Elaphocera* (of *Gilia* Ruiz & Pav.), whose type is *Ipomopsis congesta*, is available for this section (Table 4). Interspecific relationships among species of the *Elaphocera* clade are poorly resolved. ITS and combined data have significant support for the sister relationship between *I. pumila*, of the intermountain western United States, and *I. gossypifera* (Figs. 4, 5), of the Andes Mountains of Argentina, Bolivia, and Chile, a relationship proposed by Grant (1959: 246). *Ipomopsis gossypifera* represents a particularly obvious case of amphitropical dispersal from North America to South America. There is also significant support from *trnL–F* and combined data for monophyly of *I. gunnisonii*, of the intermountain western United States, *I. wrightii*, a little known, narrow endemic species of western trans-Pecos Texas and adjacent Chihuahua, Mexico, and *I. roseata*, the only known tetraploid member of this section. *Ipomopsis roseata* is usually a woody perennial and morphologically similar to *I. congesta* and *I. spicata*. The range of *I. roseata* overlaps with the annual species, *I gunnisonii*. These facts may make the hypothesis of allotetraploid origin of *I. roseata*, involving *I. gunnisonii* and either *I. congesta* or *I. spicata* attractive; however, the combined data infer more recent common ancestry between *I. gunnisonii* and *I. wrightii* than between *I. gunnisonii* and *I. roseata*, implying that *I. roseata* diverged prior to the origin of *I. gunnisonii*.

Ipomopsis section *Ipomopsis*, as circumscribed by Grant (1959), is largely supported, with the inclusion of *Ipomopsis multiflora*, *I. pinnata*, and *I. polyantha*, formerly in section *Phloganthea*. As observed with the previous group, most interspecific relationships within section *Ipomopsis* are without significant support (Figs. 3–5).

The relationships of *Ipomopsis polycladon*, a widespread annual species of the intermountain and desert western United States, are not clear. Chloroplast *trnL–F* data (Fig. 3) unambiguously place *I. polycladon* within section *Ipomopsis*. ITS data place this annual as the sister group to a clade composed of both sections *Ipomopsis* and *Elaphocera* (Fig. 4). The combined *trnL–F* and ITS analysis finds *I. polycladon* as the sister group to section *Ipomopsis* (Fig. 5). This latter placement has significant statistical support and is therefore the preferred hypothesis. Even so, the great morphological similarity between *I. polycladon* and members of section *Elaphocera* coupled with its anomalous morphology relative to section *Ipomopsis*, leaves us reluctant to place this species within section *Ipomopsis*.

Nomenclatural Changes—The sectional classification proposed in Table 4 requires that two nomenclatural innovations be made. This change involves the transfer of *Loeselia* section *Giliopsis* A. Gray and *Gilia* sect. *Elaphocera* Nutt. into *Ipomopsis*.

Ipomopsis section **Giliopsis** (Gray) J. M. Porter, L. A. Johnson & D. Wilken, comb. nov.

Loeselia sect. *Giliopsis* A. Gray, *Proc. Am. Acad. Arts* 11: 86. 1876. *Loeselia* subgen. *Giliopsis* (A. Gray) Peter, *Nat. Pflanzenfam*. 4(3a): 54. 1891. LECTOTYPE (Grant 1956: 353): *Ipomopsis tenuifolia* (A. Gray) V. E. Grant.

Ipomopsis section **Elaphocera** (Nutt.) J. M. Porter, L. A. Johnson & D. Wilken, comb. nov. *Gilia* sect. *Elaphocera* Nuttall, *J. Acad. Nat. Sci. Philadelphia ser. 2*, 1: 155. 1848. *Gilia* subgen. *Elaphocera* (Nutt.) Milliken, *Univ. Calif. Publ. Bot.* 2: 24. 1904. LECTOTYPE (Grant 1956: 357): *Ipomopsis congesta* (Hook.) V. E. Grant.

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TABLE 1. The sectional classification of *Ipomopsis* Michx., following Grant (1956, 1959). Taxa with an asterisk were not included in Grant's synopsis, but are placed based upon other authors' suggestions.

Section Group Species Infraspecific taxon
Section *Phloganthea* (Gray) V. E. Grant (type = *Ipomopsis pinnata*)
Ipomopsis havardi (A. Gray) V. E. Grant
Ipomopsis multiflora (Nutt.) V. E. Grant
Ipomopsis pinnata (Cav.) V. E. Grant
Ipomopsis polyantha (Rydb.) V. E. Grant
"*Giliopsis* Group"
**Ipomopsis effusa* (A. Gray) Moran
**Ipomopsis guttata* (A. Gray) Moran
Ipomopsis tenuifolia (A. Gray) V. E. Grant
Section *Ipomopsis* (type = *Ipomopsis rubra*)
Ipomopsis aggregata (Pursh) V. E. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *attenuata* (A. Gray) V. E. Grant & A. D. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *bridgesii* (A. Gray) V. E. Grant & A. D.

Grant

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *candida* (Rydberg) V. E. Grant & A. D. Grant

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *carmenensis* Henr.

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *collina* (Greene) Wilken & Allard

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *formosissima* (Greene) Wherry

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *texana* (Greene) Wherry

Ipomopsis aggregata (Pursh) V. E. Grant subsp. *weberi* V. E. Grant & Wilken

Ipomopsis arizonica (Greene) Wherry

Ipomopsis laxiflora (J. M. Coult.) V. E. Grant

Ipomopsis longiflora (Torr.) V. E. Grant

Ipomopsis longiflora (Torr.) V. E. Grant subsp. *australis* R. A. Fletcher & W. L. Wagner

Ipomopsis longiflora (Torr.) V. E. Grant subsp. *neomexicana* Wilken

Ipomopsis macombii (Torr.) V. E. Grant

Ipomopsis macrosiphon (Kearney & Peebles) V. E. Grant & Wilken

**Ipomopsis monticola* J. M. Porter & L. A. Johnson

**Ipomopsis pringlei* (Gray) Henr.

Ipomopsis rubra (L.) Wherry

**Ipomopsis sancti-spiritus* Wilken & R. A. Fletcher

Ipomopsis tenuituba (Rydb.) V. E. Grant

Ipomopsis tenuituba (Rydb.) V. E. Grant subsp. *latiloba* V. E. Grant & Wilken

Ipomopsis thurberi (Torr.) V. E. Grant

**Ipomopsis wendtii* Henr.

Section *Microgilia* (Benth.) V. E. Grant (type = *Ipomopsis minutiflora*)

Ipomopsis congesta (Hook.) V. E. Grant

Ipomopsis congesta (Hook.) V. E. Grant subsp. *crebrifolia* (Nutt.) A. G. Day,

Ipomopsis congesta (Hook.) V. E. Grant subsp. *frutescens* (Rydb.) A. G. Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *montana* (A. Nelson & P. B. Kenn.) V. E. Grant

Ipomopsis congesta (Hook.) V. E. Grant subsp. *nevadensis* (Tidestr.) Kartesz & Gandhi

Ipomopsis congesta (Hook.) V. E. Grant subsp. *palmifrons* (Brand) A. G. Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *pseudotypica* (Constance & Rollins) A. G. Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *viridis* (Cronquist) A. G. Day

Ipomopsis depressa (A. Gray) V. E. Grant

Ipomopsis gossypifera (Gillies ex Benth.) V. E. Grant

Ipomopsis gunnisonii (Torr. & A. Gray) V. E. Grant

Ipomopsis minutiflora (Benth.) V. E. Grant

Ipomopsis polycladon (Torr.) V. E. Grant

Ipomopsis pumila (Nutt.) V. E. Grant

Ipomopsis roseata (Rydb.) V. E. Grant

Ipomopsis sonorae (Rose) A. Grant

Ipomopsis spicata (Nutt.) V. E. Grant

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *capitata* (A. Gray) V. E. Grant

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm.

var. *orchidacea* (Brand) Dorn

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm.

var. *cephaloidea* (Rydb.) Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *robruthiae* Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *tridactyla* (Rydb.) Wilken & R. L. Hartm.

**Ipomopsis wrightii* (A. Gray) Shinners

TABLE 2. Shimodaira-Hasegawa Tests contrasting a priori hypotheses of the monophyly of the generic circumscription of *Ipomopsis* Michx. sensu Grant (1956, 1959; = Grant) and sensu Porter and Johnson (2000; = P&J) based on *trnL–F*, nrITS, and combined *trnL–F* + nrITS sequence data. The null hypothesis (H_o) is represented by the maximum likelihood tree(s) with the imposed constraint representing Grant's circumscription of *Ipomopsis*. The alternative hypothesis (H_A) is the maximum likelihood tree(s) with the imposed constraint representing P&J's circumscription of *Ipomopsis*. A 1-tailed test was used to determine if the P&J hypothesis showed significant improvement in the likelihood over Grant. Negative log-likelihood (-ln(*L*)), likelihood difference (diff), and probabilities (*P*) are provided for the a priori alpha value of 0.05. Bonferroni correction for the alpha value, given the three tests involving nrITS, yields a significance level of 0.0167. Asterisks following the probabilities indicate comparisons that display a significant improvement in likelihood.

