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Phylogenetic Relationships within *Phacelia* subgenus *Phacelia* (Hydrophyllaceae) Inferred From Nuclear rDNA ITS Sequence Data

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ABSTRACT. We examined infrageneric relationships within *Phacelia* subgenus *Phacelia* based on phylogenetic hypotheses derived from internal transcribed spacers (ITS) and the 5.8S gene of nuclear ribosomal DNA. Maximum parsimony (MP) and maximum likelihood (ML) phylogenies support the monophyly of sects. *Euglypta* and *Miltitzia*, previously hypothesized based on seed characters and ecological distribution. Tree branching patterns within the *Euglypta/Miltitzia* clade indicate three principle lineages: 1) the *P. pachyphylla* complex, 2) a clade of *P. brachyloba* and 3) a clade consisting of two sister groups, the *P. affinis* complex and the *P. fremontii* complex. An aneuploid increase in chromosome number occurs from the *P. pachyphylla* complex ($n = 11$) to *P. brachyloba* ($n = 12$) while the pattern within the *P. fremontii* complex includes both aneuploid increases and decreases. There is high bootstrap support for monophyly of taxa representing both the *P. magellanica* polyploid complex and the *P. humiles* species group; the former contains the only known tetraploid taxa in subgenus *Phacelia*.

Phacelia is the largest genus of Hydrophyllaceae with approximately 200 species in North and South America. The center of diversity is in California where 93 taxa occur, of which 39 are endemic (Raven and Axelrod 1978). *Phacelia* range from small annuals to large perennials (Wilken et al. 1993). Constance (1963) put forward an infrageneric classification for *Phacelia*, naming three subgenera: *Cosmanthus*, *Howellanthus*, and *Phacelia*. Within subg. *Cosmanthus* 14 species occur mainly in eastern North America with several ranging into Mexico; subg. *Howellanthus* is monotypic, represented by *Phacelia dalesiana* in the Klamath Range of California (Constance 1949, 1952). Constance (1963) subdivided the annuals of subg. *Phacelia* into eight species groups based primarily on chromosome numbers. Ferguson (1999) provided a recent analysis of subg. *Phacelia* listing five sections, six species groups, and 50 unassigned taxa. Sections *Euglypta* and *Miltitzia* are distributed in and around the Great Basin and Mojave desert with one disjunct taxon in South America.

No comprehensive phylogeny exists for *Phacelia*. There have been numerous partial revisions and floras suggesting affinities based upon morphological characters, ecological distribution, and/or cytological data (Constance 1963). For example, Howell (1942, 1944, 1946) suggested common ancestry between sects. *Euglypta* and *Miltitzia* based upon the shared seed character of transverse corrugation. Further studies by Constance and Chuang (1982) corroborated the relationship using overall similarity of pollen grains. Howell (1944) noted the only discriminating character of sect. *Miltitzia* from sect. *Euglypta*, a yellow marcescent corolla, had arisen in other *Phacelia*.

Several species groups within sect. *Phacelia* have been investigated. Heckard (1960) examined the *P. magellanica* polyploid complex, noting many polymorphic characters accompanying the diploid or tetraploid condition, while Lee (1986) assessed the *P. humiles* species group using corolla venation patterns.

More recent studies have incorporated molecular data for a phylogenetic assessment of *Phacelia* (Dempcy 1996; Ferguson 1999; Ganong 2002). Ferguson (1999) utilized *ndhF* sequence data to infer infrageneric and interspecific relationships within Hydrophyllaceae, including 19 *Phacelia* species. The analyses resolved a paraphyletic *Phacelia* + *Romanzoffia* clade and affirmed sectional status in subgenus *Phacelia* for sects. *Euglypta*, *Miltitzia*, *Gymnobythus*, and *Whitlavia*. Dempcy (1996) utilized ITS sequence data in combination with morphological characters to examine interspecific and intraspecific relationships within sect. *Euglypta*. He proposed a monophyletic origin with three main lineages. Following up on this work, Ganong (2002) analyzed relationships of sects. *Miltitzia* and *Euglypta* using nuclear rDNA ITS sequence data and suggested weak support for a monophyletic *Miltitzia* and stronger evidence for *Miltitzia* nesting within sect. *Euglypta*.

Our molecular analysis using ITS data incorporates taxa from several infrageneric groups proposed by Constance (1963) within subgenus *Phacelia* to infer evolutionary relationships. Our specific objectives were: (1) to evaluate the monophyly of sects. *Euglypta* and *Miltitzia*, (2) to examine interspecific and intraspecific relationships within sect. *Euglypta* and sect. *Miltitzia*, and (3) to assess the monophyly of the *P. magellanica* polyploid complex.

MATERIALS AND METHODS

Samples. The dataset includes nuclear rDNA ITS sequence data for 55 species including 51 from subg. *Phacelia*, two from subg. *Cosmanthus*, and two outgroup genera: *Draperia* and *Romanzoffia* (Appendix 1). Taxa representing subg. *Phacelia* include 26 from sect. *Phacelia*, 12 from sect. *Euglypta* (Dempcy 1996), 10 from

sect. *Miltitzia* (Ganong 2002), two from sect. *Whitlavia*, and one from sect. *Gymnobythus*. There are 17 species with more than one population sample. Among the 56 species, 48 represent new data and eight were previously published. Material used for DNA extraction was field collected or obtained from herbarium specimens. DNA was extracted from 10–30 mg of leaf tissue using either the CTAB protocol (Doyle and Doyle 1987) modified by Cullings (1992) or with a DNeasy Plant Kit (Qiagen). In some cases the CTAB DNA extractions were further purified with a Prep-A-Gene DNA Purification Kit (Bio-Rad).

