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# PHYLOGENIES AND CHROMOSOME EVOLUTION OF *PHACELIA* (BORAGINACEAE: HYDROPHYLLOIDEAE) INFERRED FROM NUCLEAR RIBOSOMAL AND CHLOROPLAST SEQUENCE DATA

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## Abstract

This project sampled throughout *Phacelia* using the internal transcribed spacer region (ITS-1, ITS-2, and 5.8S gene) of nuclear ribosomal DNA (nrITS) and the chloroplast DNA gene (*ndh*F) to infer phylogenies for nuclear and plastid partitions. Nuclear and plastid partitions were incongruent in our analyses. Phylogenetic analyses (maximum parsimony, maximum likelihood, and Bayesian inference) recovered gene tree topologies similar to previous molecular studies. We corroborate incongruence between nuclear and plastid topologies for placement of some problematic groups (e.g., *Draperia, Romanzoffia* and "core" *Phacelia* subg. *Pulchellae*, *Phacelia* sect. *Baretiana*). Combined analyses resulted in better resolution than separate analyses, and in a topology that favored the separate plastid topologies. *Romanzoffia* was sister to a monophyletic *Phacelia* in the combined analyses. Our results support combining incongruent partitions in a combined analysis to seek support for internal nodes. Maximum likelihood analyses were used to infer ancestral chromosome numbers and identify gains, losses, polyploid doubling, and whole genome duplication events from published chromosome counts in the genus. The predicted base number for the genus was $x = 9$, $x = 11$, or $x = 12$.

Key Words: Boraginaceae, California flora, chromosome evolution, Hydrophylloideae, incongruence, molecular phylogenetics, *Phacelia*, *Romanzoffia*.

*Phacelia* Juss. is the largest genus (207 spp.) in Hydrophylloideae (Boraginaceae). The majority of species (176 spp.) are distributed in western North America and an additional 16 species occur into Central America, with an amphitropical disjunct group of nine species in southwestern South America. The center of diversity for the genus is the California Floristic Province (CFP); a third of described taxa occur within the CFP (ca. 70 spp., 40 spp. endemic) and ca. 90 spp. occur within the political boundaries of the state (Raven and Axelrod 1978; Patterson et al. 2012). In California, 33 taxa in *Phacelia* are ranked in the California Native Plant Society Rare and Endangered Plant Inventory (CNPS 2011). These include one of three federally endangered taxa (*P. insularis* Munz var. *insularis*) and one candidate taxon considered for federal protection (*P. stellaris* Brand) (U.S. Department of the Interior, Fish and Wildlife Service 1978, 1982, 1997, 2004, 2011). Thus, *Phacelia* is one of ten largest genera and Boraginaceae one of ten largest flowering plant families occurring in the CFP and in California (Beard et al. 2000; Baldwin et al. 2012). *Phacelia,* as the largest and most diverse genus in Hydrophylloideae, is often used as an example of the diversity of the California flora (Stebbins and Major 1965; Raven and Axelrod 1978; Ackerly 2009; Kraft et al. 2010). However, research regarding evolution and diversification in the genus and its significance in the California flora has been limited due to the lack of a well-resolved, broadly sampled molecular phylogeny with congruent nuclear and plastid partitions.

## Recent Molecular Studies

Relationships of major lineages in *Phacelia* have been previously studied using molecular phylogenetic methods. Gilbert et al. (2005) combined thesis work in *Phacelia* sect. *Euglypta* S. Watson by Dempcy (1996) and in *Phacelia* sect. *Miltitzia* (A. de Candolle) J. T. Howell by Ganong (2002), along with sequences from dissertation work by Ferguson (1998), to publish an nrITS partition of 51 taxa (84 accessions) in *Phacelia*. Gilbert et al. (2005) recovered *Romanzoffia* Cham. sister to a monophyletic *Phacelia* and recovered a monophyletic *Phacelia* subg. *Microgenetes* (A. de Candolle) A. Gray that included a paraphyletic *Phacelia* sect. *Euglypta* and a paraphyletic *Phacelia* sect. *Miltitzia*.

Hansen et al. (2009) published an nrITS partition of 56 taxa (91 accessions) and a *rpl16* intron partition of 22 taxa (37 accessions) for *Phacelia* sect. *Gymnobytha* (A. de Candolle) Benth. & Hook.f. and *Phacelia* sect. *Whitlavia* (Harv.) Benth. & Hook.f. Hansen et al. (2009) recovered *Romanzoffia* sister to a monophyletic *Phacelia* and recovered a paraphyletic *Phacelia* sect. *Whitlavia* in the separate nuclear partition. *Phacelia* was paraphyletic in the separate plastid partition. Both *Phacelia* sect. *Euglypta* and *Phacelia* subg. *Pulchellae* (Rydb.) Walden & Patt. were recovered as basal lineages with *Romanzoffia* nested within the genus. The combined nrITS and *rpl16* intron analysis recovered *Romanzoffia* sister to a monophyletic *Phacelia* and monophyletic infrageneric sections (Hansen et al. 2009). Ferguson (1998 [1999]) included 19 taxa (19 accessions) of *Phacelia* within a larger analysis of Hydrophylloideae for *ndh*F, recovering a paraphyletic *Phacelia*. *Phacelia* subg. *Pulchellae* was sister to a nested *Romanzoffia* and remaining sampled *Phacelia*.

Collectively, researchers have published 124 accessions within *Phacelia* using the internal transcribed spacer region (ITS-1, ITS-2, and 5.8S gene) of nuclear ribosomal DNA (nrITS), published 20 accessions for the chloroplast (cpDNA) gene *ndh*F, and published 37 accessions for the chloroplast (cpDNA) marker *rpl16* intron (Ferguson 1998 [1999]; Olmstead et al. 2000; Gilbert et al. 2005; Hansen et al. 2009; Glass and Levy 2011). We combined previously published nrITS sequences with thesis work in *Phacelia* sect. *Glandulosae* (Rydb.) Walden & R. Patt. and *Phacelia* sect. *Ramosissimae* (Rydb) Walden & R. Patt. of Garrison (2007) in an expanded nuclear phylogeny to infer inter- and infraspecific evolutionary relationships in *Phacelia*. We combined previously published *ndh*F sequences with thesis work in *Phacelia* of Walden (2010) in an expanded cpDNA phylogeny to infer infrageneric relationships in the genus.

Previous molecular phylogenetic studies in *Phacelia* and Hydrophylloideae have identified significant phylogenetic incongruence between nuclear and plastid partitions (Ferguson 1998; Moore and Jansen 2006; Hansen et al. 2009; Weeks et al. 2010; Nazaire and Hufford 2012; Taylor 2012). For an extensive discussion on incongruence between nuclear (nrITS) and plastid (*ndh*F) partitions in *Phacelia* and Hydrophylloideae see Ferguson (1998). When tests for homogeneity between partitions (e.g., incongruence length test [Farris et al. 1995]) reject the null hypothesis, a combined analysis is inappropriate and partitions are analyzed separately using the conditional combination approach (Bull et al. 1993; Huelsenbeck et al. 1996). Some researchers combine incongruent partitions using simultaneous analyses, arguing that these combined analyses provide greater resolution than separate analyses of incongruent partitions (Nixon and Carpenter 2005). Our goal was to determine if increased sampling for a respective molecular marker (nrITS, *ndh*F) within *Phacelia* recovered similar gene tree topologies to previous studies for separate analyses of partitions, to compare nuclear and plastid partitions for character homogeneity and combinability (Cunningham 1997), and to determine if simultaneous analyses provided enhanced resolution for a reduced subset of samples for which both nrITS and *ndh*F sequences were available.

## Chromosome Evolution

*Phacelia* occupies a range of habitats and exhibits a variety of life history traits and ecological adaptations. Species differences have been traditionally based on morphological (e.g., seed shape and number) and cytological characters (e.g., chromosome numbers), and less so on ecological factors (e.g., edaphic factors). These characters have been used to diagnose infra- and interspecific taxa, and to delimit infrageneric groups within the genus (for a review and current infrageneric classification in *Phacelia*, see Walden and Patterson [2012]). Both Constance's (1963) and Gillett's (1968) classifications were based largely upon chromosome numbers, drawing upon Constance's extensive collaborations into chromosome number differences in Hydrophyllaceae with Marion Cave (see Cave and Constance [1942, 1944, 1947, 1950, 1959]). *Phacelia* benefits from published chromosome counts for approximately two thirds of the genus, ranging from $n = 5$ (*P. dubia* [L.] Trel. & Small, *P. maculata* Wood) to $n = 33$ (*P. hastata* Douglas ex Lehm. var. *compacta* [Greene ex Brand] Cronquist, *P. leptosepala* Rydb.) (Cave and Constance 1947, 1950; Kruckeberg 1956; Kovanda 1978).

Constance (1963); Heckard (1963), and Gillett (1968) hypothesized that $n = 11$ was the ancestral condition for the genus and noted it was also the most common haploid count for extant taxa. Hypotheses proposed for the base number for the genus have not been tested in a broad phylogenetic context. Previous studies considering evolution of chromosome numbers in a molecular context in *Phacelia* include mapping of chromosome numbers to nrITS tree topologies by Gilbert et al. (2005) and to *ndh*F sequence data using maximum parsimony by Walden (2010). Reconstructing ancestral states using a maximum parsimony approach without an explicit framework (e.g., biosystematic studies of chromosomal rearrangements across the genus) allows only for coding with a categorical character matrix using the unordered states assumption in Mesquite version 2.74 (Maddison and Maddison 2010), regardless of whether a transition represents an increasing or decreasing dysploidy event or a doubling polyploid event (Mayrose et al. 2010). Although the maximum parsimony approach has real merit, the unordered states assumption option offers little resolution for this dataset at the present time. We were interested in determining the ancestral base number for *Phacelia* to better understand patterns of chromosome evolution within infrageneric groups and within the genus using a maximum likelihood approach (Mayrose et al. 2010; Hallinan and Lindberg 2011a). chromEvol version 1.3 (Mayrose et al. 2010) and GDCN (Hallinan and Lindberg 2011a) use explicit likelihood models of evolution to infer ancestral states for chromosome numbers at nodes in phylogenies from rooted ultrametric trees. These analyses offer the ability to test hypotheses for the base number for infrageneric groups and the genus using results from our expanded nuclear and plastid phylogenies. chromEvol v.1.3 and GDCN estimate probabilities of chromosome evolution events at nodes to explore patterns of gains, losses, polyploid doubling, and whole genome duplication (WGD) events within a known phylogeny (Mayrose et al. 2010; Hallinan and Lindberg 2011a). Saltational speciation has been an important factor in cladogenesis in the California flora; we were interested in identifying any recent genome duplication events within *Phacelia* phylogenies using a maximum likelihood approach (Stebbins and Major 1965; Raven and Axelrod 1978; Wood et al. 2009; Hallinan and Lindberg 2011a).

## Materials and Methods

### Chromosome Numbers in *Phacelia*

We reviewed the literature for published chromosome counts for taxa in *Phacelia* and outgroups sampled in this study (Table 1). Taxa are presented alphabetically for outgroups (*Euploca* Nutt., *Eriodictyon* Benth., *Draperia* Torr., *Hesperochiron* S. Watson, *Howellanthus* [Constance] Walden & R. Patt., *Nama* L., *Romanzoffia, Tricardia* Torr. ex S. Watson) and ingroup taxa within *Phacelia.* Original names for published counts are noted for synonyms or where different from the current accepted name or specimen determination. Chromosome numbers for haploid ($n$) or diploid ($2n$) counts are given as published. We chose not to include references reporting unpublished counts or citing personal communications for these analyses. We note if a voucher specimen was not cited in the notes column of the table. No attempt was made to locate and examine all voucher specimens cited for each published count for this study. References may include one or more counts for an individual taxon, we do not include summary numbers of the individual counts for each reference or a comprehensive list of voucher specimens and karyotype figures. Constance (1963) reported a count for *Phacelia pauciflora* S. Watson without reference to a voucher specimen. We included this count and corresponding voucher specimen examined at the University of California Herbarium (UC) in Table 1. Chromosome counts originally published as taxa in *Phacelia* but corrected or redetermined in later publication were excluded. Citations are listed chronologically within each taxon and a list of full references follows the table. Chromosome counts were not directly obtained from individuals or populations included in the direct sequence analyses for this study. This limitation may obscure cryptic diversity in sampled populations. This list should be considered a working draft of chromosome numbers for *Phacelia* and Boraginaceae. A future comprehensive review of chromosome counts in Boraginaceae is anticipated as a useful resource for workers in the family (G. K. Walden, unpublished manuscript).

### Taxon Sampling

This study represents a joint publication of thesis work from Garrison (2007) and Walden (2010). The expanded nuclear partition (176-accession) included 89 taxa (42% genus) and the expanded plastid partition (126-accession) included 90 taxa (43% genus). Sampling within *Phacelia* for the expanded nuclear (176-accession) and plastid (126-accession) partitions included representatives from all subgenera and sections; the reduced (61-accession) partition lacked a representative from *Phacelia* sect. *Pachyphyllae* Walden & R. Patt. For the nuclear partition 48 taxa were sampled from California, 44 taxa sampled from western North America, 5 taxa were sampled from Central America, and one taxon was sampled from South America. For the plastid partition 53 taxa were sampled from California, 37 taxa were sampled from western North America, five taxa were sampled from Central America, and one taxon was sampled from South America. Accessions of *Romanzoffia* were included to assess the relationship to and monophyly of *Phacelia.* We included accessions from Hydrophylloideae to briefly assess relationships between *Phacelia* and exemplar taxa and for purposes of chromosomal evolution. *Euploca* (Heliotropioideae: Boraginaceae) was included as the diploid outgroup to root the tree.

Field collections of fresh plant material were preserved in silica gel for molecular work and voucher specimens were deposited in the Harry D. Thiers Herbarium at San Francisco State University (SFSU). Additional material was sequenced from banked molecular vouchers with herbarium vouchers received from the William L.