Gene	-ln(L_{Ho})	-ln(L_{HA})	diff	*P*
trnL–F	7328.984	7251.522	77.46207	0.002*
nrITS	9614.920	9620.42697	5.50651	0.360
trnL–F + nrITS	17948.416	17893.017	55.39948	0.011*

TABLE 3. Estimated ages of common ancestry of the genus *Ipomopsis* and clades within the genus, based on nonparametric rate smoothing of a random samples of 100 trees from Bayesian posterior distributions, from analyses of chloroplast *trnL–F* region, nuclear ribosomal internal transcribed spacer region (nr ITS), and combined DNA sequences. Age estimates are reported in million of years before present (MYBP). These measures include mean, standard error of the mean (SEM), and 95% confidence interval of the mean. Clade A = common ancestor of *Ipomopsis*, excluding *Ipomopsis havardii* and *I. sonorae* (this clade was never recovered in analyses of nrITS sequences alone, therefore for nrITS we report Clade A' = common ancestor of *Ipomopsis*, excluding *Ipomopsis havardii*, *I. sonorae* and *Ipomopsis* subgenus *Giliopsis*; Clade B = common ancestor of all subspecies of *Ipomopsis aggregata*; Clade C = common ancestor of all subspecies of *Ipomopsis aggregata*, however, subsp. *bridgesii* need not be a member of the clade; Clade D = common ancestor of *Ipomopsis* subgenus *Giliopsis*; Clade E = common ancestor of *Ipomopsis* sect. *Elaphocera*.

DNA Region Clade	mean (MYBP)	SEM	lower 95% confidence limit	upper 95% confidence limit
trnL–F				
Clade A	31.22	0.33	31.87	30.57
Clade B	15.39	0.30	15.97	14.82
Clade C	15.39	0.30	15.97	14.82
Clade D	13.51	0.64	14.76	12.25
Clade E	28.34	0.33	28.99	27.69
nrITS				
Clade A'	38.76	0.46	39.66	37.86
Clade B	26.99	0.45	27.86	26.11
Clade C	20.30	0.40	21.08	19.52

Clade D	14.46	0.67	15.76	13.15
Clade E	24.84	0.36	25.54	24.14
trnL–F + nrITS				
Clade A	36.64	0.31	37.25	36.03
Clade B	19.07	0.26	19.58	18.56
Clade C	15.97	0.25	16.45	15.48
Clade D	14.39	0.54	15.46	13.33
Clade E	22.33	0.32	22.95	21.71

TABLE 4. A new, revised sectional classification of *Ipomopsis* Michx.

Section Species Infraspecific taxon
Section *Giliopsis* (Gray) J. M. Porter, L. A. Johnson & D. Wilken. (type = *Ipomopsis tenuifolia*)
Ipomopsis effusa (A. Gray) Moran
Ipomopsis guttata (A. Gray) Moran
Ipomopsis tenuifolia (A. Gray) V. E. Grant
Section *Ipomopsis* (type = *Ipomopsis rubra*)
Ipomopsis aggregata (Pursh) V. E. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *attenuata* (A. Gray) V. E. Grant & A. D. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *bridgesii* (A. Gray) V. E. Grant & A. D. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *candida* (Rydberg) V. E. Grant & A. D. Grant
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *carmenensis* Henr.
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *collina* (Greene) Wilken & Allard
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *formosissima* (Greene) Wherry
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *texana* (Greene) Wherry
Ipomopsis aggregata (Pursh) V. E. Grant subsp. *weberi* V. E. Grant & Wilken
Ipomopsis arizonica (Greene) Wherry
Ipomopsis laxiflora (J. M. Coult.) V. E. Grant
Ipomopsis longiflora (Torr.) V. E. Grant

Ipomopsis longiflora (Torr.) V. E. Grant subsp. *australis* R. A. Fletcher & W. L. Wagner

Ipomopsis macombii (Torr.) V. E. Grant

Ipomopsis macrosiphon (Kearney & Peebles) V. E. Grant & Wilken

Ipomopsis monticola J. M. Porter & L. A. Johnson

Ipomopsis multiflora (Nutt.) V. E. Grant

Ipomopsis pinnata (Cav.) V. E. Grant

Ipomopsis polyantha (Rydb.) V. E. Grant

Ipomopsis pringlei (Gray) Henr.

Ipomopsis rubra (L.) Wherry

Ipomopsis sancti-spiritus Wilken & R. A. Fletcher

Ipomopsis tenuituba (Rydb.) V. E. Grant

Ipomopsis tenuituba (Rydb.) V. E. Grant subsp. *latiloba* V. E. Grant & Wilken

Ipomopsis thurberi (Torr.) V. E. Grant

Ipomopsis wendtii Henr.

Section *Elaphocera* (Benth.) V. E. Grant (type = *Ipomopsis congesta*)

Ipomopsis congesta (Hook.) V. E. Grant

Ipomopsis congesta (Hook.) V. E. Grant subsp. *crebrifolia* (Nutt.) A. G. Day,

Ipomopsis congesta (Hook.) V. E. Grant subsp. *frutescens* (Rydb.) A. G. Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *montana* (A. Nelson & P. B. Kenn.) V. E. Grant

Ipomopsis congesta (Hook.) V. E. Grant subsp. *nevadensis* (Tidestr.) Kartesz & Gandhi

Ipomopsis congesta (Hook.) V. E. Grant subsp. *palmifrons* (Brand) A. G. Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *pseudotypica* (Constance & Rollins) A. G.

Day

Ipomopsis congesta (Hook.) V. E. Grant subsp. *viridis* (Cronquist) A. G. Day

Ipomopsis gossypifera (Gillies ex Benth.) V. E. Grant

Ipomopsis gunnisonii (Torr. & A. Gray) V. E. Grant

Ipomopsis pumila (Nutt.) V. E. Grant

Ipomopsis roseata (Rydb.) V. E. Grant

Ipomopsis spicata (Nutt.) V. E. Grant

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *capitata* (A. Gray) V. E. Grant

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm. var. *orchidacea* (Brand) Dorn

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *orchidacea* (Brand) Wilken & R. L. Hartm. var. *cephaloidea* (Rydb.) Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *robruthiae* Wilken & R. L. Hartm.

Ipomopsis spicata (Nutt.) V. E. Grant subsp. *tridactyla* (Rydb.) Wilken & R. L. Hartm.

Ipomopsis wrightii (A. Gray) Shinners

Insertae sedis:

Ipomopsis polycladon (Torr.) V. E. Grant

Species excluded from *Ipomopsis*:

Ipomopsis havardi (A. Gray) V. E. Grant

Ipomopsis sonorae (Rose) A. Grant

APPENDIX 1. Collections sampled for nrITS and cp *trnL–F* DNA sequence variation. Information is provided in the following order: Taxon [sample number if more than one for that taxon], Locality; *collector and collection number* (herbarium where specimen is housed): GenBank accession numbers for nrITS and *trnL–F* regions.

Ipomopsis*—Ipomopsis aggregata* (Pursh) V. E. Grant subsp. *aggregata*, U.S.A., CO, Gunnison Co., Avery Park; *Wilken 13420* (RSA): EU339808, EU348458. *Ipomopsis aggregata* subsp. *attenuata* (A. Gray) V. E. Grant & A. D. Grant, U.S.A., WY, Sweetwater Co., Rock Springs; *Elias 8839* (RSA): EU339809, EU348459. *Ipomopsis aggregata* subsp. *bridgesii* (A. Gray) V. E. Grant & A. D. Grant, U.S.A., CA, Fresno Co., Burns Meadow; *Ross 3100a* (RSA): EU339811, EU348461. *Ipomopsis aggregata* subsp. *candida* (Rydb.) V. E. Grant & A. D. Grant, U.S.A., CO, Gilpin Co., Rollinsville; *Wilken 13371* (RSA): EU339768, EU348418. *Ipomopsis aggregata* subsp. *candida* [2], U.S.A., CO, Douglas Co., Larkspur; *Porter 13705* (RSA): EU339812, EU348462. *Ipomopsis aggregata* subsp. *collina* (Greene) Wilken & Allard, U.S.A., CO, Custer Co., McKenzie; *Wilken 13604* (RSA): EU339813, EU348463. *Ipomopsis aggregata* subsp. *formosissima* (Greene) Wherry, U.S.A., CO, Wayne Co., Canyonlands Nat. Park; *Porter 8042* (SJNM): EU339814, EU348464. *Ipomopsis aggregata* subsp. *weberi* V. E. Grant & Wilken, U.S.A., CO, Routt Co., Steamboat Springs; *Wolf & Wolf 195* (RSA): EU339810, EU348460. *Ipomopsis arizonica* (Greene) Wherry, U.S.A., AZ, Coconino Co., Bonita; *Wilken 14843* (RSA): EU339807, EU348457. *Ipomopsis congesta* (Hook.) V. E. Grant subsp. *congesta* [102] , U.S.A., UT, Uintah Co., Jones Hole; *Porter & Machen 9102* (RSA): EU339781, EU348431. *Ipomopsis congesta* subsp. *congesta* [784], U.S.A., NV, Elko Co., Eureka; *Tiehm 7847* (RSA): EU339780, EU348430. *Ipomopsis congesta* subsp. *crebrifolia* (Nutt.) A. G. Day, U.S.A., UT, Beaver Co., Wahwah Mtns.; *Franklin 7078* (RSA): EU339779, EU348429.