PCR Amplification and DNA Sequencing. Amplification of the ITS1, 5.8S, and ITS2 regions of the nuclear ribosomal DNA included the following primer sets: (1) ITS5 (White et al. 1990) with c28KJ (Cullings 1992), (2) ITSLEU (Baum et al. 1998) with ITS4 (White et al. 1990), and (3) ITS5 with ITS4 (White et al. 1990). All taxa in *Phacelia* sect. *Euglypta* were amplified using the polymerase chain reaction (PCR) in 12.5 μL final volumes followed by manual sequencing (Dempcy 1996). The remaining taxa were PCR amplified as in Ganong (2002). Positive amplifications were purified with a QIAquick PCR purification kit (Qiagen) and sequenced using a BigDye Terminator kit (Perkin-Elmer) and an ABI 377 automated sequencer (Applied Biosystems). The final consensus contig was aligned by eye in Sequencher 3.0 (Gene Codes Corporation).

Data Analysis. Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford 2002). Most parsimonious (MP) trees were obtained using the heuristic search algorithm. Starting trees were obtained by random stepwise addition of taxa followed by TBR branch swapping. All characters were weighted equally, character state transitions were treated as unordered, and gaps were treated as missing data. A separate analysis using gaps as a fifth character yielded a tree that was almost identical to that obtained with gaps missing. The data set is available from TreeBASE (study accession S1267; matrix accession M2211). Bootstrap resampling was used to assess nodal support in the parsimony analysis (Felsenstein 1985) with a heuristic search, closest stepwise addition of taxa, TBR branch swapping, MAXTREES = 5,000, and 500 replicates.

To establish an appropriate nucleotide substitution model for maximum likelihood (ML) analysis we used Modeltest 3.06 (Posada and Crandall 1998). For ML tree estimation the best-fit model was used in an initial heuristic search with parameter estimates obtained from a NJ tree in PAUP* 4.0b10 (Swofford 2002). Once a better tree was found, the parameters were re-estimated and the search repeated until the trees converged on the same maximum likelihood tree.

RESULTS

The aligned ITS dataset contained 617 sites; 189 (31%) were parsimony-informative, 81 (13%) were parsimony-uninformative and 347 (56%) were invariant. Fragment sizes for 80 taxa ranged from 594–609 bases and 0.5% data were missing.

Parsimony analysis produced 52,506 MP trees (L = 940). A strict consensus tree with bootstrap values is presented in Fig. 1. The tree is partially resolved with low bootstrap support for a monophyletic *Phacelia* clade (65%). Two taxa represent subg. *Cosmanthus* (*P. hirsuta* and *P. patulifora*; 100%) and nest within subg. *Phacelia*. There is high bootstrap support for three of the five sections in subg. *Phacelia* including *Whitlavia* (*P. parryi* and *P. minor*; 100%) and a fully sampled *Euglypta/Miltitzia* (93%). The well-supported lineages within *Euglypta/Miltitzia* are the *P. pachyphylla* (99%) complex followed by the divergence of *P. brachyloba* (91%); *P. brachyloba* is sister to the somewhat unresolved *P. affinis* and *P. fremontii* complexes (95%). Within sect. *Phacelia* high bootstrap support associates taxa from the *P. magellanica* polyploid complex together with members of the *P. humiles* species group (99%). Two additional associations with high bootstrap support contain unassigned species from sect. *Phacelia*. The first includes *P. procera*, *P. bolanderi*, and *P. hydrophylloides* (100%); the second includes *P. distans*, *P. cryptantha*, *P. crenulata* var. *minutifolia*, and *P. ramosissima* (97%).

The best-fit model generated from using Modeltest 3.06 (Posada and Crandall 1998) on our dataset was GTR+Γ+I. The maximum likelihood (ML) search in PAUP*4.0b10 (Swofford 2002) incorporating this model resulted in a maximum likelihood tree with a $-\ln L = 5845.49375$ (Fig. 2). The parameter values as estimated from this tree were: A⇔C: 1.470, A⇔G: 1.761, A⇔T: 1.372, C⇔G: 0.677, C⇔T: 4.180, G⇔T: 1.0. The estimated base composition was A = 0.216, C = 0.294, G = 0.288, T = 0.202, with $\alpha = 0.630$ for the shape of the Γ distribution, I = 0.330 for the proportion of invariable sites, and 8 rate categories.

There was concordance between the ML and MP trees for subg. *Cosmanthus* (MPBS and MLBS = 100%) as well as sect. *Euglypta/Miltitzia* (MLBS = 97%; Fig. 2). and sect. *Whitlavia* (MLBS = 100%; Fig. 2). Within the *Euglypta/Miltitzia* clade ML bootstrap support was similar to the MP outcome for the *P. pachyphylla* complex (99%) and the sister groups *P. affinis* and *P. fremontii* (95%) but lower for *P. brachyloba* (76%). Section *Phacelia* was not monophyletic in both the MP and the ML analyses. However, there was high ML bootstrap for the combined *P. magellanica* polyploid complex and the *P. humiles* species group (*magellanica/humiles*: 95%) in sect. *Phacelia* and the two new groups, one including *P. procera P. bolanderi*, and *P. hydrophylloides* (100%); the second includes *P. distans*, *P. cryptantha*, *P. crenulata* var. *minutifolia*, and *P. ramosissima* (97%). There was no support for a monophyletic *Phacelia* clade.