TABLE 1. CHROMOSOME COUNTS (HAPLOID [*N*], DIPLOID [2*N*]) FOR SAMPLED TAXA IN THIS STUDY AND REFERENCE CITATIONS. Counts lacking a voucher specimen are indicated in the notes column. Coding for analyses in chromEvol version 1.3 and GDCN are given. Abbreviations: *M.* = *Miltitzia*, *P.* = *Phacelia*, *R.* = *Romanzoffia*, VNC = Voucher Not Cited. Full references follow the table.

| Taxon | Haploid count (*n*) | Diploid count (2*n*) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| OUTGROUPS | | | | | | | |
| *Euploca campestris* (Griseb.) Diane & Hilger | 7 | | Di Fulvio 1969 | *Heliotropium campestre* Griseb. | | 7 | 7 |
| *Eriodictyon californicum* (Hook. & Arn.) Torr. | 14 | | Cave and Constance 1947 | | | 14 | 14 |
| *Draperia systyla* (A. Gray) Torr. ex A. Gray | 9 | | Cave and Constance 1947 | | | 9 | 9 |
| *Hesperochiron pumilus* (Griseb.) Porter | 8 | | Cave and Constance 1959 | | | 8 | 8 |
| *Howellanthus dalesianus* (J. T. Howell) Walden & R. Patt. | 8 | | Cave and Constance 1950 | *P. dalesiana* J.T. Howell | | 8 | 8 |
| *Nama demissum* A. Gray var. *demissum* | 7 | | Cave and Constance 1947 | *Nama demissum* A. Gray | | 7 | 7 |
| | 7 | | Bacon 1974 | | | | |
| *R. californica* Greene | 11 | | Cave and Constance 1942 | *R. suksdorfii* Greene | | 11 | 11 |
| *R. thompsonii* Marttala | | | | | | X | 11 |
| *Tricardia watsonii* Torr. ex S. Watson | ca. 8, 8 | | Cave and Constance 1959 | | | 8 | 8 |
| INGROUPS | | | | | | | |
| *P. adenophora* J.T. Howell | 12 | | Cave and Constance 1947 | *M. glandulifera* (Torr.) A. Heller | | 12 | 12 |
| | 12 | | Halse 1981 | | | | |
| *P. affinis* A. Gray | 11 | | Cave and Constance 1947 | | | 11_12 | 12 |
| | ca. 12, 12 | | Cave and Constance 1959 | | | | |
| *P. argentea* A. Nelson & J. F. Macbr. | 22 | | Cave and Constance 1942 | | | 22 | 22 |
| *P. arizonica* A. Gray | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| | 11 | | Ward 1983 | | | | |
| *P. artemisioides* Griseb. | 11 | | Covas and Schnack 1947 | | VNC | 11 | 11 |
| *P. bicolor* Torr. ex S. Watson | 13 | | Cave and Constance 1950 | | | 13 | 13 |
| *P. bolanderi* A. Gray | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. bombycina* Wooton & Standl. | 11 | | Ward 1983 | | | 11 | 11 |
| | 11 | | Ward 1984 | | | | |
| *P. brachyloba* (Benth.) A. Gray | 12 | | Cave and Constance 1944 | | | 12 | 12 |
| | 12 | | Cave and Constance 1947 | *P. cooperae* A. Gray | | | |
| *P. aff. brannanii* Kellogg | | | | | | X | 13 |
| *P. breweri* A. Gray | 11 | | Cave and Constance 1942 | | | 11 | 11 |
| *P. californica* Cham. | 22 | | Cave and Constance 1942 | | | 22 | 22 |
| *P. calthifolia* Brand | 11 | | Cave and Constance 1950 | | | 11_12 | 11 |
| | 12 | | Cave and Constance 1959 | | | | |
| *P. campanularia* A. Gray var. *campanularia* | 11 | | Cave and Constance 1947 | *P. campanularia* A. Gray | | 11 | 11 |
| *P. campanularia* A. Gray var. *vasiformis* (G. W. Gillett) Walden & R. Patt. | 11 | | Sugiura 1936 | *P. campanularia* A. Gray | VNC | 11 | 11 |

TABLE 1. CONTINUED.

| Taxon | Haploid count (*n*) | Diploid count (2*n*) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| | | 22 | Zhao and Turner 1993 | *P. campanularia* A. Gray subsp. *vasiformis* G. W. Gillett | | | |
| *P. capitata* Kruckeb. | 11 | | Kruckeberg 1956 | | | 11_22 | 11 |
| | 11, 22 | | Shelly 1989 | | | | |
| *P. cephalotes* A. Gray | 11 | | Spellenberg 1979 | | | 11 | 11 |
| *P. cicutaria* Greene var. *cicutaria* | 11 | | Cave and Constance 1944 | *P. cicutaria* Greene | | 11 | 11 |
| *P. cicutaria* Greene var. *hispida* (A. Gray) J. T. Howell | 11 | | Cave and Constance 1942 | *P. eximia* Eastw. | | 11 | 11 |
| *P. coerulea* Greene | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| | 11 | | Reveal and Spellenberg 1976 | | | | |
| | 11 | | Schaack et al. 1984 | | | | |
| | $11_{II}$ | | Powell and Turner 2005 | | | | |
| *P. congesta* Hook. | 9 | | Sugiura 1928 | | VNC | 9_11 | 11 |
| | 11 | | Cave and Constance 1947 | | | | |
| *P. cookei* Constance & Heckard | | 22 | Constance and Heckard 1970 | | | 11 | 11 |
| *P. corrugata* A. Nelson | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| *P. cottamii* N. D. Atwood | | | | | | X | 11 |
| *P. crenulata* Torr. ex S. Watson var. *ambigua* (M.E. Jones) J.F. Macbr. | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. crenulata* Torr. ex S. Watson var. *angustifolia* N. D. Atwood | | | | | | X | 11 |
| *P. crenulata* Torr. ex S. Watson var. *crenulata* | | 24 | Zhao and Turner 1993 | | | 12 | 12 |
| *P. cryptantha* Greene | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. cumingii* (Benth.) A. Gray | 12 | | Cave and Constance 1959 | | | 12 | 12 |
| *P. curvipes* Torr. ex S. Watson | 11 | | Cave and Constance 1947 | *P. pratensis* A. Heller | | 11 | 11 |
| *P. davidsonii* A. Gray | 10 | | Cave and Constance 1944 | | | 10 | 10 |
| *P. demissa* A. Gray | ca. 12, 12 | | Cave and Constance 1959 | | | 12 | 12 |
| *P. distans* Benth. *sensu lato* | 11 | | Cave and Constance 1942 | *P. ammophila* Greene in Baker, *nom. nud.* | | 11_12 | 11 |
| | 11 | | Cave and Constance 1942 | *P. distans* Benth. | | | |
| | 11 | | Cave and Constance 1942 | *P. distans* Benth. var. *australis* Brand | | | |
| | | 24 | Zhao and Turner 1993 | *P. distans* Benth. | | | |
| *P. divaricata* (Benth.) A. Gray | 10 | | Sugiura 1940 | *P. dwarecata* [*sic*] | VNC | 10 | 10 |
| | 10 | | Cave and Constance 1942 | | | | |
| *P. douglasii* (Benth.) Torr. | 11 | | Cave and Constance 1950 | | | 11 | 11 |
| *P. dubia* (L.) Trel. & Small var. *dubia* | 5 | | Cave and Constance 1947 | *P. dubia* (L.) Trel. | | 5 | 5 |
| | 5 | | Kovanda 1978 | | | | |
| *P. egena* (Greene ex Brand) J. T. Howell | 22 | | Cave and Constance 1944 | *P. californica* Cham. var. ? | | 22 | 22 |
| | 22 | | Cave and Constance 1944 | *P. virgata* Greene var. *bernardina* Greene ? | | | |
| *P. eisenii* Brandegee | 9 | | Cave and Constance 1947 | | | 9 | 9 |

TABLE 1. CONTINUED.

| Taxon | Haploid count (*n*) | Diploid count (*2n*) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| *P. formosula* Osterh. | | | | | | X | 11 |
| *P. franklinii* (R. Br.) A. Gray | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| | 11 | | Gillett 1960 | | | | |
| | 11 | | Mosquin 1968 | | | | |
| | | 22 | Löve and Löve 1982 | | | | |
| *P. fremontii* Torr. | 13 | | Cave and Constance 1942 | | | 13 | 13 |
| | 13 | | Cave and Constance 1944 | | | | |
| *P. glaberrima* (Torr. ex S. Watson) J. T. Howell | 13 | | Cave and Constance 1950 | | | 13 | 13 |
| *P. glandulifera* Piper | 13 | | Cave and Constance 1950 | | | 13 | 13 |
| *P. glandulosa* Nutt. | | | | | | X | 11 |
| *P. glechomifolia* A. Gray | 12 | | Cave and Constance 1959 | | | 12 | 12 |
| *P. grandiflora* (Benth.) A. Gray | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. greenei* J. T. Howell | 10 | | Cave and Constance 1950 | | | 10 | 10 |
| *P. grisea* A. Gray | 9 | | Cave and Constance 1959 | | | 9 | 9 |
| *P. gymnoclada* Torr. ex S. Watson | 13 | | Cave and Constance 1950 | | | 13 | 13 |
| *P. gypsogenia* I. M. Johnst. | 11 | | Powell and Powell 1977 | | | 11 | 11 |
| *P. hastata* Douglas ex Lehm. var. *compacta* (Greene ex Brand) Cronquist | 22 | | Cave and Constance 1942 | *P. compacta* (Greene ex Brand) J. T. Howell | | 22_33 | 22 |
| | 22 | | Cave and Constance 1944 | *P. leucophylla* Torrey in Fremont var. *?* | | | |
| | 22 | | Cave and Constance 1947 | *P. compacta* (Greene ex Brand) J. T. Howell | | | |
| | 22 | | Cave and Constance 1947 | *P. frigida* Greene | | | |
| | 22, 33 | | Kruckeberg 1956 | *P. frigida* Greene | | | |
| | 22 | | Kruckeberg 1956 | *P. leucophylla* Torrey in Fremont var. *?* | | | |
| *P. heterophylla* Pursh | 11, 22 | | Cave and Constance 1942 | | | 11_22 | 11 |
| | 22 | | Cave and Constance 1947 | *P. urticacea* Greene ex Brand *in syn, nom. invalid.* | | | |
| | 11 | | Cave and Constance 1950 | *P. urticacea* Greene ex Brand *in syn, nom. inval.* | | | |
| | 11 | | Kruckeberg 1956 | | | | |
| *P. heterophylla* Pursh var. *virgata* (Greene) R. D. Dorn | 11 | | Cave and Constance 1944 | *P. virgata* Greene | | 11 | 11 |
| | 11 | | Cave and Constance 1950 | *P. piersoniae* L. O. Williams | | | |
| | 11 | | Shelly 1989 | | | | |
| *P. hirsuta* Nutt. | 9 | | Cave and Constance 1947 | | | 9 | 9 |
| | 9 | | Smith 1968 | | | | |
| *P. howelliana* N. D. Atwood | | | | | | X | 11 |

TABLE 1. CONTINUED.

| Taxon | Haploid count (*n*) | Diploid count (*2n*) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| *P. hubbyi* (J. F. Macbr.) L. M. Garrison | 11 | | Cave and Constance 1947 | *P. cicutaria* Greene var. *hubbyi* (J. F. Macbr.) J. T. Howell | | 11 | 11 |
| *P. humilis* Torr. ex A. Gray | 11 | | Cave and Constance 1942 | | | 11 | 11 |
| | 11 | | Cave and Constance 1947 | | | | |
| *P. hydrophylloides* Torr. ex A. Gray | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| *P. imbricata* Greene var. *imbricata* | 11, 22 | | Cave and Constance 1942 | *P. imbricata* Greene | | 11_22 | 11 |
| | 11 | | Cave and Constance 1944 | *P. imbricata* Greene | | | |
| *P. infundibuliformis* Torr. var. *infundibuliformis* | | | | | | X | 11 |
| *P. insularis* Munz var. *insularis* | | | | | | X | 10 |
| *P. integrifolia* Torr. | 11 | | Cave and Constance 1950 | | | 11_12 | 11 |
| | 11 | | Powell and Powell 1977 | | | | |
| | | ca. 24 | Zhao 1996 | | | | |
| | 11 | | Spellenberg 1986 | | | | |
| | $11_{II}$ | | Powell and Turner 2005 | | | | |
| *P. integrifolia* Torr. var. *texana* (J. W. Voss) N. D. Atwood | 11 | | Cave and Constance 1950 | *P. integrifolia* Torr. | | 11 | 11 |
| *P. inundata* J. T. Howell | 12 | | Cave and Constance 1947 | *M. parviflora* Brand | | 12 | 12 |
| *P. inyoensis* (J. F. Macbr.) J. T. Howell | 12 | | Cave and Constance 1959 | *M. inyoensis* J. F. Macbr. | | 12 | 12 |
| *P. ivesiana* Torr. var. *pediculoides* J. T. Howell | 23 | | Cave and Constance 1959 | *P. pediculoides* (J.T Howell) Constance | | 23 | 23 |
| *P. keckii* Munz & I. M. Johnst. | | | | | | X | 12 |
| *P. laxiflora* J. T. Howell | 10 | | Cave and Constance 1959 | | | 10 | 10 |
| *P. leibergii* Brand | 13 | | Cave and Constance 1950 | | | 13 | 13 |
| *P. linearis* (Pursh) Holz. | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| | 11 | | Gillett 1962 | | | | |
| | | 22 | Chinnappa and Chmielewski 1988 | | | | |
| | | 22 | Taylor and Taylor 1977 | | | | |
| *P. longipes* Torr. ex A. Gray | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. lutea* (Hook. & Arn.) J. T. Howell var. *lutea* | 12 | | Cave and Constance 1950 | *M. lutea* (Hook. & Arn.) A. DC. | | 12 | 12 |
| | 12 | | Halse 1981 | | | | |
| *P. lutea* (Hook. & Arn.) J. T. Howell var. *calva* Cronquist | 12 | | Halse 1981 | | | 12 | 12 |
| *P. mammarillensis* N. D. Atwood | | | | | | X | 11 |
| *P. marshal-johnstonii* N. D. Atwood var. *marshall-johnstonii* | | | | | | X | 11 |
| *P. minor* (Harv.) Thell. ex F. Zimm. | 11 | | Sugiura 1940 | *P. whitlavia* A. Gray | VNC | 11 | 11 |
| | 11 | | Cave and Constance 1942 | | | | |
| *P. minor* × *P. parryi* | 11 | | Gillett 1955 | | $F_1$, $F_2$ counts | 11 | 11 |
| *P. monoensis* Halse | 12 | | Cave and Constance 1947 | *M. lutea* (Hook. & Arn.) A.DC. var ? | | 12 | 12 |

TABLE 1. CONTINUED.

| Taxon | Haploid count (*n*) | Diploid count (2*n*) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| *P. mutabilis* Greene | 22 | | Cave and Constance 1942 | | | 11_22 | 22 |
| | 11 | | Cave and Constance 1944 | *P. californica* Cham. var. *jacintensis* Dundas | | | |
| | 22 | | Cave and Constance 1947 | | | | |
| | 22 | | Kruckeberg 1956 | | | | |
| *P. nashiana* Jeps. | 11 | | Gillett 1955 | | | 11 | 11 |
| *P. neglecta* M. E. Jones | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| *P. nemoralis* Greene var. *nemoralis* | 11 | | Cave and Constance 1944 | *P. nemoralis* Greene | | 11 | 11 |
| | 11 | | Cave and Constance 1947 | *P. nemoralis* Greene | | | |
| *P. nemoralis* Greene var. *oregonensis* (Heckard) Walden & R. Patt. | 22 | | Cave and Constance 1942 | *P. nemoralis* Greene | | 22 | 22 |
| | 22 | | Kruckeberg 1956 | *P. nemoralis* Greene | | | |
| *P. neomexicana* Thurb. ex Torr. | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| *P. novenmillensis* Munz | | | | | | X | 10 |
| *P. pachyphylla* Coville | 11 | | Cave and Constance 1950 | | | 11 | 11 |
| *P. palmeri* Torr. ex S. Watson | 11 | | Cave and Constance 1944 | *P. palmeri* Torr. ex S. Watson var. *foetida* Brand | | 11 | 11 |
| *P. parryi* Torr. | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| *P. patuliflora* (Engelmann & A. Gray) A. Gray | 9 | | Cave and Constance 1950 | *P. patuliflora* (Engelm. & A. Gray) A. Gray var. *patuliflora* | | 9 | 9 |
| *P. pauciflora* S. Watson | 11 | | Constance 1963 | | UC1116172! | 11 | 11 |
| *P. pedicellata* A. Gray | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| *P. perityloides* Coville var. *perityloides* | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| *P. petrosa* N. D. Atwood | | | | | | X | 11 |
| *P. popei* Torr. & A. Gray | 11 | | Cave and Constance 1944 | | | 11 | 11 |
| | $11_{II}$ | | Powell and Turner 2005 | | | | |
| *P. pringlei* A. Gray | 11 | | Cave and Constance 1947 | | | 11 | 11 |
| *P. procera* A. Gray | 11 | | Cave and Constance 1942 | | | 11 | 11 |
| *P. pulchella* A. Gray var. *pulchella* | 12 | | Cave and Constance 1959 | | | 12 | 12 |
| *P. purpusii* Brandegee | 9 | | Cave and Constance 1947 | | | 9 | 9 |
| *P. rafaelensis* N. D. Atwood | | | | | | X | 11 |
| *P. ramosissima* Douglas ex Lehm. | 11 | | Cave and Constance 1942 | | | 10_11 | 11 |
| | 11 | | Cave and Constance 1944 | | | | |
| | 11 | | Cave and Constance 1947 | | | | |
| | 10 | | Cave and Constance 1947 | *P. suffrutescens* Parry | | | |
| *P. rotundifolia* Torr. | ca. 12, 12 | | Cave and Constance 1959 | | | 12 | 12 |
| *P. rupestris* Greene | 11 | | Cave and Constance 1944 | *P. congesta* Hook. var. *rupestris* (Greene) J. F. Macbr. | | 11 | 11 |
| | 11 | | Spellenberg 1986 | | | | |
| *P. salina* (A. Nelson) J. T. Howell | | | | | | X | 12 |
| *P. scariosa* Brandegee | 11 | | Cave and Constance 1950 | | | 11 | 11 |