Ipomopsis congesta subsp. *frutescens* (Rydb.) A. G. Day, U.S.A., UT, Washington Co., Zion National Park; *Craig1416* (RSA): EU339777, EU348427. *Ipomopsis congesta* subsp. *nevadensis* (Tidestr.) Kartesz & Gandhi, U.S.A., NV, Lander Co., Toiyabe Mtns.; *Neese & Goodrich 10723* (RSA): EU339782, EU348432. *Ipomopsis congesta* subsp. *palmifrons* (Brand) A. G. Day, U.S.A., NV, Humboldt Co., Santa Rosa Range; *Tiehm 8654* (RSA): EU339778, EU348428. *Ipomopsis effusa* (A. Gray) Moran, Mexico, Baja Calif., Laguna Hanson; *Thorne 55940* (RSA): EU339769, EU348419. *Ipomopsis gossypifera* (Gillies ex Benth.) V. E. Grant, Argentina, Mendoza, La Heras *Porter 11928* (RSA): EU339773, EU348423. *Ipomopsis gunnisonii* (Torr. & A. Gray) V. E. Grant, U.S.A., NM, San Juan Co., Navajo Mine; *Porter 9295* (RSA): EU339776, EU348426. *Ipomopsis guttata* (A. Gray) Moran, Mexico, Baja Calif., El Bashisha; *Thorne 62474* (RSA): EU339770, EU348420. *Ipomopsis havardi* (A. Gray) V. E. Grant, U. S. A, TX, Presidio Co., W Redford; *Porter 11350* (RSA): EU339753, EU348403. *Ipomopsis laxiflora* (J. M. Coult.) V. E. Grant, U.S.A., NM, Torrance Co., Santa Rosa; *Waterfall 11794* (RSA): EU339796, EU348446. *Ipomopsis longiflora* (Torr.) V. E. Grant subsp. *australis* R. A. Fletcher & W. L. Wagner, U.S.A., AZ, Cochise Co., Peloncillo Mtns.; *Porter 7142* (RSA): EU339794, EU348444. *Ipomopsis longiflora* subsp. *longiflora* , U.S.A., NM, Cibola Co., Villa de Cubero; *Helmkmap 7-9* (RSA): EU339795, EU348445. *Ipomopsis longiflora* subsp. *neomexicana* Wilken, U.S.A., AZ, Apache Co., Navajo; *Norris 2688* (RSA): EU339793, EU348443. *Ipomopsis macombii* (Torr.) V. E. Grant, U.S.A., AZ, Santa Cruz Co., Ft. Huachuca; *Columbus 2518* (RSA): EU339799, EU348449. *Ipomopsis macrosiphon* (Kearney & Peeb.) V. E. Grant & Wilken, U.S.A., AZ, Pima Co., Mount Lemon; *Wolf 165* (RSA): EU339805, EU348455. *Ipomopsis monticola* Porter & Johnson, Mexico, Sinaloa, Los Ornos; Breedlove & Thorne 18342 (RSA): EU339792, EU348442. *Ipomopsis multiflora* (Nutt.) V. E. Grant, U.S.A., AZ, Greenlee Co., Clifton; *Porter*

& Machen 8052 (RSA): EU339798, EU348448. *Ipomopsis pinnata* (Cav.) V. E. Grant, Mexico, Sonora, Yecora; *Porter & Columbus 11230* (RSA): EU339790, EU348440. *Gilia polyantha* Rydb. var *whitingii* Kearney & Peebles, U.S.A., AZ, Coconino Co., Walnut Canyon; *Atwood 16912* (RSA): EU339797, EU348447. *Ipomopsis polyantha* (Rydb.) V. E. Grant, U.S.A., CO, Archuleta Co., Pagosa Springs; *Grant & Grant 9468* (RSA): EU339800, EU348450. *Ipomopsis polycladon* (Torr.) V. E. Grant, U.S.A., CA, Inyo Co., Antelope Spring; *Porter 10907* (RSA): EU339772, EU348422. *Ipomopsis pringlei* (Gray) Henr., Mexico, Chihuahua, Cuauhtemoc*; Porter & Columbus 11246* (RSA): EU339791, EU348441. *Ipomopsis pumila* (Nutt.) V. E. Grant, U.S.A., NM, San Juan Co., Navajo Mine; *Porter 9290* (RSA): EU339775, EU348425. *Ipomopsis roseata* (Rydb.) V. E. Grant (C), U.S.A., UT, Uintah Co., Hickman Bridge; *Porter s. n.* (RSA): EU339784, EU348434. *Ipomopsis roseata* (M), U.S.A., UT, San Juan Co., Moqui Dugway; *Porter s. n.* (RSA): EU339783, EU348433. *Ipomopsis rubra* (L.) Wherry, U.S.A., TX, Smith Co., Winona; *Thomas 23214* (RSA): EU339801, EU348451. *Ipomopsis sancti-spiritus* Wilken & R. A. Fletcher, U.S.A., NM, San Miguel Co., Holy Ghost Canyon; *Wolf 173* (RSA): EU339803, EU348453. *Ipomopsis sancti-spiritus* (b), U.S.A., NM, San Miguel Co., Holy Ghost Canyon; *Wolf 173* (RSA): EU339804, EU348454. *Ipomopsis sonorae* (Rose) A. Grant, Mexico, Sonora; *vanDevender 93-20* (RSA): EU339752, EU348402. *Ipomopsis spicata* (Nutt.) V. E. Grant subsp. *capitata* (A. Gray) V. E. Grant, U.S.A., CO, Park Co., South Park; *Grant 9485* (RSA): EU339786, EU348436. *Ipomopsis spicata* subsp. *capitata* (2), U.S.A., CO, Summit Co., Hoosier Ridge; *Grant & Grant 9486* (RSA): EU339786, EU348436. *Ipomopsis spicata* subsp. *tridactyla* (Rydb.) Wilken & R. L. Hartm. (W), U.S.A., UT, Iron Co., Cedar Breaks; *Spencer 57-11* (RSA): EU339787, EU34843. *Ipomopsis spicata* subsp. *tridactyla* (Y), U.S.A., UT, Iron Co., Cedar Breaks; *Spencer 57-11* (RSA): EU339788, EU348438. *Ipomopsis tenuifolia* (A. Gray) V. E.

Grant, Mexico, Sonora; *McLaughlin 2465* (RSA): EU339771, EU348421. *Ipomopsis tenuituba* (Rydb.) V. E. Grant subsp. *latiloba* V. E. Grant & Wilken, U.S.A., UT, Iron Co., Cedar City; *Holmgren 10586* (RSA): EU339806, EU348456. *Ipomopsis thurberi* (Torr.) V. E. Grant, U.S.A., AZ, Santa Cruz Co., Flux Canyon; *Steinmann 802* (RSA): EU339789, EU348439. *Ipomopsis wendtii* Hendr., Mexico, Coahuila, Sierra del Jardin; *Porter 11564* (RSA): EU339802, EU348452. *Ipomopsis wrightii* (A. Gray) Shinners, U.S.A., TX, Presidio Co., McNary; *Porter 11358* (RSA): EU339774, EU348424.