Many species included in the dataset have $n = 11$ while several tetraploids ($n = 22$) occurred in the *magellanica/humiles* complex (Fig. 2). Within sect. *Euglypta/Miltitzia*, chromosome numbers vary between 11 to 13.

DISCUSSION

Within subg. *Phacelia*, we noted minimal ITS variation at the population level in the *Euglypta/Miltitzia* clade based on the extensive population sampling of Dempcy (1996) for sect. *Euglypta*. Phylogenetic inaccuracies due to unhomogenized copies of a gene family like ITS are problematic for inferring evolutionary relationships (Alvarez and Wendel 2003). However, we saw no evidence of this problem in our population level work (e.g., *P affinis* and *P. leibergii*). We did find that the data did not provide sufficient variation for resolv-

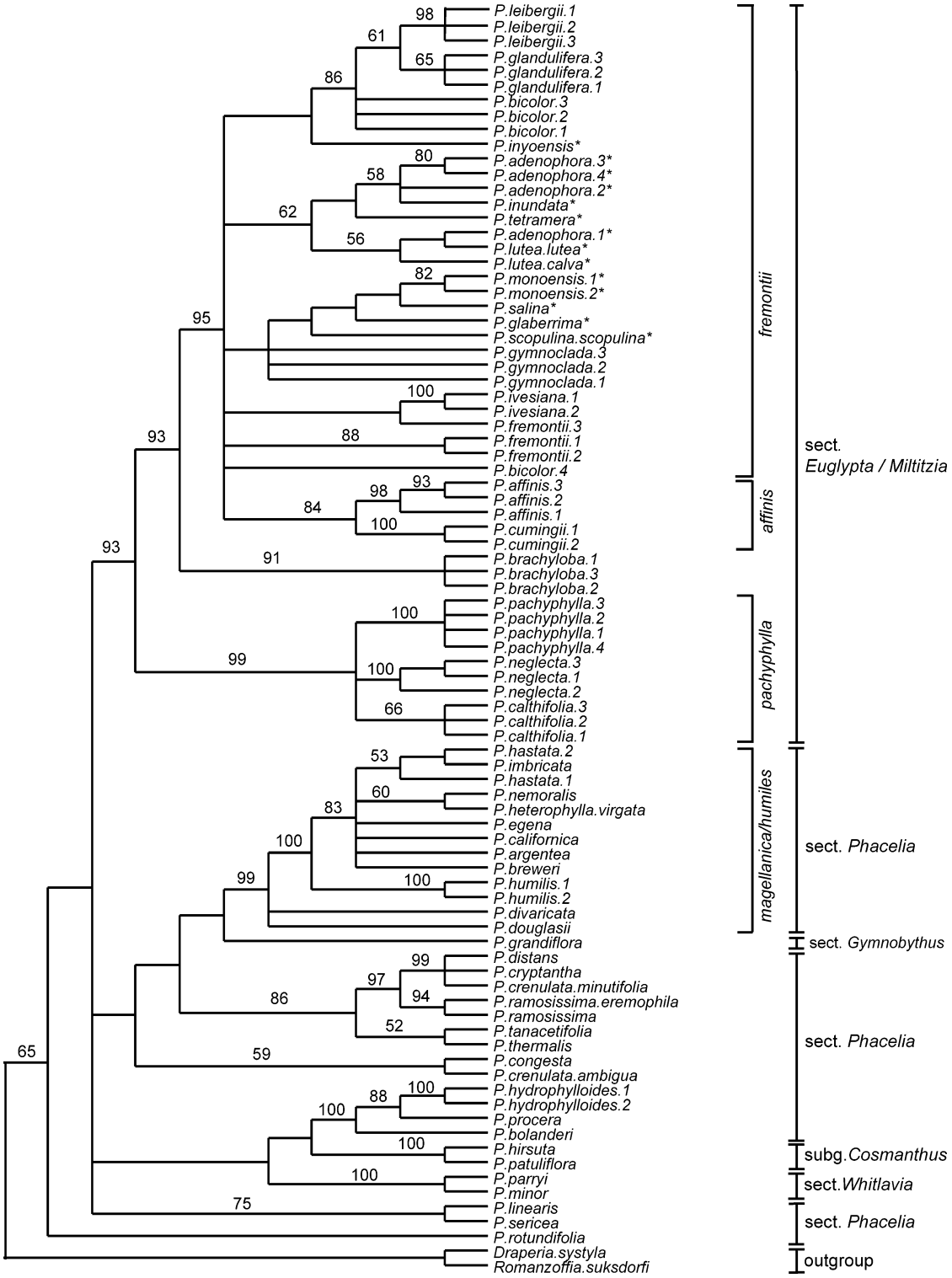

FIG. 1. Strict consensus of 52,506 MP trees using nrITS sequence data. The numbers above the branches are the bootstrap percentages (≥ 50%). Section and species complex names represent subg. *Phacelia* with the exception of two taxa from subg. *Cosmanthus* (Constance 1963). Within sect. *Euglypta/Miltitzia*, asterisks represent taxa in sect. *Miltitzia*.