TABLE 1. CONTINUED.

| Taxon | Haploid count ($n$) | Diploid count ($2n$) | Reference | Original publication name | Notes | chromEvol | GDCN |
|---|---|---|---|---|---|---|---|
| *P. scopulina* A. Nels. | 12 | | Cave and Constance 1959 | *M. scopulina* Rydb. | | 12 | 12 |
| *P. sericea* (Graham) A. Gray var. *sericea* | 11 | | Cave and Constance 1942 | *P. sericea* (Graham) A. Gray | | 11 | 11 |
| | 11 | | Gillett 1961 | | | | |
| | | 22 | Dawe and Murray 1979 | | | | |
| | | 22 | Chinnappa and Chmielewski 1988 | | | | |
| *P. splendens* Eastw. | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| *P. stebbinsii* Constance & Heckard | | 16 | Constance and Heckard 1970 | | | 8 | 8 |
| *P. stellaris* Brand | 11 | | Cave and Constance 1959 | | | 11 | 11 |
| *P. suaveolens* Greene | 12 | | Cave and Constance 1950 | | | 12 | 12 |
| *P. tanacetifolia* Benth. | 9 | | Tjebbe 1928 | | VNC | 9_11 | 11 |
| | 11 | | Sugiura 1936 | | | | |
| | 11 | | Cave and Constance 1942 | | | | |
| | 11 | | Uhríková 1978 | | | | |
| | 11 | | Feráková 1974 | | | | |
| | 11 | | Izmailow 1991 | | | | |
| *P. tetramera* J. T. Howell | | 22 | Cave and Constance 1959 | *M. pusilla* (A. Gray) Brand | | 11 | 11 |
| *P. thermalis* Greene | 11 | | Cave and Constance 1950 | | | 11 | 11 |
| *P. vallis-mortae* J. W. Voss | 11 | | Cave and Constance 1950 | | | 11 | 11 |
| *P. viscida* (Benth.) Torr. var. *albiflora* (Nutt.) A. Gray | | | | | | X | 11 |
| *P. viscida* (Benth.) Torr. var. *viscida* | 11 | | Cave and Constance 1947 | *P. viscida* (Benth.) Torr. | | 11 | 11 |
| | 11 | | Cave and Constance 1950 | *P. viscida* (Benth.) Torr. var. *albiflora* (Nutt.) A. Gray | | | |
| *P. vossii* N. D. Atwood | | | | | | X | 11 |
| *P. welshii* N. D. Atwood | | | | | | X | 11 |

BACON, J. D. 1974. Chromosome numbers and taxonomic notes on the genus *Nama* (Hydrophyllaceae). Brittonia 26:101–105.

CAVE, M. S. AND L. CONSTANCE. 1942. Chromosome numbers in the Hydrophyllaceae. University of California Publications in Botany 18:205–216.

———. 1944. Chromosome numbers in the Hydrophyllaceae, II. University of California Publications in Botany 18:293–298.

———. 1947. Chromosome numbers in the Hydrophyllaceae, III. University of California Publications in Botany 18:449–465.

———. 1950. Chromosome numbers in the Hydrophyllaceae, IV. University of California Publications in Botany 23:363–382.

———. 1959. Chromosome numbers in the Hydrophyllaceae, V. University of California Publications in Botany 30:233–258.

CHINNAPPA, C. AND J. CHMIELEWSKI. 1988. Documented plant chromosome numbers 1987: 1. Miscellaneous counts from western North America. SIDA: Contributions to Botany 12:409–417.

CONSTANCE, L. 1963. Chromosome number and classification in Hydrophyllaceae. Brittonia 15:273–285.

——— AND L. R. HECKARD. 1970. Two new species of *Phacelia* (Hydrophyllaceae) from California. Brittonia 22:25–30.

COVAS, G. AND B. SCHNACK. 1947. Estudios cariológicos en Antófitas. II Parte. Revista Argentina de agronoma 14:224–231.

DAWE, J. C. AND D. F. MURRAY. 1979. Pp. 265–268 *in* Áskell Löve (ed.), IOPB chromosome number reports LXIII. Taxon 28:265–279.

DI FULVIO, T. 1969. Recuentos cromosómicos en *Heliotropium* (Boraginaceae). Kurtziana 5:89–95.

TABLE 1. CONTINUED.

FERÁKOVÁ, V. 1974. *In* J. Májovský, et al. (ed.). Index of chromosome numbers of Slovakian flora (Part 4). Acta Facultatis rerum naturalium Universitatis Comenianae, Botanica. 23:1–23.

GILLETT, G. W. 1955. Variation and genetic relationships in the *Whitlavia* and *Gymnobythus* phacelias. University of California Publications in Botany 28:19–78.

———. 1960. A systematic treatment of the *Phacelia franklinii* group. Rhodora 62:205–222.

———. 1961. An experimental study of variation in the *Phacelia sericea* complex. American Journal of Botany 48:1–7.

———. 1962. Evolutionary relationships of *Phacelia linearis*. Brittonia 14:231–236.

HALSE, R. R. 1981. Taxonomy of *Phacelia* section *Miltitzia* (Hydrophyllaceae). Madroño 28:121–132.

IZMAILOW, R. 1991. Pp. 32–33 *in* E. Pogan, A. Jankun, and H. Wcislo (eds.). Further studies in chromosome numbers of Polish angiosperms. Part XXIV. Acta Biologica Cracoviensia. Series: Botanica 33:26–43.

KOVANDA, M. 1978. Chromosome numbers of miscellaneous United States dicotyledons. Rhodora 80:431–440.

KRUCKEBERG, A. R. 1956. Notes on the *Phacelia magellanica* complex in the Pacific Northwest. Madroño 13:209–221.

LÖVE, Á. AND D. LÖVE. 1982. Pp. 344–360 *in* Á. Löve (ed.), IOPB chromosome number reports LXXV. Taxon 31:342–368.

MOSQUIN, T. 1968. Pp. 99 *in* Á. Löve (ed.), IOPB chromosome number reports. XV. Taxon 17:91–104.

POWELL, A. M. AND S. A. POWELL. 1977. Chromosome numbers of gypsophilic plant species of the Chihuahuan Desert. SIDA Contributions to Botany 7:80–90.

——— AND B. L. TURNER. 2005. Documented chromosome numbers 2005: 1. Miscellaneous counts from western Texas, mostly Trans-Pecos. SIDA: Contributions to Botany 21:1663–1664.

REVEAL, J. AND R. SPELLENBERG. 1976. Miscellaneous chromosome counts of western American plants- III. Rhodora 78:37–52.

SCHAACK, C. G., J. D. MOREFIELD, AND W. D. WINDHAM. 1984. Pp. 353–354 *in* Á. Löve (ed.), Chromosome number reports. LXXXIII. Taxon 33:351–354.

SHELLY, J. S. 1989. Biosystematic studies of *Phacelia capitata* (Hydrophyllaceae), a species endemic to serpentine soils in southwestern Oregon. Madroño 36:232–247.

SMITH, E. B. 1968. Pp. 576–577 *in* Á. Löve (ed.), IOPB chromosome number reports. XIX. Taxon 17:573–577.

SPELLENBERG, R. 1979. Chromosome numbers from some federally proposed threatened or endangered southwestern angiosperms and other miscellaneous taxa. The Southwestern Naturalist 24:187–189.

———. 1986. Pp. 197 *in* Á. Löve (ed.), Chromosome number reports. XC. Taxon 35:195–198.

SUGIURA, T. 1928. Chromosome numbers in some higher plants, 1. The Botanical Magazine, Tokyo 42:504–506.

———. 1936. Studies on the chromosome numbers in higher plants, with special reference to cytokinesis, I. Cytologia 7:544–595.

———. 1940. Studies on the chromosome numbers in higher plants. IV. Cytologia 10:324–333.

TAYLOR, R. AND S. TAYLOR. 1977. Chromosome numbers of vascular plants of British Columbia. Syesis 10:125–138.

TJEBBES, K. 1928. The chromosome numbers of some flowering plants. Hereditas 10:328–332.

UHRÍKOVÁ, A. 1978. *In* J. Májovský, et al. (ed.). Index of chromosome numbers of Slovakian flora (Part 4). Acta Facultatis rerum naturalium Universitatis Comenianae, Botanica. 26:1–42.

WARD, D. E. 1983. Chromosome counts from New Mexico and southern Colorado. Phytologia 54:302–308.

———. 1984. Chromosome counts from New Mexico and Mexico. Phytologia 56:55–60.

ZHAO, Z. 1996. Documented chromosome numbers 1996: 2. Miscellaneous USA. and Mexican species, mostly Asteraceae. SIDA: Contributions to Botany 17:259–263.

——— AND B. L. TURNER. 1993. Documented chromosome numbers 1993: 3. Miscellaneous USA. and Mexican species, mostly Asteraceae. SIDA: Contributions to Botany 15:649–653.

Brown Center at the Missouri Botanic Garden (MO) and from living collections without herbarium vouchers received from the University of California, Berkeley Botanic Garden. University of California, Davis Center for Plant Diversity (DAV), San Diego Natural History Museum (SD), SFSU, and the University of Texas at Austin (TEX/LL) granted permission to destructively sample herbarium specimens. Sample details, including voucher specimens, botanic garden accession numbers, herbarium accession numbers, and GenBank accession numbers for all sequences are included in Appendix 1. Updated identification of voucher specimens for accessions is noted parenthetically where these differ from previous studies. GenBank accessions and vouchers cited in previous studies excluded from these analyses are listed in Appendix 2.

## Gene Regions Sampled

We examined the internal transcribed spacer region (ITS-1, ITS-2, and 5.8S gene) of nuclear ribosomal DNA sequences (nrITS) for the nuclear partition (biparentally inherited character). nrITS is a highly variable and rapidly evolving region, useful for inferring species level relationships (Baldwin et al. 1995; Álvarez and Wendel 2003). nrITS is the molecular marker with the largest representative sampling of sequences published to date for the genus. For the plastid partition (maternally inherited character) we examined the single copy gene *ndh*F. The chloroplast NADH dehydrogenase gene *ndh*F codes for the F (ND5) subunit; for an expanded discussion of *ndh*F sequence evolution and phylogenetic utility see Kim and Jansen (1995). The *ndh* group of chloroplast genes is highly conserved across major plant taxa and has been used to assess and define infrageneric evolutionary relationships in plant families (Neyland and Urbatsch 1996; Olmstead et al. 2000; Martín and Sabater 2010). *ndh*F also has a broad sampling of sequences published for Hydrophylloideae and Solanales (Olmstead and Sweere 1994; Bohs and Olmstead 1997; Ferguson 1998; Moore and Jansen 2006; Taylor 2012).

## DNA Isolation and PCR Amplification

*DNA isolation.* Total genomic DNA was extracted from 0.020mg dry weight herbarium or silica-dried leaf material following Doyle and Dickson (1987) using Qiagen DNEasy Plant kits (Qiagen, Valencia, CA, USA) and grinding with liquid nitrogen for the homogenization step. For *ndh*F, the protocol was modified to use homogenization with acid-clean steel ball bearings in lieu of grinding with liquid nitrogen and substitution of NucleoSpin filters (Macherey-Nagel, Düren, Germany) for the filtration step.

*PCR amplification for nrITS.* Total genomic DNA extract was diluted 1:10 in ultra-pure $H_20$ for best amplification in PCR (Mullis et al. 1987) for the internal transcribed spacer region (ITS-1, 5.8S, and ITS-2) of nuclear ribosomal DNA (nrITS) with primers ITS-I (F) (Urbatsch et al. 2000) and ITS4 (R) (White et al. 1990). PCR was conducted with a final reaction volume of 50 μL, containing 24.8 μL ultra-pure $H_20$, 5 μL 10× GOLD buffer (containing 150mM Tris buffer at pH 8.0, 500 mM KCl), 25 μL 25 mM $MgCl_2$, 8 μL 10 mM dNTPs, 2.0 μL each of forward and reverse primer at 10mM each, 2.0 μL 100% dimethyl sulfoxide (DMSO), 2.0 μL 1:10 dilution genomic DNA template, and 0.02 μL Gold TAQ polymerase or FastStart TAQ polymerase (Takara Bio Inc., Otsu, Shiga, Japan). PCRs reaction thermo-cycle profile had a 4 min at 94°C initial denaturation step, followed by 35 cycles of 30 sec at 94°C, 45 sec at 59°C, 45 sec 59°C ramping to 72°C at 0.5° increase per second, and terminated at 72°C for 5 min extension, followed by 5 min at 4°C to snap chill.

*PCR amplification for* ndh*F.* Total genomic DNA extract was diluted 1:10 in ultra-pure $H_20$ for amplification using overlapping primer sets 1F-1318R and 972F-2110R (Olmstead and Sweere 1994; Ferguson 1998 [1999]). PCR was conducted with a final reaction volume of 15 μL, containing 5.72 μL ultra-pure $H_20$, 1.5 μL 10× Exonuclease Taq DNA polymerase $Mg2^+$ free buffer (ExTaq) (Takara Bio Inc., Otsu, Shiga, Japan), 1.5 μL 25 mM $MgCl_2$, 1.2 μL 2.5 mM dNTPs, 1.0 μL each of forward and reverse primer at 10mM, 1.0 μL bovine serum albumin (BSA), 2.0 μL 1:10 dilution genomic DNA template, and 0.08 μL ExTaq. PCRs reaction thermo-cycle profile had a 2 min at 96°C initial denaturation step, followed by 35 cycles of 30 sec at 94°C, 1 min at 61°C, 1 min at 72°C, terminated at 72°C for 5 min extension, followed by 5 min at 4°C to snap chill. This amplification protocol was followed for both sets of *ndh*F primer pairs.