Other Polemoniaceae—*Acanthogilia gloriosa* (Brandegee) A. G. Day & Moran, Mexico, Baja Calif., Punta Prieta; *Porter & Heil 7987* (SJNM): EU339722, EU348374. *Aliciella latifolia* (S. Watson) J. M. Porter, U.S.A., CA, Riverside Co., Box Canyon; *Porter & Machen 10253* (RSA): EU339745, EU339745. *Aliciella mcvickerae* (M. E. Jones) J. M. Porter, U.S.A., UT, Garfield Co, Panguitch; *Porter & Machen 7184* (RSA): EU339743,EU348394. *Aliciella triodon* (A. Eastwood) Brand, U.S.A., AZ, Apache Co., Chuska Mtns.; *Porter & Heil 7942* (RSA): EU339744, EU348395. *Allophyllum divaricatum* (Nutt.) A. D. Grant & V. E. Grant, U.S.A., CA, Lake Co., Mt. Konocti; *Porter & Machen 10819* (RSA): EU339730, EU348381. *Allophyllum glutinosum* (Benth.) A. D. Grant & V. E. Grant, U.S.A., CA, San Diego Co., ; *Johnson 93-032* (BRY): EU339728, AF208168. *Allophyllum integrifolium* (Brand) A. D. Grant & V. E. Grant, U.S.A., CA, Calavaras Co., ; *Johnson 93-111* (BRY): EU339729, EU348380. *Bonplandia geminiflora* Cav., Mexico, Michoacan, WSW La Paz; *Porter & Steinmann 13895* (RSA): EU339723, EU348375. *Bryantiella glutinosa* (Phil.) J. M. Porter, Peru, Arequipa, Nevado Chachani; *Porter 12195* (RSA): EU339754, EU348404. *Bryantiella palmeri* (S. Wats.) J. M. Porter, Mexico, Baja Calif, Isla Angel de le Guarda; *Tenorior 10949* (RSA): EU339755, EU348405. *Cantua buxifolia* Juss. ex Lam., Peru, Dept. Junin, Muquio; *Porter & Columbus*

12227 (RSA): EU339724, EU348376. *Cantua volcanica* J. M. Porter & Prather, Peru, Dept. Arequipa, Volcan Misti; *Porter & Columbus 12199* (RSA): EU339726, EU348378. *Cantua quercifolia* Juss., Peru, Dept. Amazonas, Chachapoyas; *Porter & Columbus 12165* (RSA): EU339725, EU348377. *Cobaea scandens* Cav., Peru, Dept. Amazonas, S Tingo; *Porter & Columbus 12166* (RSA): EU339727, EU348379. *Collomia heterophyla* Douglas ex Hook., U.S.A., CA, Mendocino Co., Reeves Canyon Rd.; *Smith 5742* (RSA): EU339732, EU348383. *Collomia linearis* Nutt., U.S.A., CO, Archuleta Co., Pogosa Springs, *Porter & Machen 8565* (RSA): EU339733, EU348384. *Collomia rawsoniana* Greene, U.S.A., CA, Madera Co., N. Bass Lake; *Porter 12255* (RSA): EU339734, EU348385. *Dayia grantii* J. M. Porter, Mexico, Baja Calif. Sur, Cerro Prieto; *Porter & Heil 7991*(RSA): EU339751, EU348401. *Dayia scabra* (Brandegee) J. M. Porter, Mexico, Baja Calif. Sur, Santa Rosalia; *Porter & Machen 11542* (RSA): EU339750, EU348400. *Eriastrum densifolium* (Benth.) H. Mason subsp. *mohavensis* (T. T. Craig) H. Mason, U.S.A., CA, Kern Co., Red Rock Canyon; *Wilken s. n.* (RSA): EU339764, EU348414. *Eriastrum wilcoxii* (A. Nelson) H. Mason, U.S.A., CA, Inyo Co., E Independence; *Porter & Machen 10851* (RSA): EU339765, EU348415. *Gilia cana* Jones subsp *triceps* (Brand) V. & A. Grant, U.S.A., CA, San Bernardino Co., Trona Pinacles; *Porter 14370* (RSA): EU339742, EU348393. *Gilia capitata* Sims, U.S.A., WA, Skamania Co., E Washougal; *Halse 2900* (AZ): EU339740, EU348391. *Gilia laciniata* Ruiz & Pav., Argentina, Prov. Tucuman, Tafi del Valle; *Porter & Columbus 12034* (RSA): EU339741, EU348392. *Giliastrum foetidum* (Gillies ex Benth.) J. M. Porter, Argentina, Mendoza, La Heras *Porter & Columbus 11930* (RSA): EU339748, EU348398. *Giliastrum ludens* (Shinners) J. M. Porter, U. S. A, TX, Jim Wells Co., W side FM 534; *Porter & Columbus 11732* (RSA): EU339746, EU348396. *Giliastrum purpusii* (K. Brandegee) J. M. Porter, Mexico, Coahuila, Sierra Solis; *Porter 11277*

(RSA): EU339749, EU348399. *Giliastrum rigidulum* (Benth.) Rydb., U.S.A., TX, Kimble Co., NE Junction; *Correll & Rollins 20881* (RSA): EU339747, EU348397. *Gymnosteris parvula* (Rydb.) A. Heller, U.S.A., CA, Mono Co., White Mtns, Milner Cr.; *Taylor 9151* (RSA): EU339816, EU348466. *Langloisia setosissima* (Torr. & A. Gray) Greene subsp. *punctata* (Coville) Timbrook, U.S.A., NV, Nye Co., U. S Hwy 95 & NV Hwy 160; *Porter 8840* (SJNM): EU339760, EU348410. *Lathrocasis tenerrima* (A. Gray) L. A. Johnson, U.S.A., NV, Elko Co., NW Elko; *Nichols & Lund 206* (AZ): EU339739, EU348390. *Leptosiphon ciliatus* (Benth.) Jeps., U.S.A., CA, Calaveras Co., Ebbetts Pass; *Everett & Balls 22099* (RSA): EU339821, EU348471. *Leptosiphon nuttallii* (A. Gray) J. M. Porter & L. A. Johnson, U.S.A., AZ, Greenlee Co., N Granville; *Porter & Machen 9004* (RSA): EU339822, EU348472. *Linanthus demissus* (A. Gray) Greene, U.S.A., CA, Inyo Co., Resting Spring Range; *Boyd 7739* (RSA): EU339819, EU348469. *Linanthus dichotomous* Benth., U.S.A., CA, San Bernardino Co., Mojave Desert; *Grant & Grant 8853* (RSA): EU339820, EU348470. *Linanthus filiforme* (C. Parry ex A. Gray) J. M. Porter & L. A. Johnson, U.S.A., CA, Inyo Co., E Independence; *Porter & Machen 10849* (RSA): EU339815, EU348465. *Linanthus jaegeri* (P. A. Munz) J. M. Porter & L. A. Johnson, U.S.A., CA, San Bernardino Co., San Gorgonio Mtn.; *Thorne 32357* (RSA): EU339817, EU348467. *Linanthus watsonii* (A. Gray) Wherry, U.S.A., UT, Wayne Co., Waterpocket Fold; *Porter 8571* (RSA): EU339818, EU348468. *Loeselia ciliata* L., Mexico, Veracruz, Paso de la Milpa; *Ventura 15710* (RSA): EU339759, EU348409. *Loeselia glandulosa* (Cav.) G. Don subsp. *conglomerata* (Kunth) Brand, Mexico, Mexico, Sultepec; *Moreno 26* (RSA): EU339757, EU348407. *Loeselia involucrata* G. Don, Mexico, Baja Calif Sur, Rancho La Huerta; *Harder 1104* (RSA): EU339758, EU348408. *Loeselia pumila* (M. Martens & Galeotti) Walp., Mexico, Sonora, Rio Mayo; *van Devender 95-1113* (RSA): EU339756, EU348406. *Loeseliastrum*

depressum (M. E. Jones ex A. Gray) J. M. Porter & L. A. Johnson, U.S.A., CA, Mono Co., White Mtns.; *Pierson 12462* (RSA): EU339761, EU348411. *Loeseliastrum depressum*, U.S.A., NV, Nye Co., Nevada Test Site; *Beatley 8733* (RSA): EU339762, EU348412. *Loeseliastrum matthewsii* (A. Gray) Timbrook, U.S.A., CA, Los Angeles Co., Ft. Tejon Rd.; *Porter 10560* (RSA): EU339763, EU348413. *Microgilia minutiflora* (Benth.) J. M. Porter & L. A. Johnson, U.S.A., ID, Bonneville Co., Idaho Falls; *Grant 9736* (RSA): EU339766, EU348416. *Microsteris gracilis* (Dougl. ex Hook.) Greene, U.S.A., CA, Los Angeles Co., Mt. Emma; *Porter 10566* (RSA): EU339823, EU348473. *Navarretia breweri* (A. Gray) Greene, U.S.A., CO, Montrose Co., Columbine Pass; *Porter 8599* (RSA): EU339735, EU348386. *Navarretia capillaris* (Kellogg) Kuntze, U.S.A., CA, Placer Co., Soda Springs; *Day 82-71* (RSA): EU339731, EU348382. *Navarretia mellita* Greene, U.S.A., CA, Solano Co., Blue Ridge Rd.; *Ertter 8539* (RSA): EU339736, EU348387. *Phlox glaberrima* L., U.S.A., NC, Wake Co., Co. Rte. 1127; *Leonard & Radford 1508* (RSA): EU339825, EU348475. *Phlox stansburyi* (Torr.) A. Heller, U.S.A., NV, Nye Co., U. S. Hwy, 95; *Porter 8841* (SJNM): EU339824, EU348474. *Polemonium caeruleum* L., U.S.A., AK, Brooks Range; *Welsh & Ostler 1126* (RSA): EU339828, EU348478. *Polemonium californicum* Eastw, U.S.A., CA, Sierra Co., Yuba Pass; *Gustafson 3150* (RSA): EU339826, EU348476. *Polemonium pauciflorum* S. Watson, U.S.A., AZ, Cochise Co., Chiracaua Mtns.; *Grant & Grant 61-S* (RSA): EU339827, EU348477. *Saltugilia caruifolia* (Abrams) L. A. Johnson, U.S.A., CA, San Diego Co., Aguanga Mtn.; *Boyd 8795* (RSA): EU339738, EU348389. *Saltugilia splendens* (Mason & A. G. Grant) L. A. Johnson, U.S.A., CA, Los Angeles Co., San Gabriel Mtns.; *Porter 11849* (RSA): EU339737, EU348388.