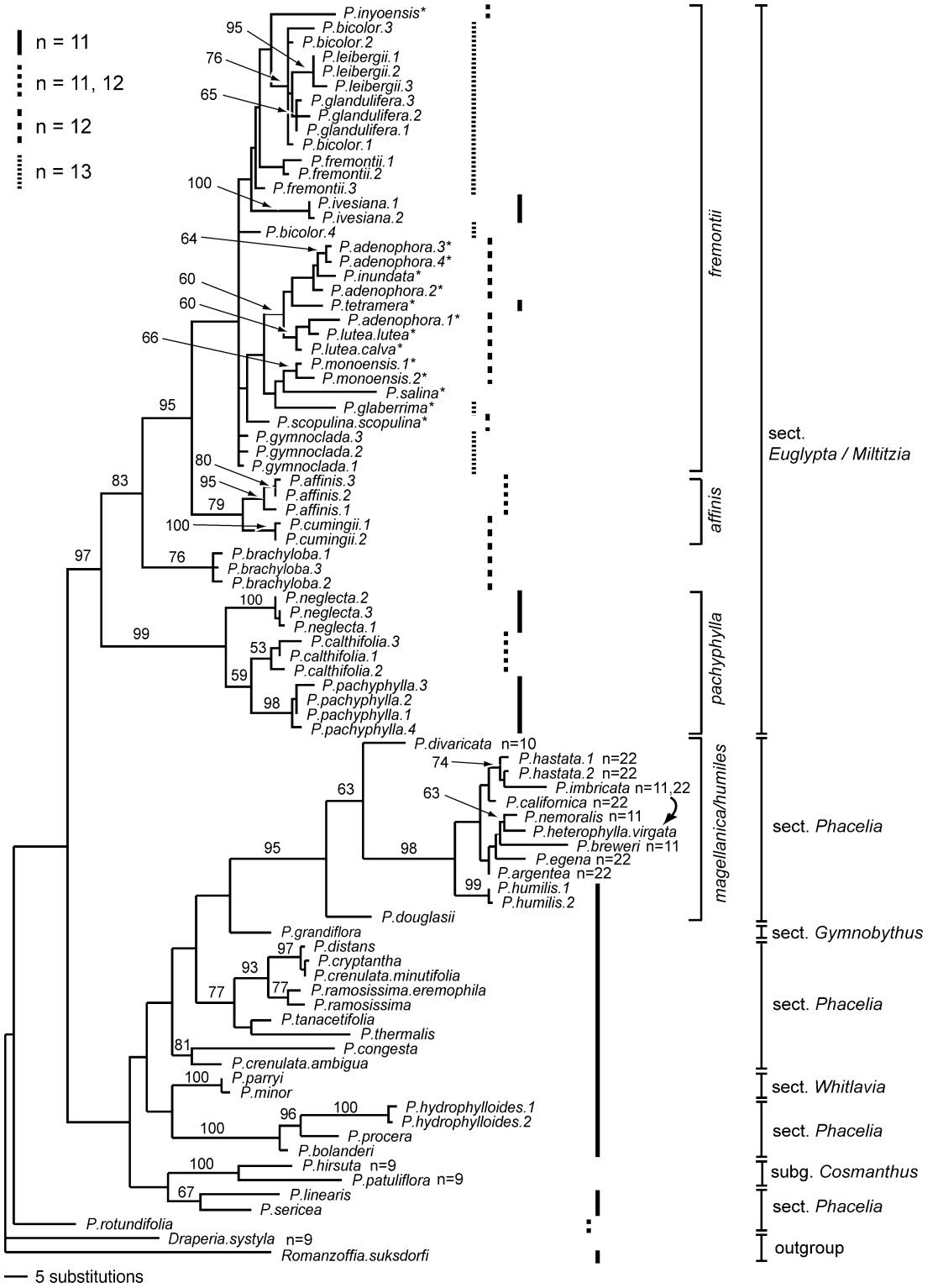

FIG. 2. Phylogram from the ML analysis of nrITS sequence data. The numbers above the branches are the bootstrap percentages (≥ 50%). Section and species complex names represent subg. *Phacelia* with the exception of two taxa from subg. *Cosmanthus* (Constance 1963). Within sect. *Euglypta/Miltitzia*, asterisks represent taxa in sect. *Miltitzia*.

ing interspecific relationships within the *P. fremontii* complex.

Our results provided a framework to examine some of the traditional taxonomic groups in *Phacelia* (Howell 1944; Heckard 1960; Constance 1963; Lee 1986) and to evaluate some the evolutionary relationships within sect. *Euglypta/Miltitzia*. Unlike Ferguson (1999), we did not find a paraphyletic *Phacelia+Romanzoffia* clade; subgenus *Phacelia* was paraphyletic containing two taxa representing subg. *Cosmanthus*. There were several interesting evolutionary patterns within subg. *Phacelia*. Sections *Euglypta* and *Miltitzia* formed a monophyletic clade (Figs. 1, 2) corroborating Howell's (1946) hypothesis about the affinity of these species based upon transversely corrugated seeds, a synapomorphy for the group. However, neither sect. *Euglypta* nor sect. *Miltitzia* were monophyletic. Within a nonmonophyletic sect. *Phacelia*, species representing the *P. magellanica* polyploid complex along within taxa representing the *P. humiles* species group (*P. humilis*, *P. breweri*, *P. douglasii*, and *P. divaricata*) formed a monophyletic clade, an association first identified by Ferguson (1999). This confirmed Lee's (1986) observation that the taxonomic unity of the *P. humiles* species group is questionable. Two additional associations arose within subg. *Phacelia*. The first, a clade consisting of *P. distans*, *P. cryptantha*, *P. crenulata*, and *P. ramosissima* (MPBS = 97%; MLBS = 93%) share the characters of 1–4 seeds per fruit and compound leaves. The second clade, *P. hydrophylloides*, *P. procera*, and *P. bolanderi* (MPBS and MLBS = 100%), share a perennial/biennial habit and flowers with deciduous corollas and exerted stamens (Wilken et al. 1993). Further sampling, particularly within subg. *Cosmanthus* and the monotypic subg. *Howellanthus* is needed for a clear phylogenetic interpretation of the subgeneric taxonomic level in *Phacelia*.