To verify amplification of nuclear and plastid PCR products a combined total of 3 μL of template PCR product and 1 μL of 6X dye was run on a 1.6% agarose gel (APEX agarose) in 1X TBE buffer at 100 volts, with a standard 100 bp (*nr*ITS) or 1kb (*ndh*F) ladder to visually size fragments. The gel was stained in an ethidium bromide bath, rinsed in deionized water, and viewed under ultra-violet light. Gel photographs were taken for reference (not shown). Sequence polymorphism in the direct sequenced PCR product was not observed for either nrITS or *ndh*F accessions.

## DNA Sequencing

*Cycle sequencing for nrITS.* Template DNA from PCR product was purified using MO BIO

UltraClean PCR Clean-Up DNA Purification Kit (MO BIO Laboratories, Inc., Solano Beach, CA). Cycle sequencing for nrITS was conducted with ITS4, ITS-I, and internal primers ITS2 and ITS3 (White et al. 1990; Urbatsch et al. 2000) in a final reaction volume of 12 μL, containing 6.45 μL ultra-pure $H_20$, 0.5 μL BigDye (Applied Biosystems, Inc., Foster City, CA, USA), 2.0 μL 5× buffer, 0.75 μL primer, and 2.0 μL template DNA. Reaction parameters were an initial 30 sec at 95°C denaturation step, followed by 25 cycles of 10 sec at 94°C, 30 sec at 59°C, and terminated at 60°C for 4 min.

*Cycle sequencing for* ndhF. PCR products were cleaned of excess nucleotides (dNTPs) and primers from the amplification reaction using 1 μL ExoSAP-IT (USB Corp., Cleveland, Ohio, USA) per 5 μL template, with an initial 37°C incubation for 30 min for digestion, followed by 80°C for 10 min to inactivate the enzymes. Cycle sequencing for *ndh*F was conducted with internal primers 1F, 536F, 536R, 972F, 1318F, 1318R, and 2110R in a final reaction volume of 12 μL containing 6.45 μL ultra-pure $H_20$, 0.5 μL BigDye (Applied Biosystems Inc., Foster City, CA, USA), 2.0 μL 5× buffer, 0.75 μL primer, and 2.0 μL template DNA. Reaction parameters were an initial 30 sec at 96°C denaturation step, followed by 25 cycles of 10 sec at 96°C, 30 sec at 60°C, and terminated at 60°C for 4 min extension.

*Sequencing for nrITS and* ndhF. Cycle sequencing products were precipitated using EDTA/Sodium acetate in ethanol protocol, and then resuspended in 15 μL Hi-Di Formamide (Applied Biosystems, Inc., Foster City, CA, USA). Products were denatured for 2 min at 95°C, followed by 5 min at 4°C to snap chill. Samples were loaded into a 96 well plate and spun down at low speed (700 rpm for 1 min). Sequencing was conducted using an ABI PRISM 377 Sequencer or ABI PRISM 3100 Sequencer (Applied Biosystems, Inc., Foster City, CA, USA). All molecular work was conducted in the SFSU Department of Biology Conservation Genetics Laboratory (now the SFSU Department of Biology Genomics/Transcriptomics Analysis Core [GTAC]).

## Data Analysis

Nucleotide sequences were edited and assembled using Sequencher 4.8 (Gene Codes Corporation, Inc., Ann Arbor, MI, USA). Base calling was straightforward for both nrITS and *ndh*F. We used the conditional combination approach to determine if partitions should be analyzed separately or could be combined (Huelsenbeck et al. 1996). This study joined 124 previously published nrITS sequences from GenBank with 52 new sequences from Garrison (2007) for a total of 176 nrITS sequences (nrITS expanded), and 28 previously published *ndh*F sequences from GenBank were joined with 98 new sequences from Walden (2010) for a total of 126 *ndh*F sequences (*ndh*F expanded). All sequences generated for this study were deposited in GenBank (Appendix 1). Multiple sequences for each molecular marker were aligned in ClustalW2 (Larkin et al. 2007; Goujon et al. 2010) using default parameters and indels edited manually in MacClade v.4.8 OSX (Sinauer Associates Inc., Sunderland, Massachusetts, [Maddison and Maddison 2001]). We identified nrITS and *ndh*F sequences derived from the same voucher specimen for a reduced subset of 61 accessions. The reduced 61-accession nrITS (nrITS reduced) and *ndh*F (*ndh*F reduced) partitions were concatenated in Mesquite v.2.74 for a combined partition following the total evidence approach (nrITS + *ndh*F 61-accession).

*Maximum parsimony.* The incongruence length difference test (ILD, as the partition homogeneity test) was implemented in PAUP* v.4.0b10 (Swofford 2002) to detect conflicting signal and assess combinability in the nrITS + *ndh*F 61-accession concatenated subset, uninformative characters excluded, using 1000 replicates, 100 random addition sequence replicates, multrees = 1, and MAXTREES increased by 100 to a limit of 10,000.

nrITS (expanded, reduced) and *ndh*F (expanded, reduced) individual partitions and the combined nrITS + *ndh*F 61-accession partition were analyzed using the maximum parsimony criterion (MP) in PAUP* v.4.0b10. MP phylogeny reconstruction was performed using a heuristic search of 1000 random addition sequence replicates with tree-bisection-reconnection (TBR) branch swapping algorithm, ACCTRAN, all characters unordered and weighted equally, gaps treated as missing data, MAXTREES increased by 100 to a limit of 100,000, and nchuck = 100. Nonparametric bootstrap analyses were performed using the starting strict consensus tree obtained via stepwise addition, using a heuristic search, including 100 random addition sequence replicates with 1000 bootstrap replicates (Felsenstein 1985).

*Model selection.* jModeltest version 0.1.1 with PHYML was used to test 88 models of evolution for best fit for the nuclear and plastid partitions (Posada and Crandall 1998; Guindon and Gascuel 2003; Posada and Buckley 2004; Posada 2008). Calculations using the Akaike information criterion (AIC) and hierarchical likelihood ratio tests (hLRTs) via jModeltest selected GTR + G (General Time Reversible model of nucleotide substitution with the gamma [Γ] model of rate heterogeneity) as the best-fit model of evolution for the nuclear (expanded, reduced), plastid (expanded, reduced), and combined nrITS + *ndh*F 61-accession partitions (-lnL score: nrITS expanded 8933.5854, nrITS reduced 5998.5051, *ndh*F expanded 10029.4265, *ndh*F reduced 7996.9681, nrITS + *ndh*F 14590.1426).

*Maximum likelihood.* File formats were converted through the CIPRES portal (Miller et al. 2010) with NCLconverter version 2.1 (Lewis and Holder 2008) to a relaxed PHYLIP format for RAxML-HPC2 version 7.4.4 analysis on XSEDE (Extreme Science and Engineering Discovery Environment). The model of evolution for the nuclear (expanded, reduced), plastid (expanded, reduced), and combined nrITS + *ndh*F 61-accession partitions was GTRGAMMA for the entire analysis, with 5000 rapid bootstrap replicates and best tree search, and gaps and undetermined values treated as missing data (Stamatakis 2006, Stamatakis et al. 2008). The combined analysis was partitioned by gene and unlinked in analysis (Brown and Lemmon 2007).

*Bayesian analysis.* Analyses were initiated in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using XSEDE through the CIPRES portal (Miller et al. 2010) using default priors, random starting trees, four independent runs, number of generations for the nrITS expanded partition was 20,00,000, all other partitions the number of generations were 10,000,000, using four chains (three hot and one cold) sampled every 1000 generations (total samples per run: nrITS expanded 20,001, all other partitions 10,001), with the general time reversible model with gamma-distributed rate variation across sites (GTR + G). The average standard deviation of split frequencies from each run was less than 0.01 at the end of the four runs (nrITS expanded 0.008313, nrITS reduced 0.006756, *ndh*F expanded 0.003820, *ndh*F reduced 0.001946, nrITS + *ndh*F 0.001142), and PSRF (Potential Scale Reduction Factor) approached one for the 95% credibility interval for the expanded partitions and equaled one for the reduced and combined partitions (Gelman and Rubin 1992). The number of trees required to reach stationarity was determined using Tracer v.1.5 (Rambaut and Drummond 2009). Convergence of posterior probabilities of split frequencies of runs was assessed in AWTY (Are We There Yet?) using the between run compare diagnostic function; graphical plots did not reject convergence (p near one for burn-in at 10%) (Wilgenbusch et al. 2004; Nylander et al. 2008). Burn-in samples (nrITS expanded 5000; all other partitions 2500) were discarded (samples included for analysis from each run: nrITS expanded 15,001, all other partitions 7501), and runs were combined with posterior probabilities of nodes ≥95% strongly supported.

*Maximum likelihood ancestral chromosome number evolution analysis in chromEvol version 1.3.* Sampled taxa were coded with haploid chromosome counts for analysis in chromEvol version 1.3 using the ML RAxML best tree from the expanded nuclear, expanded plastid, and combined nrITS + *ndh*F analyses (Mayrose et al. 2010; Mayrose 2012). Diploid counts were divided by two and input as haploid if these were the only published counts available for a taxon (see Table 1 for the coding schema). Taxa without published chromosome counts were coded "X" and treated as a gap in the ML analysis. Polymorphic chromosome counts or taxa circumscribed as polyploid were both coded as polymorphic (e.g., 11_22). It was necessary to assign the root to the outgroup (*Euploca*) manually in the control file after an initial analysis to locate the appropriate node number using FigTree 1.3.1 (Rambaut 2009), and then rerun chromEvol v.1.3 analyses (Mayrose et al. 2010). All eight models were included as there was no a priori reason to exclude one or more of them.

Accession JQ250033 is a collection from Chile received as a molecular voucher from MO and is likely *Phacelia artemisioides* Griseb. (identified in Appendix 1 as *P. aff. artemisioides*). The voucher specimen was not examined for this study. All published chromosome counts for taxa from South America are $n = 11$ (Covas and Schnack 1947; Cave and Constance 1959). Alternative coding and analysis in chromEvol v.1.3 using a missing count (X) for this accession inferred $n = 11$ as the ancestral condition at the node and did not change the best model chosen by AIC (alternate analyses not shown in results section).

*Maximum likelihood whole genome duplication event analysis in GDCN.* The ML RaXML best tree was imported into Mesquite v.2.74 and chromosome counts for sampled taxa were coded as continuous characters for analysis in GDCN as specified in the manual (Hallinan and Lindberg 2011a, b). Samples without published chromosome counts at sampled taxonomic rank (coded "X" in chromEvol v.1.3 analyses) were recoded as required in GDCN (see Table 1 for coding schema). Taxa lacking chromosome counts were coded with published counts of the variety or species (e.g., *P. insularis* Munz var. *continentis* J. T. Howell, $n = 10$ [Cave and Constance 1947] was coded for *P. insularis* var. *insularis*). Polymorphic chromosome counts coded in chromEvol were recoded with a single state in GDCN to match sampled population locations in this study and corresponding voucher specimen localities for published chromosome counts. Where sampled populations and chromosome counts overlapped in geographic distribution the more common count was used (measured by number of published vouchers). If chromosome counts differed in number the count with a cited voucher specimen was chosen. For taxa where no chromosome count was available, the inferred chromosome number from chromEvol v.1.3 analyses was used for an "informed" coding (Cusimano et al. 2012). WGD events were allowed (wgd = sto), chromosome duplications ($\lambda$ in GDCN) and losses ($\mu$ in GDCN) were equal (muset = lam), and the chromosome number for the root was determined by ML for each analysis (rootfit = ml).

chromEvol v.1.3 and GDCN use slightly different ways to symbolically represent and discuss gains, losses, and doubling chromosome events within lineages. The rate of chromosome gains is symbolized by $\delta$ in chromEvol v.1.3 and the rate of chromosome duplication is symbolized by $\lambda$ in GDCN. The rate of chromosome loss is symbolized by $\lambda$ in chromEvol v.1.3 and by $\mu$ in GDCN. The rate of demipolyploidization is symbolized by $\mu$ in chromEvol v.1.3 and polyploidization is symbolized by $\rho$ in chromEvol. In GDCN the rate of whole genome duplication is symbolized by $\delta$. We refer to the textual interpretation of the symbols used by each analysis and parenthetically indicate the symbol and corresponding analysis throughout this paper.

## Results

### Phylogenetic Analyses

Partition homogeneity test results for the combined nrITS + *ndh*F 61-accession subset rejected the null hypothesis (P = 0.001), indicating the nuclear and plastid partitions were significantly heterogeneous and should not be combined.

*Separate analyses.* Separate analyses for the expanded and reduced partitions (nuclear, plastid) resulted in similar tree topologies for each respective molecular marker. For this reason we show results for the expanded individual partitions only (nrITS expanded 176-accession Fig. 1, *ndh*F expanded 126-accession Fig. 2).

The following groups (corresponding to the infrageneric classification of Walden and Patterson [2012]) were supported as monophyletic in both the nuclear and plastid separate analyses: *Phacelia* subg. *Microgenetes, Phacelia* sect. *Phacelia, Phacelia* sect. *Eutoca* (R. Br.) Benth. & Hook.f., *Phacelia* sect. *Cosmantha* (Nolte ex A. de Candolle) Benth. & Hook.f., *Phacelia* sect. *Gymnobytha*, and *Phacelia* sect. *Ramosissimae*. *Phacelia* sect. *Eutoca* and *Phacelia* sect. *Cosmantha* were sister to each other in both the nuclear and plastid separate analyses, as were *Phacelia* sect. *Gymnobytha* and the group corresponding to *Phacelia* subsect. *Whitlaviae* (Harv.) G. W. Gillett (included within *Phacelia* sect. *Whitlavia*, subsections not labeled on figures). Although *Phacelia* subg. *Microgenetes* was supported as monophyletic, neither the nuclear nor the plastid separate analyses (expanded and reduced) recovered a monophyletic *Phacelia* sect. *Euglypta* or *Phacelia* sect. *Miltitzia*.

Incongruence occurred between the nuclear and plastid topologies for placement of *Draperia* relative to *Tricardia* + *Hesperochiron* (+ *Howellanthus* sampled in the *ndh*F expanded partition), placement of *Romanzoffia* and "core" *Phacelia* subg. *Pulchellae*, and placement of *Phacelia* sect. *Baretiana* Walden & R. Patt. (nested within *Phacelia* sect. *Glandulosae* in the nuclear partition). *Phacelia* subg. *Phacelia* was not supported as monophyletic in the nuclear partition due to recovery of a paraphyletic *Phacelia* sect. *Whitlavia*. *Phacelia* subsect. *Phacelia* and *Phacelia* subsect. *Humiles* Walden & R. Patt. were not supported as monophyletic in the nuclear partition due to placement of *P. breweri* A. Gray sister to *P. californica* Cham. *Phacelia* was not supported as monophyletic in the plastid partition ("core" *Phacelia* subg. *Pulchellae* sister to *Romanzoffia* and remaining sampled *Phacelia*).

*nrITS expanded partition.* The expanded nrITS partition included 176 sequences and contained 1973 total characters. 1612 (81.70%) characters were constant and 111 (5.62%) variable characters were parsimony uninformative. The total number of parsimony informative characters was 250 (12.67%). nrITS sequences identical to each other are as follows: FJ814643 to FJ814644, AY630311 to AY630312, FJ814633 to FJ814632 to FJ814634, FJ814651 to FJ814652. Identical nrITS sequences, and *ndh*F sequences derived from the same genomics, are indicated in Appendix 1. The total number of MP trees was 4400, with the best MP tree score = 1445. The phylogram of the 0.5 majority rule consensus tree with MP bootstrap values, ML bootstrap values, and BI posterior probabilities is shown in Figure 1.