Fouquieriaceae—*Fouquieria splendens*, U.S.A., CA, San Bernardino Co., Whipple Mtns: *Porter 11583* (RSA): EU339721, EU348373.

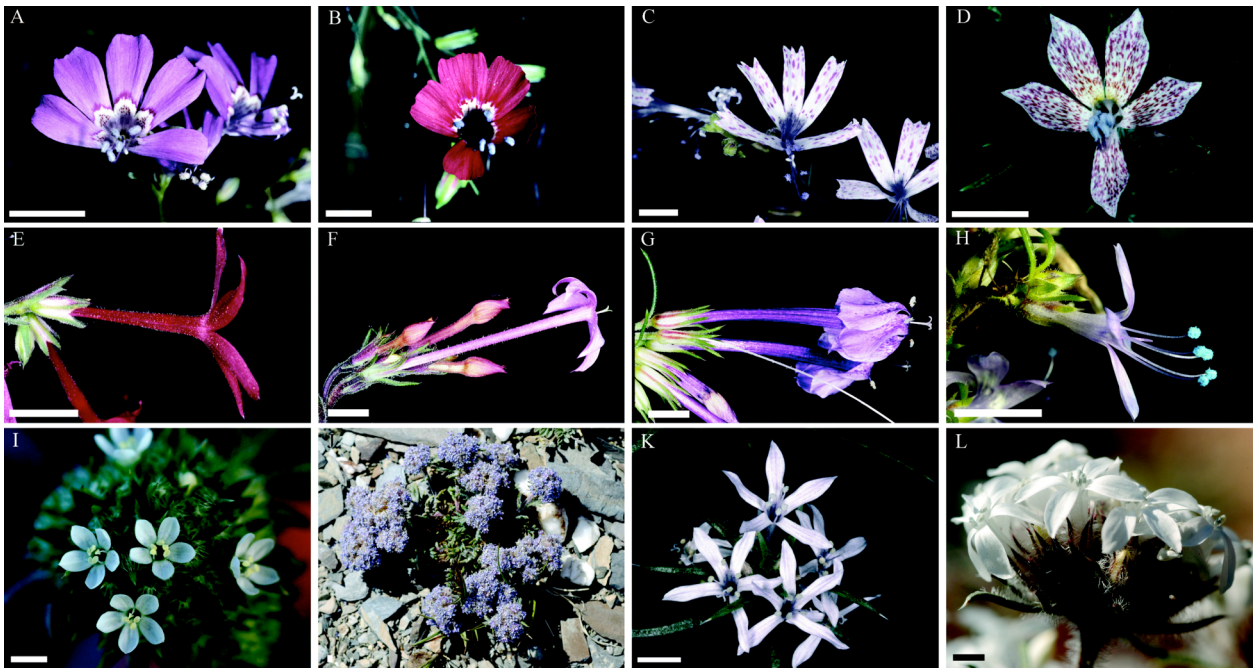


FIG. 1. Floral form and variation in *Ipomopsis*: section *Giliopsis*, (section *Phloganthea* sensu V. Grant, in part; A–C)—A. *Ipomopsis effusa* (A. Gray) Moran, B. *I. tenuifolia* (A. Gray) V. E. Grant, C. *I. guttata* (A. Gray) Moran; D. *Ipomopsis havardii* (A. Gray) V. E. Grant (section *Phloganthea* sensu V. Grant); section *Ipomopsis* (E–H)—E. *I. aggregata* (Pursh.) V. E. Grant subsp. *aggregata*, F. I. *tenuituba* (Rydb.) V. E. Grant subsp. *tenuituba*, G. *I. thurberi* (Torr. ex A. Gray) V. E. Grant, H. *Gilia polyantha* Rydb. var. *whitingii* Kearney & Peebles (section *Phloganthea* sensu V. Grant); section *Elaphocera* (sect. *Microgilia* sensu V. Grant)—I. *I. congesta* (Hook.) V. E. Grant subsp. *palmifrons* (Brand) A. G. Day, J. *I. congesta* (Hook.) V. E. Grant subsp. *nevadensis* (Tidestr.) Kartez & Gandhi, K. *I. gunnisonii* (Torr. & A. Gray) V. E. Grant, L. *I. roseata* (Rydb.) V. E. Grant. Scale bars for A–H = 5.0 mm, I–L = 2.0 mm.

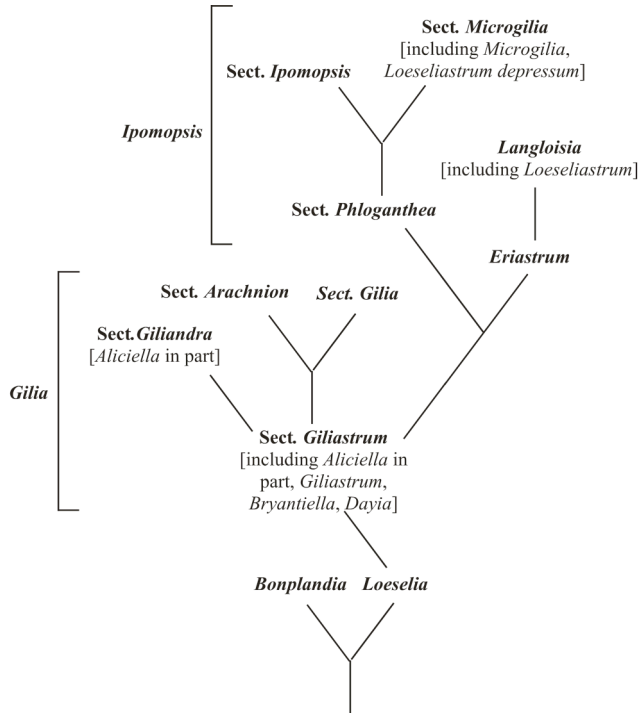
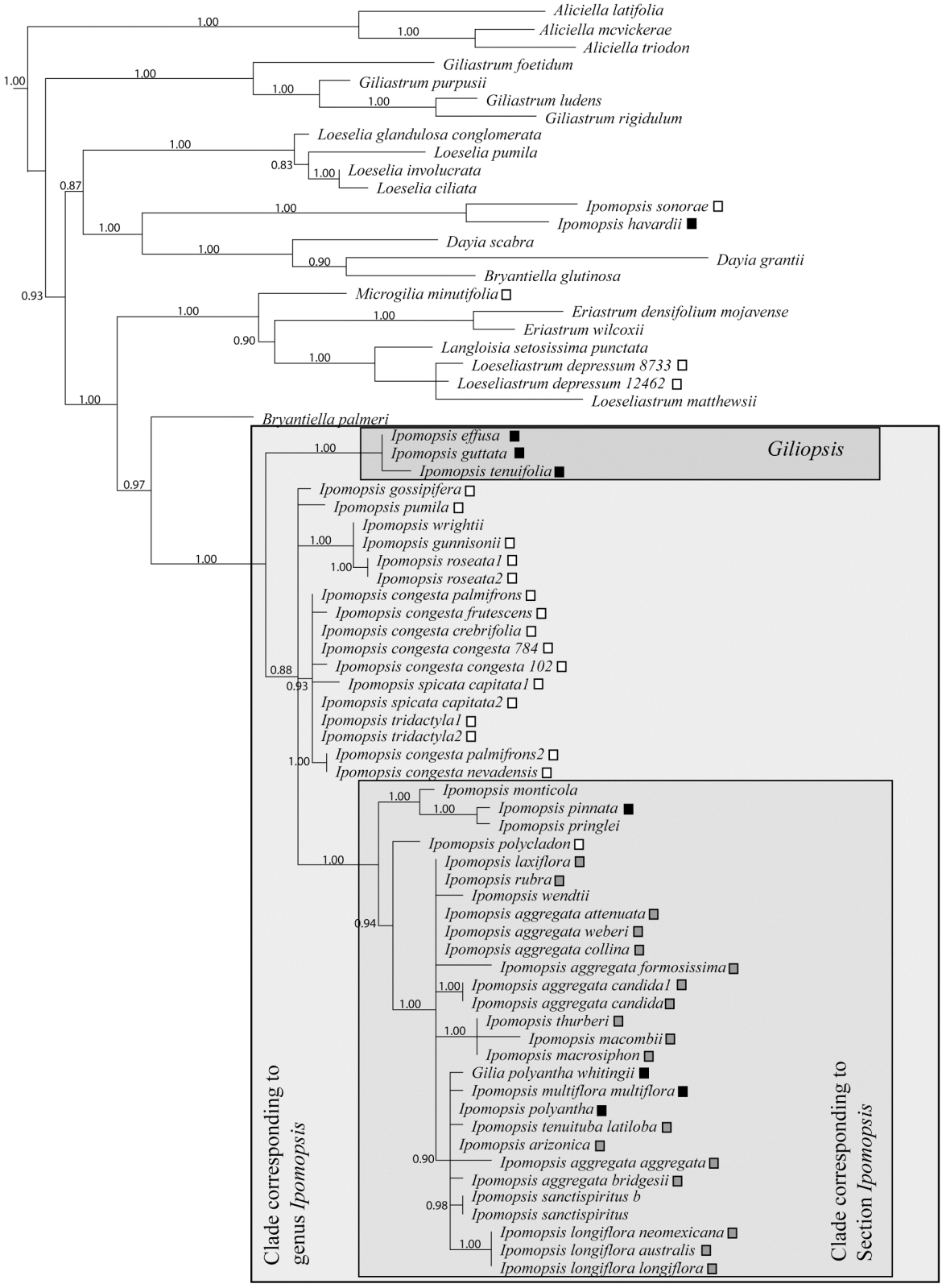