All analyses supported Howell's (1946) *P. pachyphylla* complex as a distinct lineage including *P. calthifolia*, *P. neglecta*, and *P. pachyphylla*. These three species are united by the following symplesiomorphies: characters of small roundish leaves, a capsule protruding above the calyx, presence of corolla scales, and the absence of yellow corolla tubes. Corolla scales are prevalent throughout *Phacelia* but in *Euglypta/Miltitzia* have been reduced (*P. ivesiana*, *P. inundata*) or eliminated (*P. brachyloba*, *P cumingii*, *P. gymnoclada*, *P. monoensis*, *P. glandulifera*, and *P. inyoensis*). Both *P. pachyphylla* and *P. calthifolia* bear violet/purple flowers (*P. neglecta* is white) while a yellow corolla tube has evolved in all remaining *Euglypta/Miltitzia* except in *P. tetramera* with white flowers. We remain cautious about interpreting these morphological characters pending a more thorough developmental/morphological analysis of such characters as corolla scales and corolla tube color.

Howell (1946) considered *P. pachyphylla* and *P. neglecta* uncommon and unique for their unusual ability to grow on desert mosaic (a type of desert pavement) as well as on alkaline flats. Although *P. calthifolia* is confined to the Death Valley region the other two taxa in the complex range south into the Mojave and Sonoran deserts (Fig. 3A). Howell (1946) noted sympatric populations of *P. pachyphylla* and *P. neglecta* in the Mojave Desert with no evidence of intermediate forms. Both *P. pachyphylla* and *P. neglecta* overlap geographically with *P. fremontii*, *P. ivesiana*, and *P. calthifolia* in and around the Mojave Desert but do not grow in common habitat. Howell (1946) considered *P. fremontii* and *P. ivesiana* associated more with the Great Basin (Fig. 3B); these two species nested within the *P. fremontii* complex in our phylogeny along with other taxa that grow in and around the Great Basin. Climatic patterns that contributed to the Great Basin and Mojave Deserts are recent; Raven and Axelrod (1978) suggested that *Phacelia* is one of several genera whose evolution was greatly influenced by patterns of aridification beginning in the

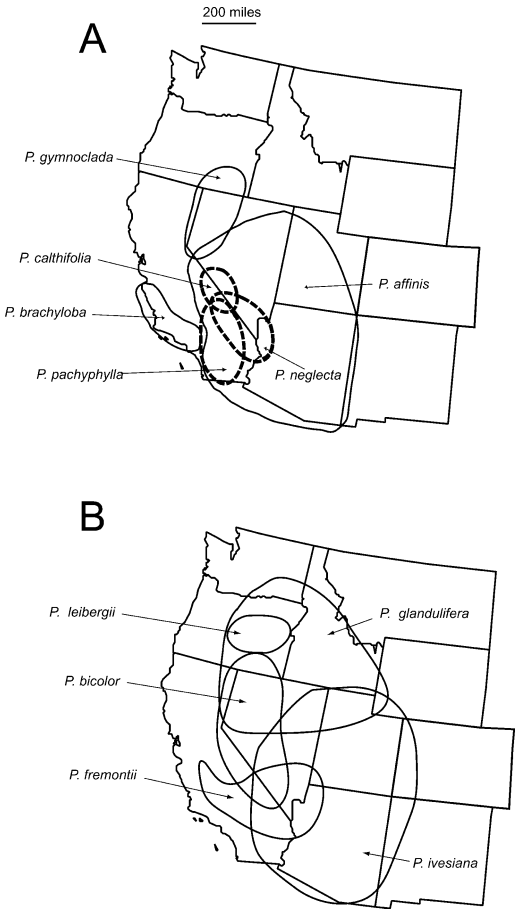

FIG. 3. Distribution of *Phacelia* species. A. The *pachyphylla* complex along with *P. affinis, P. brachyloba,* and *P. gymnoclada*. B. Distribution of *Phacelia bicolor*, P. *fremontii, P. glandulifera, P. ivesiana*, and *P. leibergii*. Redrawn with permission from J. Dempcy (1996).

Upper Tertiary. It should also be noted that *P. brachyloba* is the only taxon in *Euglypta/Miltitzia* outside the desert; it is restricted to the coast of California south to Baja California, Mexico. Geological changes occurred within the Peninsular Ranges during the Pliocene so conceivably both the evolution of the *P. pachyphylla* complex and divergence of *P. brachyloba* were influenced by Pliocene events (Raven and Axelrod 1978).