AY630269 (cited as *P. bicolor* Torr. ex S. Watson in Gilbert et al. [2005]) was recovered sister to FJ814624 (cited as *P. fremontii* Torr. in Hansen et al. [2009]). The voucher specimen for FJ814624 is the blue-throated form of *P. fremontii*, corresponding to some of the characters described in the protologue for *P. brannanii* Kellogg, especially "lobes rounded, bright blue" (Kellogg 1877). The voucher specimen for AY630269 has faded corollas. These accessions are identified as *P. aff. brannanii* in Appendix 1 and Figure 1. The name *Phacelia brannanii* is currently placed in synonymy with *P. fremontii* and the type specimen has not been located (CAS?). The type was collected "near Fresno" and was probably collected from populations in

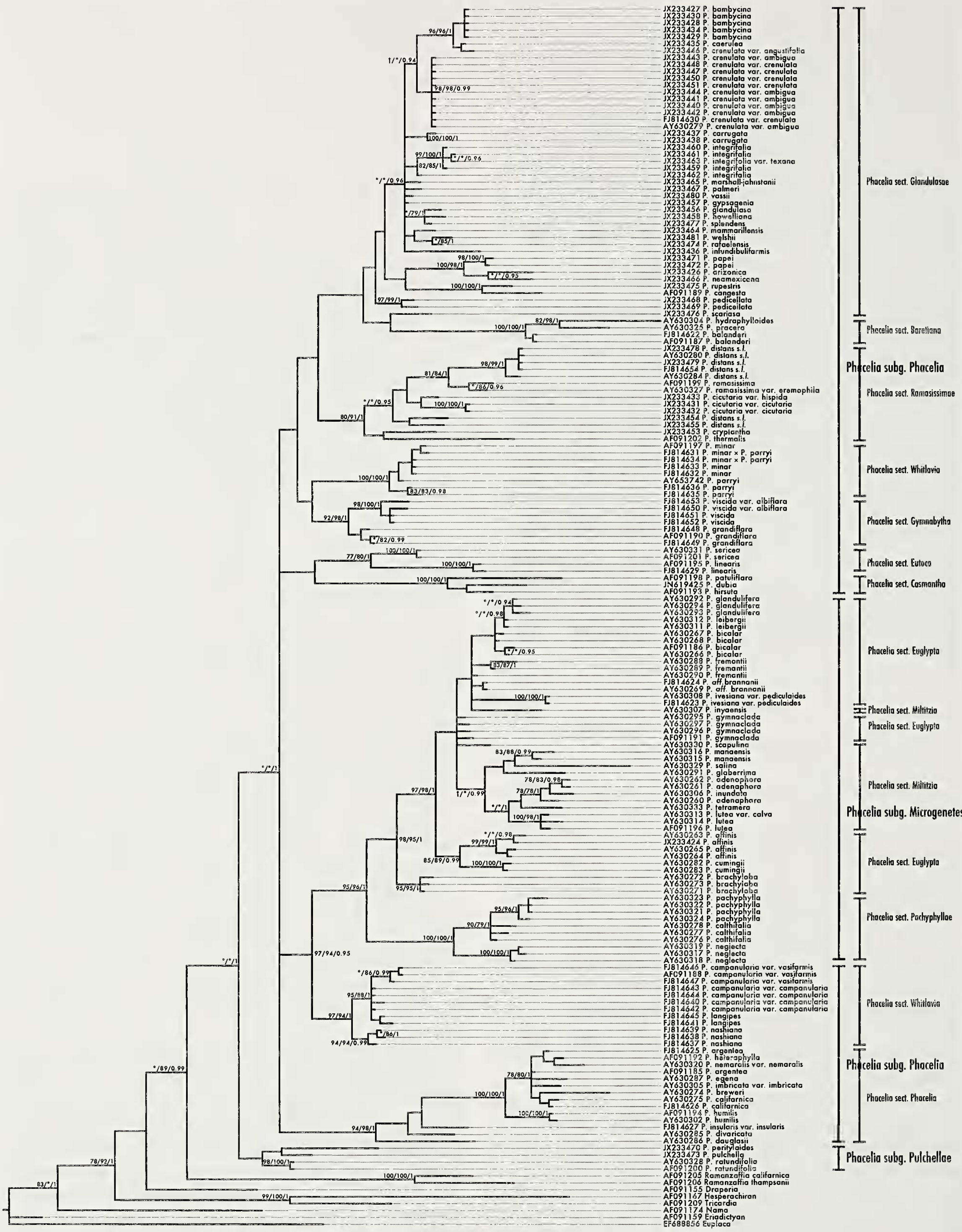


FIG. 1. Phylogram of 0.5-majority rule consensus tree for the expanded nrITS 176-accession partition from Bayesian phylogenetic analysis (scale bar = mean number of nucleotide substitutions per site). Support values are shown above branches (or at nodes if branches too short to show full text) for Maximum Parsimony bootstrap values / Maximum Likelihood bootstrap values / Bayesian posterior probabilities for nodes supported at ≥95% posterior probability. An asterisk (*) indicates clade with <75% support in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. A dagger (†) indicates a clade not resolved in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. Abbreviation: P. = *Phacelia*. Infrageneric groups labeled (subgenera, sections) following Walden and Patterson (2012).

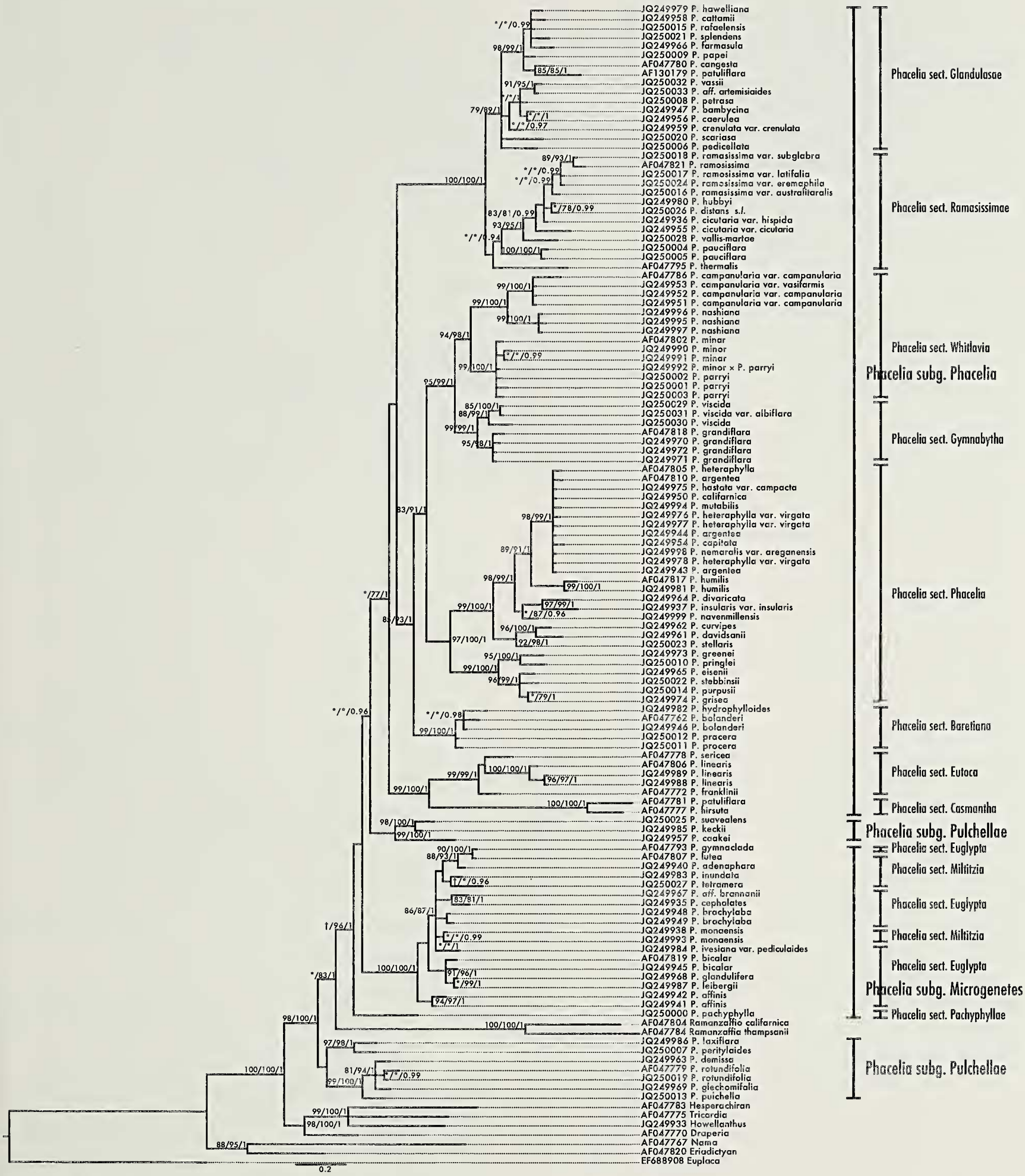


FIG. 2. Phylogram of 0.5-majority rule consensus tree for the expanded *ndh*F 126-accession partition from Bayesian phylogenetic analysis (scale bar = mean number of nucleotide substitutions per site). Support values are shown above branches (or at nodes if branches too short to show full text) for Maximum Parsimony bootstrap values / Maximum Likelihood bootstrap values / Bayesian posterior probabilities for nodes supported at ≥95% posterior probability. An asterisk (*) indicates clade with <75% support in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. A dagger (†) indicates a clade not resolved in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. Abbreviation: P. = *Phacelia*. Infrageneric groups labeled (subgenera, sections) following Walden and Patterson (2012).

the western San Joaquin Valley. Both voucher specimens were made in the southern Sierra Nevada Mountains in Kern County. Howell (1946) and Dempcy (1996) previously identified the Owens Valley and the Mohave Desert as areas of intergradation between *P. bicolor* and *P. fremontii* populations. Thorough understanding requires sampling throughout the distribution of

*P. fremontii* in the San Joaquin Valley, Transverse Ranges, and Tehachapi Mountains to determine range, characters, and relationships. Our results indicate that, at least for *P. fremontii*, there is need to reconsider the need for reconsideration of the names currently placed in synonymy with *P. fremontii* and possible recognition of *P. brannanii* as a variety. Taxonomic changes must be addressed in a future paper, pending determination of type material.

*Phacelia scariosa* is a "problematic taxon" included in these analyses (Ferguson 1998). *Phacelia* sect. *Baretiana* was still supported as sister to *Phacelia* sect. *Glandulosae* when *P. scariosa* was pruned from the partition (results not shown). *Phacelia crenulata* Torr. ex S. Watson, as currently circumscribed, was not supported as monophyletic in this analysis. A clade of eleven accessions representing two of four infraspecific varieties sampled from Arizona and California was recovered with very little nrITS variation. Accession JX233446 *P. crenulata* var. *angustifolia* N. D. Atwood was recovered sister to JX233435 *P. coerulea* Greene, a result consistent with prior work (Garrison 2007). *Phacelia crenulata* var. *angustifolia* should be treated outside of *Phacelia crenulata*, although no name is currently available. However, there is not adequate support at this time to propose taxonomic segregation due to incongruent nuclear and plastid partitions, lack of corresponding plastid sequences for *Phacelia crenulata* var. *angustifolia*, and the need for adequate sampling throughout the described varieties of *Phacelia crenulata*.

Species and varietal segregations in *Phacelia* have been made based on morphology and distribution. Some of these segregations are in need of revision. For example, *Phacelia distans sensu* lato is paraphyletic and requires revision. A group of five accessions included plants corresponding to four names currently placed in synonymy. A clade of two accessions (JX2333454, JX233455) included plants sampled from Arizona that correspond to the expanded circumscription of *Phacelia distans* var. *australis* Brand (1913). The type for that name is from the "Greenhorn Range" of California (UC63348!) and additional sampling is needed to determine relationships for the original and expanded circumscriptions for this taxon and relationship with *P. gentryi* Constance (Brand 1913; Constance 1948).

*ndhF expanded partition.* The *ndh*F dataset of 126 sequences contained 2547 total characters. 1956 (76.79%) characters were constant and 233 (9.14%) variable characters were parsimony uninformative. The total number of parsimony informative characters was 358 (14.05%). Best MP tree length was 1033. The phylogram of the 0.5 majority rule consensus tree with MP bootstrap values, ML bootstrap values, and BI posterior probabilities is shown in Figure 2.

*Phacelia* subg. *Pulchellae* is not monophyletic as currently circumscribed. Paraphyletic *Phacelia* subg. *Pulchellae* consists of a "core" group sister to *Romanzoffia* and remaining sampled *Phacelia*, and a group consisting of *Phacelia cookei, P. keckii,* and *P. suaveolens* sister to *Phacelia* subg. *Phacelia*. *Phacelia cookei*, *P. keckii*, and *P. suaveolens* were not sampled for nrITS sequences (Appendix 1) and could not be examined with a separate nuclear or combined analysis. This result would argue for a refined circumscription of "core" *Phacelia* subg. *Pulchellae* excluding *P. cookei*, *P. suaveolens*, and *P. keckii*. These taxa should be placed in an unresolved group with low support within *Phacelia* subg. *Phacelia* and the focus of future studies.

Two accessions identified as *P. patuliflora* A. Gray in GenBank were placed in separate clades. Accession AF047781 was supported in every analysis (MP, ML, BI) within *Phacelia* sect. *Cosmantha* and sister to AF047777 *P. hirsuta* Nutt., results accordant with Ferguson (1998 [1999]), while accession AF130179 (Olmstead et al. 2000) was supported in every analysis (MP, ML, BI) within *Phacelia* sect. *Glandulosae*. The voucher specimen of AF047781 (2089 bp) was received by loan and identification confirmed, but the AF130179 (2223 bp) voucher specimen was not examined for this study. Placement of AF130179 within *Phacelia* sect. *Glandulosae* could be attributed to branch length attraction as the two sequences differ by some 200 base pairs, misidentification of source material, sequencing error, lineage sorting, or the paucity of representative accessions from *Phacelia* sect. *Cosmantha* (see chromosome evolution results section for additional discussion).

*Combined analysis.* Acknowledging that combining the nuclear and plastid partitions was statistically inappropriate (as determined by ILD test result) and that the separate analyses corroborated incongruence between topologies reported in previous studies, we include results of the combined nrITS + *ndh*F 61-accession analyses (Fig. 3) in a departure from the conditional combination approach in favor of the total evidence approach. The combined nrITS + *ndh*F analyses supported a monophyletic *Phacelia* (*Romanzoffia* sister to *Phacelia*). *Phacelia* consists of a basal lineage of *Phacelia* subg. *Pulchellae* sister to *Phacelia* subg. *Microgenetes*, which is sister to *Phacelia* subg. *Phacelia*. *Phacelia* sect. *Euglypta* and *Phacelia* sect. *Miltitzia* are not monophyletic (sections not labeled in figure) within *Phacelia* subg. *Microgenetes* and require taxonomic reconsideration. *Phacelia* sect. *Eutoca* is sister to *Phacelia* sect. *Cosmantha*, and these form the basal group in *Phacelia* subg. *Phacelia*.