FIG. 2. Hypothesis for the origin and diversification of *Ipomopsis*, according to Grant (1959; 1992b; 1998b).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Aliciella mcvickerae
Aliciella triodon
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Giliastrum purpusii
Giliastrum ludens
Giliastrum rigidulum
Loeselia glandulosa conglomerata
Loeselia pumila
Loeselia involucrata
Loeselia ciliata
Ipomopsis sonorae
Ipomopsis havardii
Dayia scabra
Dayia grantii
Bryantiella glutinosa
Microgilia minutifolia
Eriastrum densifolium mojavense
Eriastrum wilcoxii
Langloisia setosissima punctata
Loeseliastrum depressum 8733
Loeseliastrum depressum 12462
Loeseliastrum matthewsii
Bryantiella palmeri
Giliopsis
Ipomopsis effusa
Ipomopsis guttata
Ipomopsis tenuifolia
Ipomopsis gossipifera
Ipomopsis pumila
Ipomopsis wrightii
Ipomopsis gunnisonii
Ipomopsis roseata1
Ipomopsis roseata2
Ipomopsis congesta palmifrons
Ipomopsis congesta frutescens
Ipomopsis congesta crebrifolia
Ipomopsis congesta congesta 784
Ipomopsis congesta congesta 102
Ipomopsis spicata capitata1
Ipomopsis spicata capitata2
Ipomopsis tridactyla1
Ipomopsis tridactyla2
Ipomopsis congesta palmifrons2
Ipomopsis congesta nevadensis
Ipomopsis monticola
Ipomopsis pinnata
Ipomopsis pringlei
Ipomopsis polycladon
Ipomopsis laxiflora
Ipomopsis rubra
Ipomopsis wendtii
Ipomopsis aggregata attenuata
Ipomopsis aggregata weberi
Ipomopsis aggregata collina
Ipomopsis aggregata formosissima
Ipomopsis aggregata candida1
Ipomopsis aggregata candida
Ipomopsis thurberi
Ipomopsis macombii
Ipomopsis macrosiphon
Gilia polyantha whitingii
Ipomopsis multiflora multiflora
Ipomopsis polyantha
Ipomopsis tenuituba latiloba
Ipomopsis arizonica
Ipomopsis aggregata aggregata
Ipomopsis aggregata bridgesii
Ipomopsis sanctispiritus b
Ipomopsis sanctispiritus
Ipomopsis longiflora neomexicana
Ipomopsis longiflora australis
Ipomopsis longiflora longiflora
Clade corresponding to genus Ipomopsis
Clade corresponding to Section Ipomopsis

FIG. 3. Phylogenetic relationships of *Ipomopsis*, in the context of members of Polemoniaceae tribe Loeselieae (Porter and Johnson 2000), inferred from maximum likelihood analysis (ML). One of two ML (-ln(*L*) = 7265. 75514) trees, based on comparative DNA sequence analysis of the chloroplast *trnL–F* region. Bayesian posterior probabilities are adjacent to the corresponding branches. The small boxes adjacent to species names reflect the placement of species in Grant's (1956, 1959) sectional classification of *Ipomopsis*: gray = section *Ipomopsis*, black = section *Phloganthea*, white = section *Microgilia*.