The remaining taxa found within *Euglypta/Miltitzia* group into an unresolved clade consisting of the *P. affinis* and *P. fremontii* complexes (MPBS and MLBS = 95%). The *P. affinis* complex contains the only South American taxon in this clade, *P. cumingii*. Dempcy (1996) suggested long distance dispersal of the common ancestor of *P. affinis* and *P. cumingii* to South America. He based this hypothesis upon a population sample of *P. affinis* var. *patens* that he included in his analysis (= *P. affinis* in Wilken 1993). This particular sample came from the mountains around Death Valley and Dempcy (1996) noted that it grouped with *P. cumingii* and not the other *P. affinis* populations in his phylogeny of sect. *Euglypta*. In addition to the phylogenetic association, Dempcy (1996) suggested that the published chromosome number for *P. affinis* of $n = 11, 12$ reflected samples that Constance (1963) collected near Death Valley ($n = 12$) and in Arizona ($n = 11$); it was possible that the chromosome number for *P. cumingii* ($n = 12$) aligned with his population sample of *P. affinis* from Death Valley. Bird dispersal is not likely with *Phacelia* as the seeds are not known to be an avian food source. Raven and Axelrod (1974) discussed various North American and South American disjunctions suggesting such events occurred in the Pleistocene when a land bridge in various form existed between the continents. This may be a more plausible explanation for the *P. affinis/P. cumingii* association. Further sampling from South America would enable an assessment of nucleotide divergence estimates and improve our understanding of this disjunction between North and South American *Phacelia*.

While the topology within the *P. fremontii* complex is somewhat unresolved, one hypothesis consistent with our phylogeny was Ganong's (2002) observation that sect. *Miltitzia* shared closest affinity to the *P. fremontii* complex associated with the Great Basin (indicated with *, Figs. 1, 2). Also, the distribution of those sect. *Euglypt*a taxa nesting within the *P. fremontii* complex are associated in and around the Great Basin (Fig. 3B; Halse 1981). The phylogenies (Figs. 1, 2) do not support a close affinity between *P. monoensis* and *P. adenophora* noted by Halse (1981) nor do they support Howell's (1944) hypothesis that *P. salina* is an intermediate between *P. scopulina* and *P. tetramera*, forming a distinct lineage.

There was moderate support for a complex including *P. leibergii*, *P. bicolor*, and *P. glandulifera* (MPBS = 86%; MLBS = 76%) consistent with a putative hybrid origin of *P. leibergii* from *P. bicolor* and *P. glandulifera* (Howell 1946). The restricted range of *P. leibergii* in central Oregon was considered by Howell (1946) as the northward extension of *P. bicolor* and the intersection of both species within the more extended distibution of *P. glandulifera* throughout the Great Basin formed his geographical framework for hybrid evolution (Fig. 3B). However, we did not detect incomplete gene conversion within ITS for *P. leibergii* in comparisons with *P. bicolor* and *P. glandulifera*, suggesting a recent hybrid origin. Dempcy (1996) described several other putative hybrid forms among his sect. *Euglypta* accessions. For example, *P. bicolor*.4 was collected in an area of geographic overlap with *P. fremontii*.3 where intermediates between the two have been documented (Howell 1946). It did not group with the other *P. bicolor* samples and the *P. bicolor*.4 ITS sequence aligned with the *P. fremontii* accessions at seven of the parsimony informative sites. The similar appearance among *P. bicolor* and *P. fremontii* and *P. ivesiana* posed a challenge for Howell (1946) in defining taxonomically useful characters. Our results confirmed this difficulty since populations of these three species did not cluster as monophyletic taxa.

Constance (1963) determined chromosome numbers for 145 species and subspecies of *Phacelia*, with haploid numbers ranging from 9 to 13. Of these, the most frequent number (45%) was $n = 11$. Within *Euglypta/Miltitzia* (Fig. 2) numbers include $n = 11, 12$, and 13. Using the current phylogeny as an hypothesis of evolutionary relationships, the distribution of chromosome numbers in this complex can be explained by one or more aneuploid progressions. For example, the *pachyphylla* complex, has a haploid number of $n = 11$ or 12. A parsimonious scenario would involve one aneuploid increase to $n = 12$ for *P. calthifolia*, and another in *P. brachyloba* followed by at least one independent increase to $n = 13$, and two independent reversals to $n = 11$. Dempcy (1996) noted a loose association between the sect. *Euglypta* taxa associated primarily with the Great Basin (*P. fremontii*, *P. leibergii*, *P. bicolor*, *P. glandulifera*, and *P. gymnoclada*) and $n = 13$. However, most of the taxa assigned to sect. *Miltitzia* have a Great Basin association but a haploid chromosome number of $n = 12$ except for $n = 11$ (*P. tetramera*) and $n = 13$ (*P. glaberrima*). It appears that either the ITS-based phylogeny for the *P. fremontii* group is not accurately based (as indicated by weak bootstrap support), or considerable chromosome evolution in the form of aneuploidy has occurred in this clade.

Our phylogenies support the evolution of a *magellanica/humiles* polyploid complex that was partially studied by Heckard (1960). He analyzed morphological attributes and conducted artificial hybridizing trials of 12 North American taxa, of which seven are represented in our dataset. He suggested allopolyploid or-

igins for tetraploids *P. argentea*, *P. californica*, *P. egena*, and *P. hastata* based on normal pairing of bivalents observed in microsporocyte analysis. He noted difficulty in assigning parentage based on plasticity in the characters scored for this group. An interesting ecological observation was that these species often occupy roadsides and disturbed areas. The branch support within the cladogram was weak (< 50%) but the ML phylogram associates *P. hastata* with *P. imbricata* and *P. californica*. The latter two are noted to have sympatric populations and this pattern was also well documented by Heckard (1960) between *P. imbricata* and *P. egena* and between *P. hastata* and *P. heterophylla*. Identifying the reticulate evolution within this complex will require careful sampling in areas of sympatry and a broader sampling from within the *magellanica/humiles* species group.