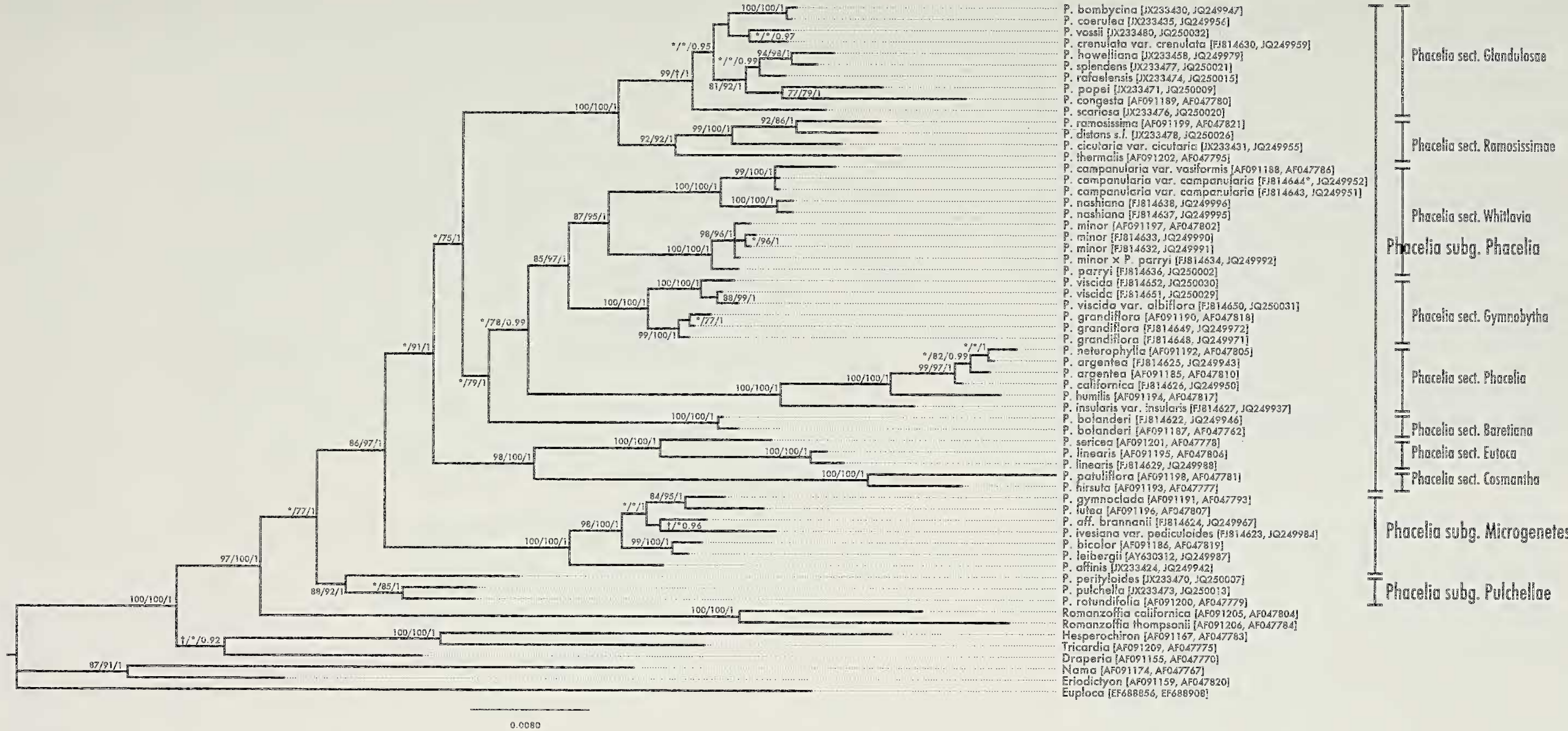


Fig. 3. Phylogram of 0.5 majority rule consensus tree for the reduced and combined nrITS+*ndh*F 61-accession dataset from Bayesian phylogenetic analysis (scale bar = mean number of nucleotide substitutions per site). Support values are shown above branches (or at nodes if branches too short to show full text) for Maximum Parsimony bootstrap values / Maximum Likelihood bootstrap values / Bayesian posterior probabilities for nodes supported at ≥95% posterior probability. An asterisk (*) indicates clade with <75% support in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. A dagger (†) indicates a clade not resolved in 0.5 majority rule MP bootstrap tree or 0.5 majority rule ML bootstrap tree. Abbreviation: P. = *Phacelia*. Infrageneric groups labeled (subgenera, sections) following Walden and Patterson (2012).

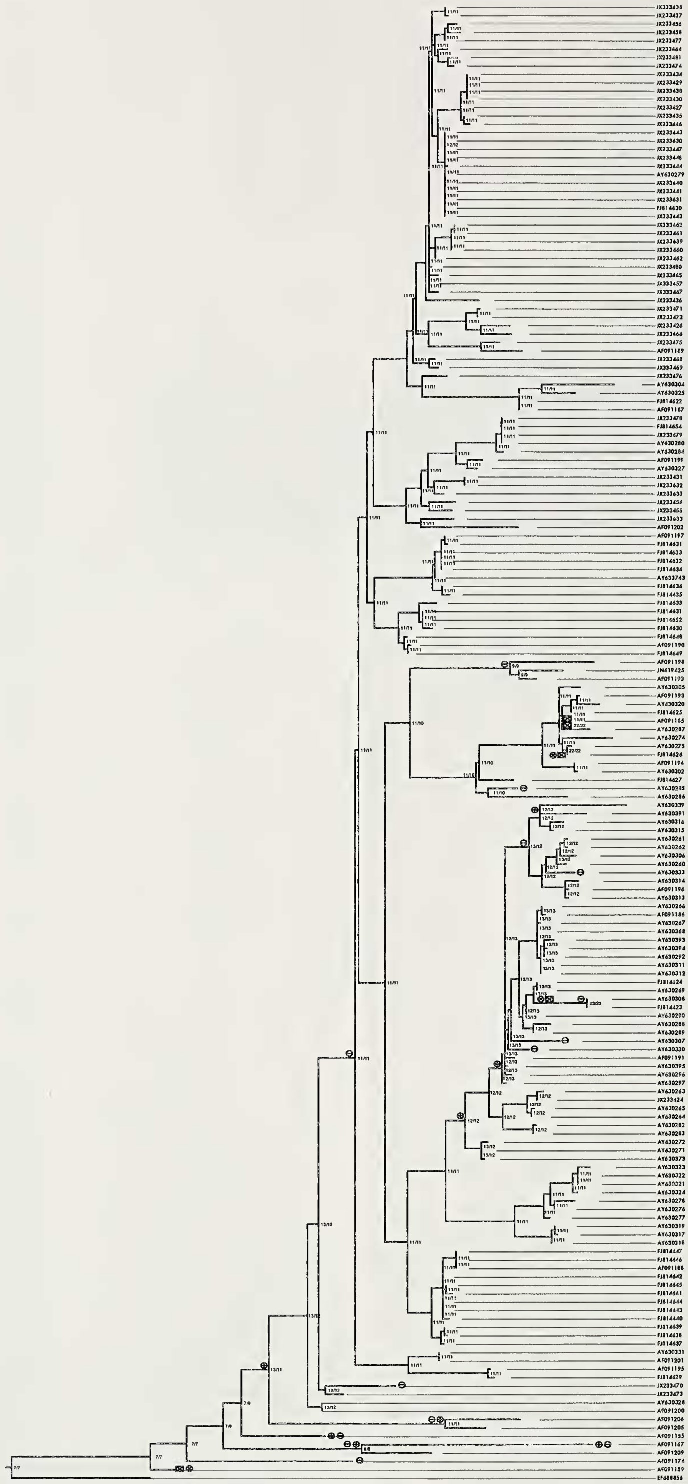

FIG. 4. RaxML phylogram of the single, best tree [-lnL = 8926.106148, tree length = 2.993179] for the expanded nrITS 176-accession partition from maximum likelihood analysis (scale bar = mean number of nucleotide substitutions per site). Tip labels are shown with GenBank accession numbers. Maximum likelihood inferred ancestral chromosome numbers are indicated at nodes for chromEvol analysis model M1 ($\lambda$, $\delta$, $\rho$, $\mu = 0$, AIC = 343.6) / GDCN analysis. Nodes and branches with expectations above 0.5 from chromEvol analysis for gains ($\lambda$ in chromEvol) are indicated with ⊕ (total number of events 13.9872), for losses ($\delta$ in chromEvol) are indicated with ⊖

*Phacelia* sect. *Baretiana* is sister to the group consisting of *Phacelia* sect. *Phacelia, Phacelia* sect. *Gymnobytha,* and (a monophyletic) *Phacelia* sect. *Whitlavia. Phacelia* sect. *Glandulosae* is sister to *Phacelia* sect. *Ramosissimae.*

*Combined nrITS +* ndh*F 61-accession partition.* The nrITS + *ndh*F dataset of 61 sequences contained 4520 total characters. 3711 (82.10%) characters were constant and 310 (6.85%) variable characters were parsimony uninformative. The total number of parsimony informative characters was 499 (11.03%). The total number of MP trees was 36, with the best MP tree score = 1772. The phylogram of the 0.5 majority rule consensus tree with MP bootstrap values, ML bootstrap values, and BI posterior probabilities is shown in Figure 3. Atwood (1975) proposed six complexes within *Phacelia* sect. *Glandulosae.* The separate nuclear and plastid partitions do not support these complexes and the combined 61-accession partition does not include adequate representation to address the circumscription of the complexes.

## Chromosome Evolution

Results from chromEvol v.1.3 and GDCN analyses for the nrITS expanded partition are shown in Figure 4, for the *ndh*F expanded partition in Figure 5, and for the nrITS + *ndh*F 61-accession partition in Figure 6. The best supported chromEvol v.1.3 model for the expanded partitions was M1 and for the combined partitions the best supported model was M2 (see figure captions for details). The results of chromEvol v.1.3 and GDCN analyses for the inferred base number and genome doubling events are dependent on sampling and input tree topology. This study is limited in ability to resolve patterns of chromosomal evolution within infrageneric groups because there is a lack of multiple accessions for every taxon sampled across the range of documented diversity. The predicted base number for the genus was $x = 9$, $x = 11$, or $x = 12$. The predicted base number for *Phacelia* excluding "core" *Phacelia* subg. *Pulchellae* (and excluding *Romanzoffia* for the *ndh*F expanded partition) was $x = 11$ or $x = 12$.

*Phacelia* subg. *Pulchellae* contains descending dysploidy ($n = 10, 11, 12$) and annual polyploid taxa ($n = 22, 24$, not sampled). The predicted base number for "core" *Phacelia* subg. *Pulchellae* was $x = 11$ or $x = 12$. There is ascending and descending dysploidy within *Phacelia* subg. *Microgenetes* ($n = 11, 12, 13$) and annual polyploid taxa ($n = 23$), discussed previously in Constance (1963), Heckard (1963), and Gilbert et al. (2005). The predicted base number for *Phacelia* subg. *Microgenetes* was $x = 11$ or $x = 12$. *Phacelia* sect. *Pachyphyllae* ($n = 11$) predicted base number was $x = 11$. The predicted base number for the paraphyletic *Phacelia* sect. *Euglypta* and *Phacelia* sect. *Miltitzia* (*Phacelia* subg. *Microgenetes* excluding *Phacelia* sect. *Pachyphyllae*) was $x = 12$. Cytological characters will be helpful in a future revision of *Phacelia* sect. *Euglypta* and *Phacelia* sect. *Miltitzia.*

There is descending dysploidy within *Phacelia* sect. *Cosmantha* ($n = 5, 6, 8, 9$) and annual polyploid taxa ($n = 14$, not sampled). Sampling throughout this group is extremely limited for the nuclear and plastid partitions. The predicted base number for *Phacelia* sect. *Cosmantha* was $x = 9$ as hypothesized by Constance (1949). The predicted base number for *Phacelia* sect. *Eutoca, Phacelia* sect. *Baretiana, Phacelia* sect. *Gymnobytha,* and *Phacelia* sect. *Whitlavia* was $x = 11$. *Phacelia* sect. *Phacelia* includes the annual *Phacelia* subsect. *Humiles* (not labeled in figures) with ascending and descending dysploidy ($n = 7, 8, 9, 10, 11$) and the perennial polyploid complex *Phacelia* subsect. *Phacelia* ($n = 11, 22, 33$). The predicted base number for *Phacelia* sect. *Phacelia* was $x = 11$.

There is descending dysploidy within *Phacelia* sect. *Ramosissimae* ($n = 10, 11$), although accessions of *P. suffrutescens* Parry ($n = 10$) were not included in analysis. The predicted base number for *Phacelia* sect. *Ramosissimae* was $x = 11$. There is ascending dysploidy in *Phacelia* sect. *Glandulosae* ($n = 11, 12$) and a putative annual polyploid taxon (AF130179). Our review of published chromosome counts for sampled taxa indicates wider variation than has previously has been noted for *Phacelia* sect. *Glandulosae* (e.g., *Phacelia congesta, Phacelia crenulata* var. *crenulata*) (Atwood 1975; Walden and Patterson 2012). These counts may represent infraspecific cytotypes that should be the focus of future studies of cryptic diversity in the section. The predicted base number for *Phacelia* sect. *Glandulosae* was $x = 11$. Coding of AF130179 *P. patuliflora* with the published count of $n = 9$ for the taxon or with X did not change the inferred ancestral state of the nearest ancestral node ($n = 11$) in chromEvol v.1.3 analysis (alternate analyses not shown in results section, see phylogenetic analysis results section for discussion regarding this accession). A loss event was inferred along the branch with coding of $n = 9$ (expectation 1.89974). It is possible that, due to our coding schema, we did

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(total number of events 21.5156), for polyploidization (ρ in chromEvol) are indicated with ⊗ (total number of events 4.24671). A whole genome duplication (WGD) event supported at ≥95% posterior probability in GDCN analysis is indicated with ⊠ at nodes and branches (symbol shown at tip for WGD event along branch).