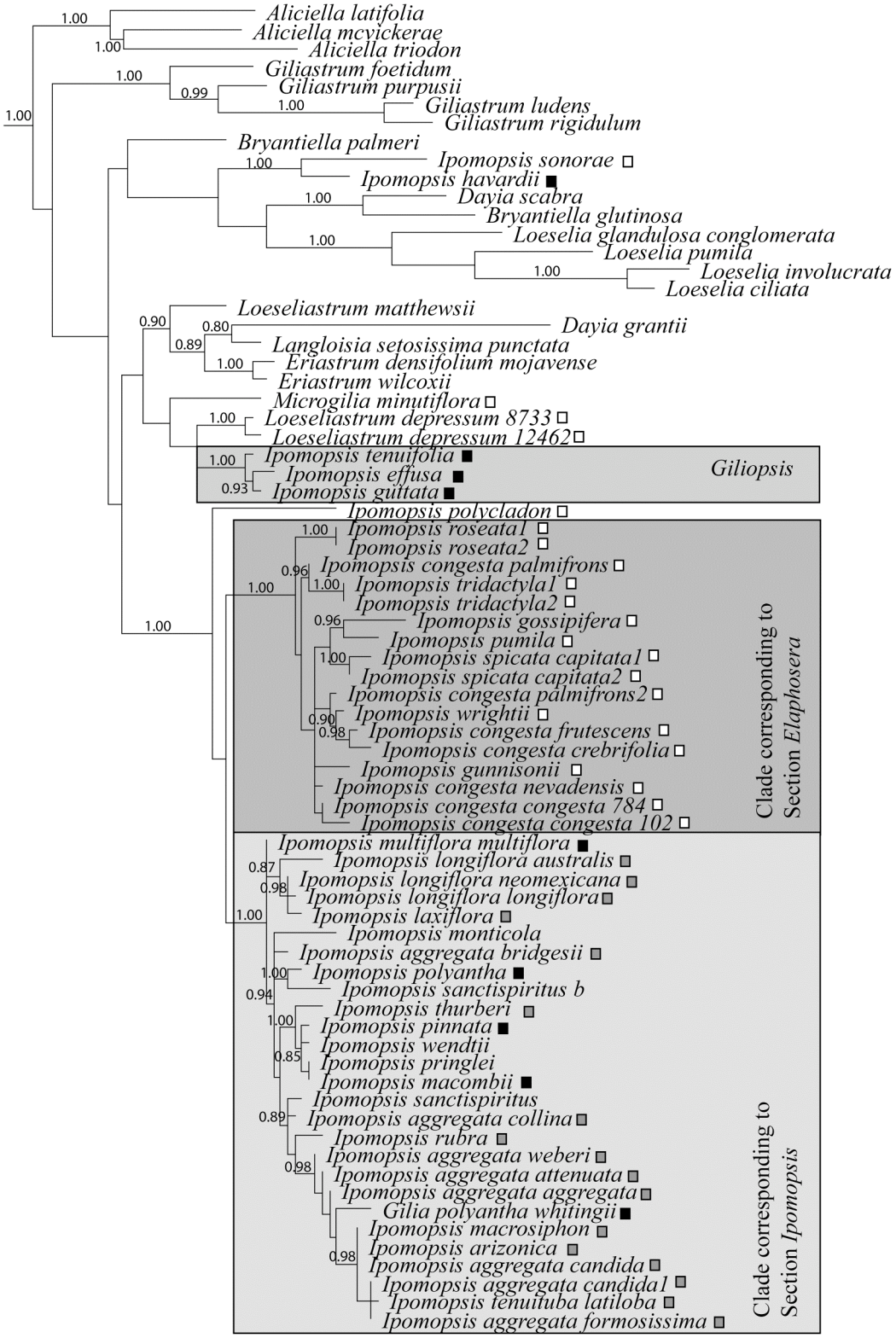

Aliciella latifolia
Aliciella mcvickerae
Aliciella triodon
Giliastrum foetidum
Giliastrum purpusii
Giliastrum ludens
Giliastrum rigidulum
Bryantiella palmeri
Ipomopsis sonorae
Ipomopsis havardii
Dayia scabra
Bryantiella glutinosa
Loeselia glandulosa conglomerata
Loeselia pumila
Loeselia involucrata
Loeselia ciliata
Loeseliastrum matthewsii
Dayia grantii
Langloisia setosissima punctata
Eriastrum densifolium mojavense
Eriastrum wilcoxii
Microgilia minutiflora
Loeseliastrum depressum 8733
Loeseliastrum depressum 12462
Ipomopsis tenuifolia
Ipomopsis effusa
Ipomopsis guttata
Giliopsis
Ipomopsis polycladon
Ipomopsis roseata1
Ipomopsis roseata2
Ipomopsis congesta palmifrons
Ipomopsis tridactyla1
Ipomopsis tridactyla2
Ipomopsis gossipifera
Ipomopsis pumila
Ipomopsis spicata capitata1
Ipomopsis spicata capitata2
Ipomopsis congesta palmifrons2
Ipomopsis wrightii
Ipomopsis congesta frutescens
Ipomopsis congesta crebrifolia
Ipomopsis gunnisonii
Ipomopsis congesta nevadensis
Ipomopsis congesta congesta 784
Ipomopsis congesta congesta 102
Clade corresponding to Section Elaphosera
Ipomopsis multiflora multiflora
Ipomopsis longiflora australis
Ipomopsis longiflora neomexicana
Ipomopsis longiflora longiflora
Ipomopsis laxiflora
Ipomopsis monticola
Ipomopsis aggregata bridgesii
Ipomopsis polyantha
Ipomopsis sanctispiritus b
Ipomopsis thurberi
Ipomopsis pinnata
Ipomopsis wendtii
Ipomopsis pringlei
Ipomopsis macombii
Ipomopsis sanctispiritus
Ipomopsis aggregata collina
Ipomopsis rubra
Ipomopsis aggregata weberi
Ipomopsis aggregata attenuata
Ipomopsis aggregata aggregata
Gilia polyantha whitingii
Ipomopsis macrosiphon
Ipomopsis arizonica
Ipomopsis aggregata candida
Ipomopsis aggregata candida1
Ipomopsis tenuituba latiloba
Ipomopsis aggregata formosissima
Clade corresponding to Section Ipomopsis

FIG. 4. Phylogenetic relationships of *Ipomopsis*, in the context of members of Polemoniaceae tribe Loeselieae (Porter and Johnson 2000), inferred from maximum likelihood analysis (ML). One of two ML (-ln(*L*) = 9604. 35904) trees, based on comparative DNA sequence analysis of the nrITS region. Bayesian posterior probabilities are adjacent to the corresponding branches. The small boxes adjacent to species names reflect the placement of species in Grant's (1956, 1959) sectional classification of *Ipomopsis*: gray= section *Ipomopsis*, black = section *Phloganthea*, white = section *Microgilia*.

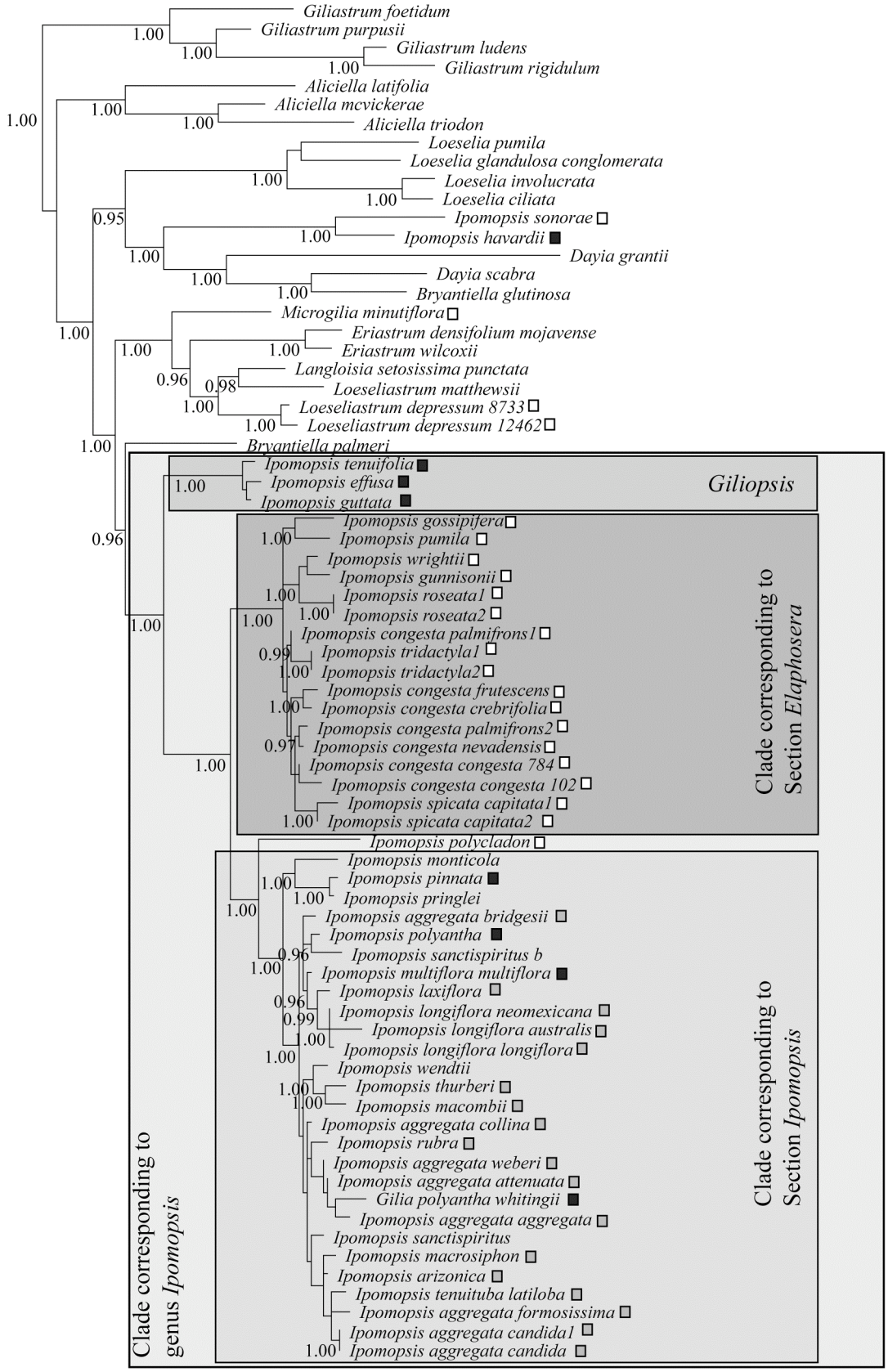
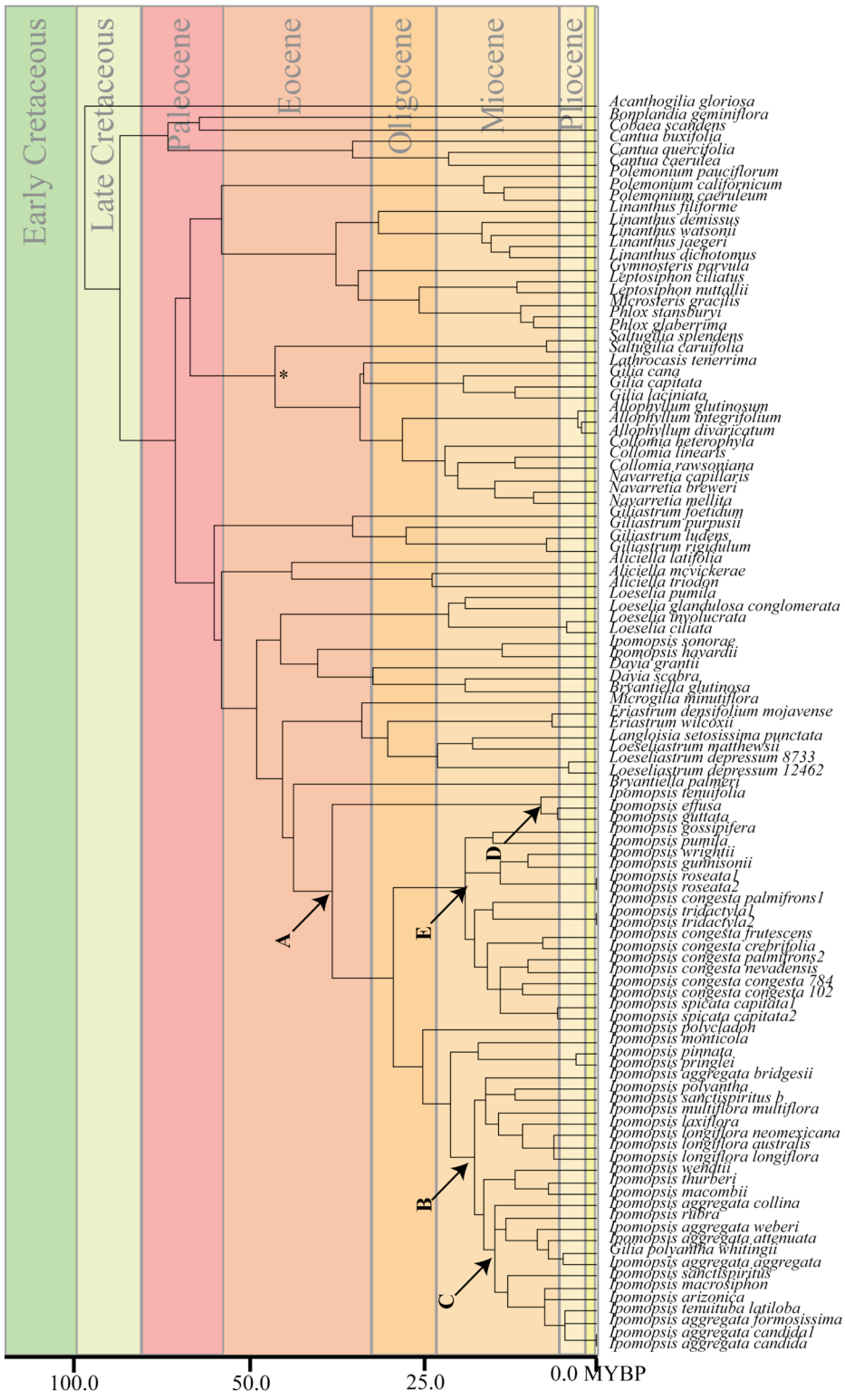