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APPENDIX 1.

DNA vouchers listed alphabetically with name, locality, collector, and GenBank accession number. *P.* = *Phacelia*, *D.* = *Draperia*, *R.* = *Romanzoffia*. Previously published Genbank accessions are noted with an asterisk. Location of vouchers in specific herbaria are abbreviated: California Academy of Sciences (CAS), Oregon State University (OSC), Rancho Santa Ana Botanic Garden (RSA), San Francisco State University (SFSU), and the Rocky Mountain Herbarium of the University of Wyoming (RM).

P. adenophora*-1** J. Howell, Lassen Co., CA, *J. Dempcy 116* (SFSU), AY630259. ***P. adenophora*-2,** Lassen Co., CA, *J. Dempcy 114* (SFSU), AY630260. ***P. adenophora*-3,** boundary of Storey and Lyon Cos., NV, *J. Dempcy 117* (SFSU), AY630261. ***P. adenophora*-4,** boundary of Storey and Lyon Cos., NV, *J. Dempcy 117* (SFSU), AY630262. ***P. affinis*-1** A. Gray, Mohave Co., AZ, *E. McClintock 52-264* (CAS), AY630263. ***P. affinis*-2,** Nye Co., NV, *J. Dempcy 138-1* (SFSU), AY630264. ***P. affinis*-3,** Nye Co., NV, *J. Dempcy 137-2* (SFSU), AY630265. ***P. argentea*,** AF091185*. ***P. bicolor*-1** S. Watson, Mono Co., CA, *J. Dempcy 89-2* (SFSU), AY630266. ***P. bicolor*-2,** Lassen Co., CA, *J. Dempcy 112-1* (SFSU), AY630267. ***P. bicolor*-3,** Humboldt Co., NV, *J. Dempcy 118-2* (SFSU), AY630268. ***P. bicolor*-4,** Kern Co., CA, J. *Shevock 8564* (CAS), AY630269. ***P. bolanderi A. Gray, Del Norte Co., CA, *C. Gilbert 54* (SFSU), AY630270. ***P. brachyloba*-1** A. Gray, Santa Barbara Co., CA, *J. Ammirati 315* (SFSU), AY630271. ***P. brachyloba*-2,** Santa Barbara Co., CA, *J. Dempcy 123-1* (SFSU), AY630272. ***P. brachyloba*-3,** Santa Barbara Co., CA, *J. Dempcy 124-1* (SFSU), AY630273. ***P. breweri*** A. Gray, Stanislaus Co., CA, *C. Condos 023* (SFSU), AY630274. ***P. californica*** Cham., San Francisco Co., CA, *M. Ely 040* (SFSU), AY630275. ***P. calthifolia*-1** Brand, Inyo Co., CA, *D. Breedlove 17289* (RSA), AY630276. ***P. calthifolia*-2,** Inyo Co., CA, *J. Thorne 42503* (RSA), AY630277. ***P. calthifolia*-3,** Inyo Co., CA, *J. Dempcy 128-1* (SFSU), AY630278. ***P. congesta*,** AF091189*. ***P. crenulata* var. *ambigua*** J.F. Macbr., San Bernadino Co., CA, *K. Whitney 028*