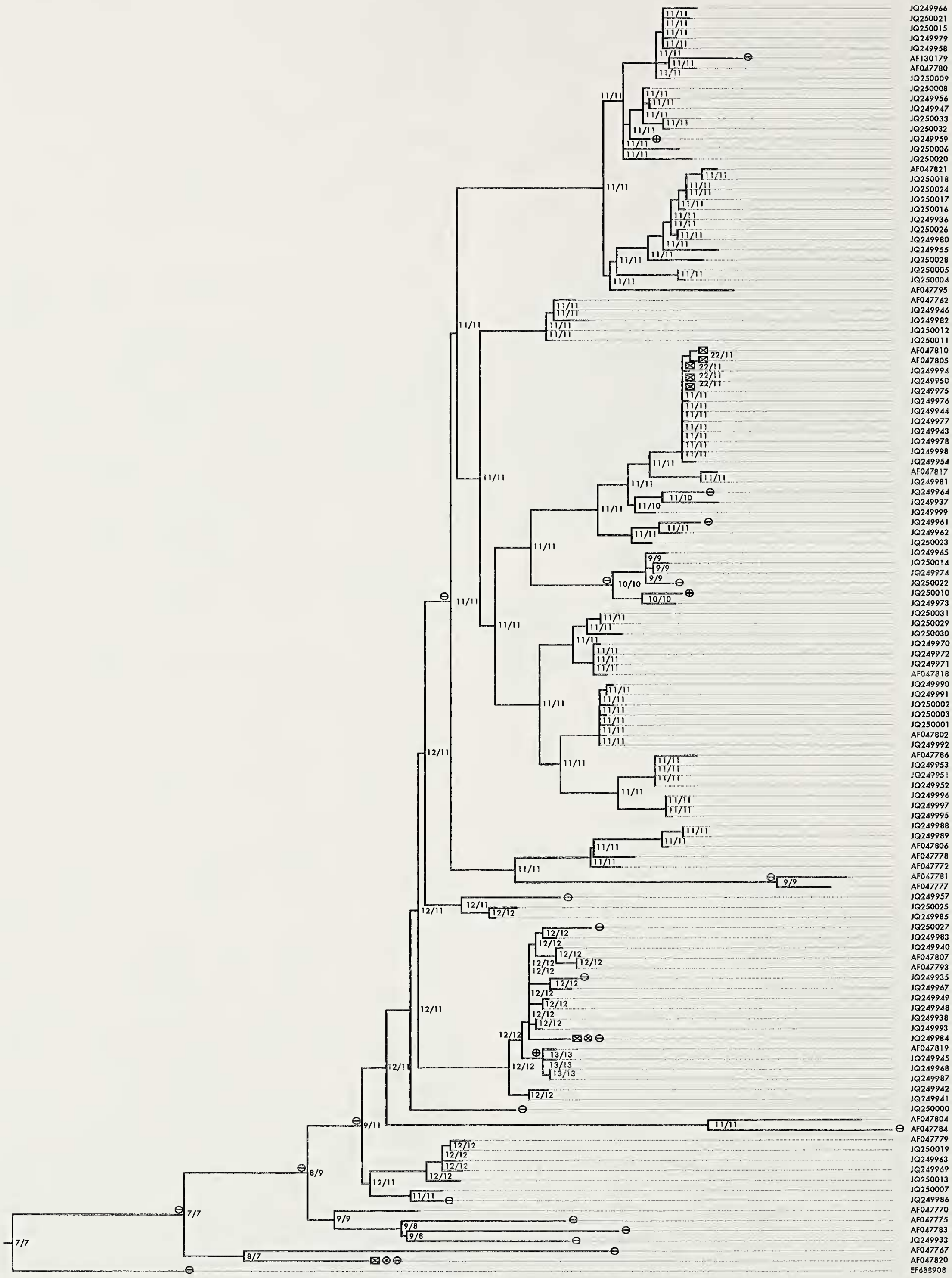


FIG. 5. RaxML phylogram of the single, best tree [-lnL = 10029.632275, tree length = 0.533931] for the expanded *ndh*F 126-accession partition from maximum likelihood analysis (scale bar = mean number of nucleotide substitutions per site). Tip labels are shown with GenBank accession numbers. Maximum likelihood inferred ancestral chromosome numbers are indicated at nodes for chromEvol analysis model M1 ($\lambda$, $\delta$, $\rho$, $\mu$ = 0, AIC = 285.6) / GDCN analysis. Nodes and branches with expectations above 0.5 from chromEvol analysis for gains ($\lambda$ in chromEvol) are indicated with ⊕ (total number of events 7.04247), for losses ($\delta$ in chromEvol) are indicated with

not capture a cryptic polyploidization event for this accession like that for *P. ivesiana* var. *pediculoides*.

Results from chromEvol v.1.3 and GDCN infer polyploidization and whole genome duplication events occurring within *Phacelia*, although this is dependent upon sampling, coding of the chromosome counts at tips, choice of provided tree topology, and AIC selection of the best model. All analyses identified a polyploidization event (for chromEvol v.1.3) and a whole genome duplication (WGD) event (for GDCN) for *Eriodictyon* and *Phacelia ivesiana* var. *pediculoides*. The plastid expanded analyses identified five WGD events (for GDCN) in *Phacelia* subsect *Phacelia* and the combined nrITS + *ndh*F 61-accession analyses identified one polyploidization event (for chromEvol v.1.3) and three WGD events (for GDCN). Tentatively, *P. humilis* ($n$ = 11) appears to be the diploid ancestor for the perennial polyploid complex and *P. breweri* ($n$ = 11) is the diploid ancestor for *P. californica* ($n$ = 22). Future research requires sampling throughout *Phacelia* sect. *Phacelia* (North and South American members) and consideration of Heckard's hypotheses of ploidy intergradations in the complex (Fig. 7 in Heckard [1960]).

## Discussion

### Phylogenetic Relationships

This project sampled throughout *Phacelia* using the internal transcribed spacer region (ITS-1, ITS-2, and 5.8S gene) of nuclear ribosomal DNA (nrITS) and the chloroplast DNA gene (*ndh*F) to infer expanded phylogenies for the nuclear and plastid partitions. An objective of this study was to determine if additional sampling within the genus for each nuclear and plastid molecular marker would recover similar topologies as in previous analyses. We recovered similar nrITS topologies and support values for this study as in Gilbert et al. (2005) and as in Hansen et al. (2009) (Fig. 2 in Gilbert et al. [2005], Fig. 2 in Hansen et al. [2009]). We recovered similar *ndh*F topologies and MP support values for this study as in Ferguson (1998) (Fig. 3 in Ferguson [1998 (1999)]), and note that we show only the MP support values in Figure 2 (MP tree not shown). The nuclear partition resulted in weaker support for deeper nodes along the backbone of trees when compared with the plastid partition. Overall, MP analyses for both the nuclear and plastid partitions (expanded, reduced) resulted in weaker support for deeper nodes along the backbone of trees and these were collapsed as polytomies and reported in the figure with a dagger or with an asterisk indicating low support.

Within group relationships were well supported for both nuclear and plastid partitions in separate analyses, except for *Phacelia* sect. *Glandulosae* and for *Phacelia* subsect. *Phacelia*. nrITS and *ndh*F were minimally variable for resolving relationships in these sections and indicate recent adaptive radiations in these sections. There was better resolution within groups where taxa were represented by multiple accessions rather than singletons. This sort of intensive sampling was balanced by practical concerns. *Phacelia* sect. *Glandulosae* includes taxa of conservation concern, some represented by extremely limited populations and few herbarium collections, including two federally endangered taxa (*P. argillacea* N. D. Atwood [not sampled] and *P. formosula* Osterh. [*ndh*F expanded only]). The gypsophilic and gypsovag taxa (*P. gypsogenia* I. M. Johnst., *P. marshall-johnstonii* N. D. Atwood & Pinkava, *P. palmeri* Torr. ex S. Watson, *P. vossii* N. D. Atwood) were similarly unresolved in the section (Turner 2011). It is unfortunate that this study is inadequate to provide much needed answers regarding evolutionary relationships for these taxa, but indicates that an explicit sampling regime is required for future systematic studies.

Phylogenetic incongruence between plastid and nuclear partitions was previously noted in molecular studies in *Phacelia* (Ferguson 1998, 1998 [1999]; Gilbert et al. 2005; Hansen et al. 2009). The nuclear and plastid partitions were significantly incongruent in our analyses. We corroborate incongruence between nuclear and plastid topologies for placement of some groups (e.g., *Draperia, Romanzoffia* and "core" *Phacelia* subg. *Pulchellae*, *Phacelia* sect. *Baretiana*). Long-branch attraction may be a factor in the incongruent results at the base of the tree for *Draperia, Romanzoffia*, and *Phacelia*, although this remains to be exhaustively tested (Bergsten 2005). The low support for groups for the nuclear partition may partially account for incongruence between the topologies. Different sampling between nuclear and plastid partitions could partially account for incongruent topologies in the expanded analyses. For example, the expanded nuclear topology recovered a paraphyletic *Phacelia* subsect. *Humiles* (*P. breweri* sister to *P. californica* within *Phacelia* subsect. *Phacelia*). *Phacelia breweri* was not sampled for *ndh*F. However, incongruence between the nuclear

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⊖ (total number of events 36.2782), for polyploidization (ρ in chromEvol) are indicated with ⊗ (total number of events 2.42607). A whole genome duplication (WGD) event supported at ≥95% posterior probability in GDCN analysis is indicated with ⊠ at nodes and branches (symbol shown at tip for WGD event along branch).

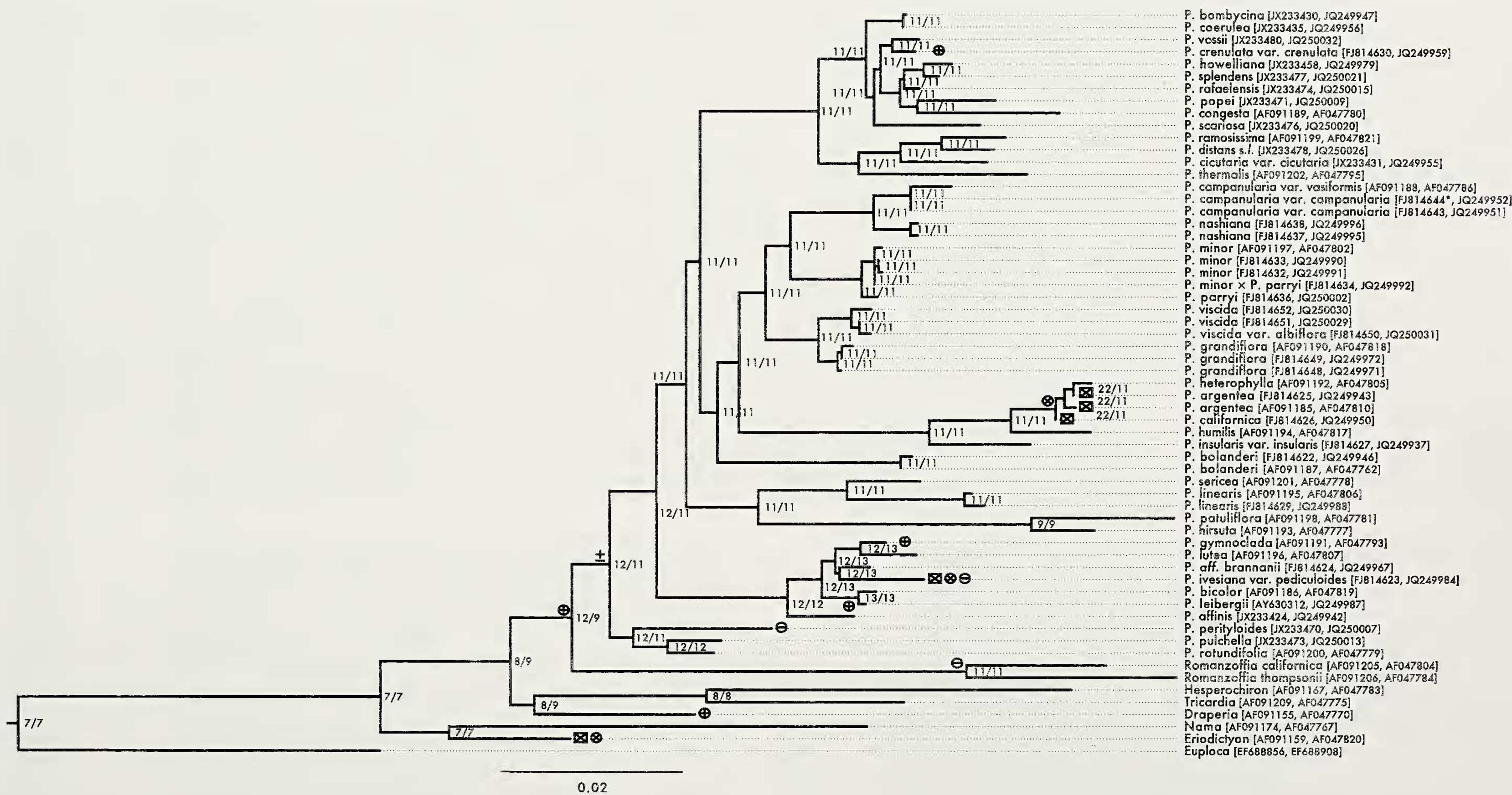


FIG. 6. RaxML phylogram of the single, best tree [-lnL = 14591.525588, tree length = 0.834130] for the reduced and combined nrITS + *ndh*F 61-accession dataset from maximum likelihood analysis (scale bar = mean number of nucleotide substitutions per site). Tip labels are shown with GenBank accession numbers. Maximum likelihood inferred ancestral chromosome numbers are indicated at nodes for chromEvol analysis model M2 ($\lambda$, $\delta$, $\rho = \mu$, AIC = 121.5) / GDCN analysis. Nodes and branches with expectations above 0.5 from chromEvol analysis for gains ($\lambda$ in chromEvol) are indicated with $\oplus$ (total number of events 8.12033), for losses ($\delta$ in chromEvol) are indicated with $\ominus$ (total number of events 7.21463), for polyploidization ($\rho$ in chromEvol) are indicated with $\otimes$ (total number of events 3.02863), for demipolyploidization ($\mu$ in chromEvol) are indicated with $\pm$ (total number of events 1.23939). A whole genome duplication (WGD) event supported at $\geq$95% posterior probability in GDCN analysis is indicated with $\boxtimes$ at branches (symbol shown at tip for WGD event along branch).

(Fig. 1) and the plastid (Fig. 2) topologies remained for the 61-accession partitions in separate analyses (results not shown), indicating that sampling of representatives using sequences from different specimens was not contributing to the incongruence between partitions.

Incongruent topologies between the separate nuclear partition (expanded, reduced) separate and plastid partition (expanded, reduced) indicated that simultaneous analyses of the combined nrITS + *ndh*F 61-accession partition would be statistically inappropriate but potentially beneficial (Nixon and Carpenter 2005). Combined analyses resulted in better resolution than separate analyses, and in a topology that favored the separate plastid topologies (expanded, reduced). Where nuclear and plastid topologies were congruent in separate analyses, the combined nrITS + *ndh*F 61-accession topology was also congruent and internal nodes were recovered with support. Our results support combining incongruent partitions in a combined analysis to seek support for internal nodes.

Previous molecular phylogenetic studies in Hydrophylloideae have identified significant phylogenetic incongruence between nuclear and plastid partitions (Ferguson 1998; Hansen et al. 2009; Taylor 2012). Studies in Cordioideae (Weeks et al. 2010), Ehretioideae (Smith 2003; Moore and Jansen 2006), and broad sampling across Boraginaceae that included "problematic" subfamilies (Ferguson 1998 [1999]; Nazaire and Hufford 2012) have also identified significant phylogenetic incongruence between nuclear and plastid partitions. Broad sampling across Boraginaceae with limited sampling from Cordioideae, Hydrophylloideae, and Ehretioideae and excluding "problematic" taxa found nuclear and plastid partitions to be congruent (Cohen 2013). Studies focusing on Boraginoideae (Winkworth et al. 2002; Hilger et al. 2004; Hasenstab-Lehman and Simpson 2012) and Heliotropioideae (Luebert and Wen 2008) have found the nuclear and plastid partitions to be congruent for those subfamilies. It is clear that a comprehensive examination of these "problematic" but interesting lineages within the larger context of Boraginaceae is needed. Particularly necessary is a comparative, statistical examination of partition homogeneity, incomplete lineage sorting, and analysis of hidden support within datasets of Boraginaceae (Huelsenbeck and Bull 1996; Gatesy et al. 1999; Leigh et al. 2008; Sarkar et al. 2008; Simon et al. 2009).