Giliastrum foetidum
Giliastrum purpusii
Giliastrum ludens
Giliastrum rigidulum
Aliciella latifolia
Aliciella mcvickerae
Aliciella triodon
Loeselia pumila
Loeselia glandulosa conglomerata
Loeselia involucrata
Loeselia ciliata
Ipomopsis sonorae
Ipomopsis havardii
Dayia grantii
Dayia scabra
Bryantiella glutinosa
Microgilia minutiflora
Eriastrum densifolium mojavense
Eriastrum wilcoxii
Langloisia setosissima punctata
Loeseliastrum matthewsii
Loeseliastrum depressum 8733
Loeseliastrum depressum 12462
Bryantiella palmeri
Ipomopsis tenuifolia
Ipomopsis effusa
Ipomopsis guttata
Giliopsis
Ipomopsis gossipifera
Ipomopsis pumila
Ipomopsis wrightii
Ipomopsis gunnisonii
Ipomopsis roseata1
Ipomopsis roseata2
Ipomopsis congesta palmifrons1
Ipomopsis tridactyla1
Ipomopsis tridactyla2
Ipomopsis congesta frutescens
Ipomopsis congesta crebrifolia
Ipomopsis congesta palmifrons2
Ipomopsis congesta nevadensis
Ipomopsis congesta congesta 784
Ipomopsis congesta congesta 102
Ipomopsis spicata capitata1
Ipomopsis spicata capitata2
Clade corresponding to Section Elaphosera
Ipomopsis polycladon
Ipomopsis monticola
Ipomopsis pinnata
Ipomopsis pringlei
Ipomopsis aggregata bridgesii
Ipomopsis polyantha
Ipomopsis sanctispiritus b
Ipomopsis multiflora multiflora
Ipomopsis laxiflora
Ipomopsis longiflora neomexicana
Ipomopsis longiflora australis
Ipomopsis longiflora longiflora
Ipomopsis wendtii
Ipomopsis thurberi
Ipomopsis macombii
Ipomopsis aggregata collina
Ipomopsis rubra
Ipomopsis aggregata weberi
Ipomopsis aggregata attenuata
Gilia polyantha whitingii
Ipomopsis aggregata aggregata
Ipomopsis sanctispiritus
Ipomopsis macrosiphon
Ipomopsis arizonica
Ipomopsis tenuituba latiloba
Ipomopsis aggregata formosissima
Ipomopsis aggregata candida1
Ipomopsis aggregata candida
Clade corresponding to Section Ipomopsis
Clade corresponding to genus Ipomopsis

FIG. 5. Phylogenetic relationships of *Ipomopsis*, in the context of members of Polemoniaceae tribe Loeselieae (Porter and Johnson 2000), inferred from maximum likelihood analysis (ML). The single ML (-ln*L* = 17862. 76502) tree, based on comparative DNA sequence analysis of combined chloroplast *trnL–F* and nrITS regions. Bayesian posterior probabilities are adjacent to the corresponding branches. The small boxes adjacent to species names reflect the placement of species in Grant's (1956, 1959) sectional classification of *Ipomopsis*: gray = section *Ipomopsis*, black = section *Phloganthea*, white = section *Microgilia*.


Early Cretaceous
Late Cretaceous
Paleocene
Eocene
Oligocene
Miocene
Pliocene
Acanthogilia gloriosa
Bonplandia geminiflora
Cobaea scandens
Cantua buxifolia
Cantua quercifolia
Cantua caerulea
Polemonium pauciflorum
Polemonium californicum
Polemonium caeruleum
Linanthus filiforme
Linanthus demissus
Linanthus watsonii
Linanthus jaegeri
Linanthus dichotomus
Gymnosteris parvula
Leptosiphon ciliatus
Leptosiphon nuttallii
Microsteris gracilis
Phlox stansburyi
Phlox glaberrima
Saltugilia splendens
Saltugilia caruifolia
Lathrocasis tenerrima
Gilia cana
Gilia capitata
Gilia laciniata
Allophyllum glutinosum
Allophyllum integrifolium
Allophyllum divaricatum
Collomia heterophyla
Collomia linearis
Collomia rawsoniana
Navarretia capillaris
Navarretia breweri
Navarretia mellita
Giliastrum foetidum
Giliastrum purpusii
Giliastrum ludens
Giliastrum rigidulum
Aliciella latifolia
Aliciella mcvickerae
Aliciella triodon
Loeselia pumila
Loeselia glandulosa conglomerata
Loeselia involucrata
Loeselia ciliata
Ipomopsis sonorae
Ipomopsis havardii
Davia grantii
Davia scabra
Bryantiella glutinosa
Microgilia minutiflora
Eriastrum densifolium mojavense
Eriastrum wilcoxii
Langloisia setosissima punctata
Loeseliastrum matthewsii
Loeseliastrum depressum 8733
Loeseliastrum depressum 12462
Bryantiella palmeri
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Ipomopsis effusa
Ipomopsis guttata
Ipomopsis gossypifera
Ipomopsis pumila
Ipomopsis wrightii
Ipomopsis gunnisonii
Ipomopsis roseata1
Ipomopsis roseata2
Ipomopsis congesta palmifrons1
Ipomopsis tridactyla1
Ipomopsis tridactyla2
Ipomopsis congesta frutescens
Ipomopsis congesta crebrifolia
Ipomopsis congesta palmifrons2
Ipomopsis congesta nevadensis
Ipomopsis congesta congesta 784
Ipomopsis congesta congesta 102
Ipomopsis spicata capitata1
Ipomopsis spicata capitata2
Ipomopsis polycladon
Ipomopsis monticola
Ipomopsis pinnata
Ipomopsis pringlei
Ipomopsis aggregata bridgesii
Ipomopsis polyantha
Ipomopsis sanctispiritus b
Ipomopsis multiflora multiflora
Ipomopsis laxiflora
Ipomopsis longiflora neomexicana
Ipomopsis longiflora australis
Ipomopsis longiflora longiflora
Ipomopsis wenditi
Ipomopsis thurberi
Ipomopsis macombii
Ipomopsis aggregata collina
Ipomopsis rubra
Ipomopsis aggregata weberi
Ipomopsis aggregata attenuata
Gilia polyantha whitingii
Ipomopsis aggregata aggregata
Ipomopsis sanctispiritus
Ipomopsis macrosiphon
Ipomopsis arizonica
Ipomopsis tenuituba latiloba
Ipomopsis aggregata formosissima
Ipomopsis aggregata candida1
Ipomopsis aggregata candida
*
A
B
C
D
E
100.0
50.0
25.0
0.0 MYBP

FIG. 6. Chronogram of Polemoniaceae, based upon maximum likelihood (ML) analysis of combined chloroplast *trnL–F* and nrITS regions. ML branch lengths were rescaled using nonparametric rate smoothing (Sanderson 1997). The tree was calibrated at the node indicated with an asterisk, using the fossil *Gilisenium hueberii*. Clades A–E indicate the target clades for dating using a sample from the posterior distributions of trees from trnL–F region, ITS region and combined data Bayesian analyses. Epochs of the geological time scale are indication; however, the Pleistocene (yellow) and Holocene (white) are not labeled.