(SFSU), AY630279. ***P. crenulata* var. *minutifolia*** Jepson, San Diego Co., CA, *R. Peters 001* (SFSU), AY630280. ***P. cryptantha*** E. Greene, San Diego Co., CA, *S. Pense 002* (SFSU), AY630281. ***P. cumingii*-1** (Benth.) Gray, Chile, *L. Constance 3502* (CAS), AY630282. ***P. cumingii*-2,** Chile, *Werdeman 1042* (CAS), AY630283. ***P. distans*** Benth., Marin Co., CA, *P. Wharton 024* (SFSU), AY630284. ***P. divaricata*** A. Gray, San Mateo Co., CA, *M.A. Hewlett 581* (SFSU), AY630285. ***P. douglasii*** (Benth.) Torrey, Monterey Co., CA, *L. S. Rose 69018* (SFSU), AY630286. ***P. egena*** (Brand) J. Howell, Tehama Co., CA, *M.A. Showers 1679* (SFSU), AY630287. ***P. fremontii*-1** Torrey, San Bernadino Co., CA, *J. Dempcy 102-2* (SFSU), AY630288. ***P. fremontii*-2,** Nye Co., NV, *J. Dempcy 101-1* (SFSU), AY630289. ***P. fremontii*-3,** Inyo Co., CA, *J. Dempcy 105-13* (SFSU), AY630290. ***P. glaberrima*** (Torr.) J.T. Howell, Pershing Co., NV, *A. Tiehm 11666* (OSC)**,** AY630291. ***P. glandulifera*-1** Piper, Harney Co., OR, *A. Tiehm 11063* (CAS), AY630292. ***P. glandulifera*-2,** Lake Co., OR, *J. Dempcy 119-3* (SFSU), AY630293. ***P. glandulifera*-3,** Harney Co., OR, *J. Dempcy 120-2* (SFSU), AY630294. ***P. grandiflora*,** AF091190*. ***P. gymnoclada*-1** S. Watson, Washoe Co., NV, *J. Dempcy 115-1* (SFSU), AY630295. ***P. gymnoclada*-2,** Esmeraldo Co., NV, *Holmgren 11352* (CAS), AY630296. ***P. gymnoclada*-3,** Nye Co., NV, *J. Dempcy 136-1* (SFSU), AY630297. ***P. hastata*-1** Lehm., Inyo Co, CA, *C. Gilbert 106* (SFSU), AY630298. ***P. hastata*-2,** Mono Co, CA, *C. Gilbert 109* (SFSU), AY630299. ***P. heterophylla*** (Pursh) *ssp. virgata* (E. Green) Heckard, Alpine Co, CA, *C. Gilbert 101* (SFSU), AY630300. ***P. hirsuta*,** AF091193*. ***P. humilis*-1** Torrey & A. Gray, Sonoma Co. CA, *C. Gilbert 3A* (SFSU), AY630301. ***P. humilis*-2,** Sierra Co., CA, *R. Patterson 1795* (SFSU), AY630302. ***P. hydrophylloides*-1** A. Gray, Sierra Co., CA, *J. Dempcy 126* (SFSU), AY630303. ***P. hydrophylloides*-2,** Sierra Co., CA, *J. Shevock 5140* (CAS), AY630304. ***P. imbricata*** E. Greene, Lake Co., CA, *D. Toren 3582* (SFSU), AY630305. ***P. inundata*** J. Howell, Lassen Co., CA, *B. Bartholomew 6559* (CAS), AY630306. ***P. inyoensis*** (J.F. Macbr.) J. Howell, Inyo Co., CA, *M. DeDecker 6444* (RSA)**,** AY630307. ***P. ivesiana*-1** Torrey, San Bernadino Co., CA, *Priggle 2952* (RSA), AY630308. ***P. ivesiana*-2,** San Bernadino Co., CA, *J. Dempcy 08* (SFSU), AY630309. ***P. leibergii*-1** Brand, Deschutes Co., OR, *J. Dempcy 99-2* (SFSU), AY630310. ***P. leibergii*-2,** Deschutes Co., OR, *J. Dempcy 100-2* (SFSU), AY630311. ***P. leibergii*-3,** Deschutes Co., OR, *J. Dempcy 121-2* (SFSU), AY630312. ***P. linearis*,** AF091195*. ***P. lutea* var. *calva*** *Cronquist*, Humbolt Co., NV, *A. Tiehm 12085* (CAS), AY630313. ***P. lutea* var. *lutea*** (H. and A.) J.T. Howell, Washoe Co., NV, *A. Tiehm 10617* (CAS), AY630314. ***P. minor*,** AF091197*. ***P. monoensis*-1,** R. Halse, Lyon Co, NV, *J. Dempcy 116-1* (SFSU), AY630315. ***P. monoensis*-2,** Lyon Co., NV, *J. Dempcy 116-2* (SFSU), AY630316. ***P. neglecta*-1** M.E. Jones, Riverside Co., CA, *Sanders 12090* (RSA), AY630317. ***P. neglecta*-2,** San Bernadino Co., CA, *Hendrickson 16473* (RSA), AY630318. ***P. neglecta*-3,** Inyo Co., CA, *Castagnoli et al. 124* (CAS), AY630319. ***P. nemoralis*** E. Greene, Marin Co., CA, *H. Leschke* (SFSU), AY630320. ***P. pachyphylla*-1** A. Gray, Kern Co., CA, *Gustafson 487* (RSA), AY630321. ***P. pachyphylla*-2,** San Bernadino Co., CA, *Sanders 227* (CAS), AY630322. ***P. pachyphylla*-3,** San Bernadino Co., CA, *J. Dempcy 130-1* (SFSU), AY630323. ***P. pachyphylla*-4,** San Bernadino Co., CA, *J. Dempcy 130-2* (SFSU), AY630324. ***P. parryi*** Torrey, San Diego Co., CA *J. Dempcy 003* (SFSU), AY653742. ***P. patulifera*,** AF091198*. ***P. procera*** A. Gray, Lake Co., CA, *D. Toren* (SFSU), AY630325. ***P. ramosissima*-1** Lehm., Alpine Co., CA, *C. Gilbert 100* (SFSU), AY630326. ***P. ramosissima*-2,** Mono Co., CA, *H.D. Thiers* (SFSU), AY630327. ***P. rotundifolia*** S. Watson, San Bernadino Co., CA, *C. Condos 009* (SFSU), AY630328. ***P. salina*** (A. Nels) J.T. Howell, Sweetwater Co., WY, *B. E. Nelson 36344* (RM), AY630329. ***P. scopulina*** *(*A. Nels.*) J.T. Howell,* Elko Co, NV, *A. Tiehm 10573* (OSC), AY630330. ***P. sericea*** (Graham) A. Gray, Modoc Co., CA, *M.A. Showers* (SFSU), AY630331. ***P. tanacetifolia*** Benth., Inyo Co., CA, *C. Gilbert 108* (SFSU), AY630332. ***P. tetramera*** J. Howell, Humbolt Co, NV, *A. Tiehm, 12133* (CAS), AY630333. ***P. thermalis*,** AF091202*. ***R. californica*** E. Green, Marin Co., CA, *M. Ely 063* (SFSU), AY630334. ***D. systyla*** (A. Gray) Torrey, Sierra Co., CA, *S. Pense 040* (SFSU), AY630335.