### Chromosome Evolution

Maximum likelihood analyses were used to infer ancestral chromosome numbers and identify gains, losses, polyploid doubling, and whole genome duplication events from published chromosome counts assembled from the literature for sampled taxa. Results of analyses from chromEvol v.1.3 and GDCN are dependent on sampling and known tree topology (Mayrose et al. 2010; Hallinan and Lindberg 2011a). Our results demonstrate the utility of using both chromEvol v.1.3 and GDCN in combination to identify patterns of chromosome evolution in *Phacelia*. Constance (1963), Heckard (1963), and Gillett (1968) hypothesized that $n = 11$ was the ancestral condition for the genus and noted it was also the most common haploid count for extant taxa. Results for the separate nuclear, separate plastid, or combined dataset did not provide a consensus. The predicted base number for the genus was $x = 9$, $x = 11$, or $x = 12$. Total evidence approaches may provide better-resolved phylogenies for *Phacelia* and Hydrophylloideae, offering resolution of inferred ancestral states for chromosome evolution, especially those that are shown as equivocal in areas of the tree with lower sampling.

## Conclusions

This study contributes to our understanding of the evolutionary relationships of *Phacelia*, an entirely New World genus with a center of diversity in California. We investigated chromosome evolution in an explicit molecular context using maximum likelihood models of evolution. *Phacelia* is an excellent group to study chromosome evolution, as it is a large genus representative of the California flora with a range of chromosome counts (Stebbins 1942; Stebbins and Major 1965; Stebbins 1971). Our study identifies patterns of gains, losses, and polyploid doubling events in lineages that likely contributed to the overall diversity in *Phacelia* and the California flora. Although an extensive dataset of chromosome counts exists for the genus and the subfamily, approximately a third of the genus (ca. 70 taxa) lack published chromosome counts.

Expanded sampling for each respective marker (nuclear nrITS and plastid *ndh*F) and phylogenetic analyses (maximum parsimony, maximum likelihood, and Bayesian inference) recovered similar topologies for separate and combined analyses as previous studies. The combined nrITS + *ndh*F analyses supported *Romanzoffia* sister to a monophyletic *Phacelia*. Our results support combining incongruent partitions in a combined analysis to seek support for internal nodes. Results indicated recent adaptive radiations in *Phacelia* sect. *Glandulosae* and *Phacelia* sect. *Phacelia*. Future work is needed to understand and compare the rate of molecular and morphological evolution in the genus.

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## Literature Cited


Ackerly, D. D. 2009. Evolution, origin and age of lineages in the Californian and Mediterranean floras. Journal of Biogeography 36:1221–1233.

Álvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution 29:417–434.

Atwood, N. D. 1975. A revision of the *Phacelia Crenulatae* group (Hydrophyllaceae) for North America. The Great Basin Naturalist 35:127–190.

AWTY (Are We There Yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Website http://ceb.csit.fsu.edu/awty (accessed 05 August 2012).

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, (eds.). 2012. The Jepson Manual: vascular plants of California, 2nd ed. University of California Press, Berkeley, CA.

———, M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82:247–277.

Beard, J. S., A. R. Chapman, and P. Gioia. 2000. Species richness and endemism in the Western Australian flora. Journal of Biogeography 27: 1257–1268.

Bergsten, J. 2005. A review of long-branch attraction. Cladistics 21:163–193.

Bohs, L. and R. G. Olmstead. 1997. Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndh*F sequences. Systematic Botany 22:5–17.

Brand, A. 1913. Hydrophyllaceae. Pp. 1–210 *in* A. Engler (ed.), Das Pflanzenreich IV, Vol. 251 (Heft 59). Verlag von Wilhelm Engelmann, Leipzig.

Brown, J. M. and A. R. Lemmon. 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Systematic Biology 56:643–655.

Bull, J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford, and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. Systematic Biology 42:384–397.

Cave, M. S. and L. Constance. 1942. Chromosome numbers in the Hydrophyllaceae. University of California Publications in Botany 18:205–216.

———. 1944. Chromosome numbers in the Hydrophyllaceae, II. University of California Publications in Botany 18:293–298.

———. 1947. Chromosome numbers in the Hydrophyllaceae, III. University of California Publications in Botany 18:449–465.

———. 1950. Chromosome numbers in the Hydrophyllaceae, IV. University of California Publications in Botany 23:363–382.

———. 1959. Chromosome numbers in the Hydrophyllaceae, V. University of California Publications in Botany 30:233–258.

chromEvol (v.1.3). 2012. Website http://www.tau.ac.il/~itaymay/cp/chromEvol/index.html (accessed 01 May 2012).

California Native Plant Society (CNPS). 2011. Inventory of Rare and Endangered Plants (online edition, v8–01a). California Native Plant Society. Sacramento, CA. Website http://www.cnps.org/inventory (accessed 30 June 2013).

Cohen, J. I. 2013. A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution. Cladistics. doi: 10.1111/cla.12036 (accessed 12 July 2013).

Constance, L. 1948. A new species of *Phacelia* from Sonora, México. Madroño 9:255–256.

———. 1949. A revision of *Phacelia* subgenus *Cosmanthus* (Hydrophyllaceae). Contributions to the Gray Herbarium 168:1–48.

———. 1963. Chromosome number and classification in Hydrophyllaceae. Brittonia 15:273–285.

Covas, G. and B. Schnack. 1947. Estudios cariológicos en Antófitas. II Parte. Revista Argentina de agronoma 14:224–231.

Cunningham, C. W. 1997. Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14:733–740.

Cusimano, N., A. Sousa, and S. S. Renner. 2012. Maximum likelihood inference implies a high, not a low, ancestral haploid chromosome number in Araceae, with a critique of the bias introduced by '*x*'. Annals of Botany 109:681–692.

Dempcy, J. M. 1996. Phylogenetic relationships in *Phacelia* section *Euglypta* (Hydrophyllaceae). M.S. Thesis, Biology, San Francisco State University, San Francisco, CA.

DOYLE, J. J. AND E. E. DICKSON. 1987. Preservation of plant samples for DNA restriction endonuclease analysis. Taxon 36:715–722.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. Systematic Biology 44:570–572.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

FERGUSON, D. M. 1998. Phylogenetic systematics of Hydrophyllaceae. Ph.D. Dissertation, Biology, Harvard, Cambridge, MA.

FERGUSON, D. 1998 (1999). Phylogenetic analysis and relationships in Hydrophyllaceae based on *ndh*F sequence data. Systematic Botany 23:253–268.

FIGTREE v.1.3.1 2006–2009. Website http://tree.bio.ed.ac.uk/software/figtree/. (accessed 28 May 2009).

GANONG, C. K. 2002. Phylogenetic analysis of *Phacelia* section *Miltitzia*. M.S. Thesis, Biology, San Francisco State University, San Francisco, CA.

GARRISON, L. M. 2007. Phylogenetic relationships in *Phacelia* (Boraginaceae) inferred from *nrITS* sequence data. M.S. Thesis, Biology, San Francisco State University, San Francisco, CA.

GATESY, J., P. O'GRADY, AND R. H. BAKER. 1999. Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. Cladistics 15:271–313.

GELMAN, A. AND D. B. RUBIN. 1992. Inference from iterative simulation using multiple sequences. Statistical Science 1:451–511.

GILBERT, C., J. M. DEMPCY, C. K. GANONG, R. PATTERSON, AND G. S. SPICER. 2005. Phylogenetic relationships within *Phacelia* subgenus *Phacelia* (Hydrophyllaceae) inferred from nuclear rDNA ITS sequence data. Systematic Botany 30:627–634.

GILLETT, G. W. 1968. Systematic relationships in the *Cosmanthus* phacelias (Hydrophyllaceae). Brittonia 20:368–374.

GLASS, P. M. AND F. LEVY. 2011. Correspondence of morphology, phylogeny and reproductive barriers in *Phacelia* subgenus *Cosmanthus* (Hydrophyllaceae). The Journal of the Torrey Botanical Society 138:341–352.

GOUJON, M., H. MCWILLIAM, W. LI, F. VALENTIN, S. SQUIZZATO, J. PAERN, AND R. LOPEZ. 2010. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Research 38:W695–W699.

GUINDON, S. AND O. GASCUEL. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

HALLINAN, N. M. AND D. R. LINDBERG. 2011a. Comparative analysis of chromosome counts infers three paleopolyploidies in the mollusca. Genome Biology and Evolution 3:1150–1163. doi: 10.1093/gbe/evr087 (accessed 25 April 2012).

———. 2011b. GDCN (Genome Duplication from Chromosome Numbers). Website http://code.google.com/p/gdcn/ (accessed 25 April 2012).

HANSEN, D. R., G. S. SPICER, AND R. PATTERSON. 2009. Phylogenetic relationships between and within *Phacelia* sections *Whitlavia* and *Gymnobythus* (Boraginaceae). Systematic Botany 34: 737–746.

HASENSTAB-LEHMAN, K. E. AND M. G. SIMPSON. 2012. Cat's eyes and popcorn flowers: phylogenetic systematics of the genus *Cryptantha* s.l. (Boraginaceae). Systematic Botany 37:738–757.

HECKARD, L. R. 1960. Taxonomic studies in the *Phacelia magellanica* polyploid complex, with special reference to the California members. University of California Publications in Botany 32:1–126.

———. 1963. The Hydrophyllaceae. The Quarterly Review of Biology 38:117–123.

HILGER, H. H., F. SELVI, A. PAPINI, AND M. BIGAZZI. 2004. Molecular systematics of Boraginaceae tribe Boragineae based on ITS1 and *trn*L sequences, with special reference to *Anchusa* s.l. Ann Bot 94:201–212.

HOWELL, J. T. 1946. A revision of *Phacelia* section *Euglypta*. American Midland Naturalist 36:381–411.

HUELSENBECK, J. P. AND J. J. BULL. 1996. A likelihood ratio test to detect conflicting phylogenetic signal. Systematic Biology 45:92–98.

———, ———, AND C. W. CUNNINGHAM. 1996. Combining data in phylogenetic analysis. Trends in Ecology & Evolution 11:152–158.

——— AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

KELLOGG, A. 1877. Notes and descriptions of some Californian plants. Proceedings of the California Academy of Sciences 7:89–94.

KIM, K. J. AND R. K. JANSEN. 1995. *ndh*F sequence evolution and the major clades in the sunflower family. Proceedings of the National Academy of Sciences 92:10379–10383.

KOVANDA, M. 1978. Chromosome numbers of miscellaneous United States dicotyledons. Rhodora 80:431–440.

KRAFT, N. J. B., B. G. BALDWIN, AND D. D. ACKERLY. 2010. Range size, taxon age and hotspots of neoendemism in the California flora. Diversity and Distributions 16:403–413.

KRUCKEBERG, A. R. 1956. Notes on the *Phacelia magellanica* complex in the Pacific Northwest. Madroño 13:209–221.

LARKIN, M. A., G. BLACKSHIELDS, N. P. BROWN, R. CHENNA, P. A. MCGETTIGAN, H. MCWILLIAM, F. VALENTIN, I. M. WALLACE, A. WILM, R. LOPEZ, J. D. THOMPSON, T. J. GIBSON, AND D. G. HIGGINS. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

LEIGH, J. W., E. SUSKO, M. BAUMGARTNER, AND A. J. ROGER. 2008. Testing congruence in phylogenomic analysis. Systematic Biology 57:104–115.

LEWIS, P. O. AND M. T. HOLDER. 2008. NEXUS Class Library (version 2.1). Website http://sourceforge.net/projects/ncl/.

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

MACCLADE v.4.08. Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, MA

MADDISON, W. AND D. MADDISON. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.74. Website http://mesquiteproject.org (accessed 3 October 2010).

MARTÍN, M. AND B. SABATER. 2010. Plastid *ndh* genes in plant evolution. Plant Physiology and Biochemistry 48:636–645.

MAYROSE, I., M. S. BARKER, AND S. P. OTTO. 2010. Probabilistic models of chromosome number evolution and the inference of polyploidy. Systematic Biology 59:132–144.

MILLER, M. A., W. PFEIFFER, AND T. SCHWARTZ. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Paper read at the Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA.

MOORE, M. J. AND R. K. JANSEN. 2006. Molecular evidence for the age, origin, and evolutionary history of the American desert plant genus *Tiquilia* (Boraginaceae). Molecular Phylogenetics and Evolution 39:668–687.

MULLIS, K. B., F. FALOONA, S. SCHARF, R. SAIKI, G. HORN, AND H. A. ERLICH. 1987. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology 51:263–273.

NAZAIRE, M. AND L. HUFFORD. 2012. A broad phylogenetic analysis of Boraginaceae: implications for the relationships of *Mertensia*. Systematic Botany 37:758–783.

NEYLAND, R. AND L. E. URBATSCH. 1996. The *ndh*F chloroplast gene detected in all vascular plant divisions. Planta 200:273–277.

NIXON, K. C. AND J. M. CARPENTER. 2005. On simultaneous analysis. Cladistics 12(3): 221–241.

NYLANDER, J. A. A., J. C. WILGENBUSCH, D. L. WARREN, AND D. L. SWOFFORD. 2008. AWTY (Are We There Yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–583.

OLMSTEAD, R. G., K.-J. KIM, R. K. JANSEN, AND S. J. WAGSTAFF. 2000. The phylogeny of the Asteridae *sensu* lato based on chloroplast *ndh*F gene sequences. Molecular Phylogenetics and Evolution 16:96–112.

——— AND J. A. SWEERE. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology 43:467–481.

PATTERSON, R., L. M. GARRISON, AND D. R. HANSEN. 2012. *Phacelia*. Pp. 485–501 *in* B. G. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken (eds.), The Jepson Manual: vascular plants of California, 2nd ed. University of California Press, Berkeley, CA.

POSADA, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

——— AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53:793–808.

——— AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Modeltest 3.7. Bioinformatics 14:817–818.

RAVEN, P. H. AND D. AXELROD. 1978. Origin and relationships of the California flora. University of California Publications in Botany 72:1–134.

RONQUIST, F. AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SARKAR, I. N., M. G. EGAN, G. CORUZZI, E. K. LEE, AND R. DESALLE. 2008. Automated simultaneous analysis phylogenetics (ASAP): an enabling tool for phlyogenomics. BMC Bioinformatics 9(1): 103.

SIMON, J., P. A. MCLENACHAN, AND P. J. LOCKHART. 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. The American Naturalist 174:E54–E70. doi: 10.1086/600082.

SMITH, R. A. 2003. The systematics and molecular evolution of Lennoaceae. Ph.D. Dissertation, Biology, Vanderbilt University, Nashville, TN.

STAMATAKIS, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

———, P. HOOVER, AND J. ROUGEMONT. 2008. A fast bootstrapping algorithm for the RAxML webservers. Systematic Biology 57:758–771.

STEBBINS, G. L., JR. 1942. Polyploid complexes in relation to ecology and the history of floras. American Naturalist 76:36–45.

———. 1971. Chromosomal evolution in higher plants. Edward Arnold Ltd, London.

——— AND J. MAJOR. 1965. Endemism and speciation in the California Flora. Ecological Monographs 35:1–35.

PAUP* Phylogenetic analysis using parsimony (*and other methods), version 4.0 beta 10. Sinauer Associates Inc., Sunderland, MA.

TAYLOR, S. 2012. Molecular systematics and the origin of gypsophily in *Nama* L. (Boraginaceae). Ph.D. Dissertation, Plant Biology, University of Texas at Austin, Austin, TX.

TRACER v.1.5.0. 2009. Website http://tree.bio.ed.ac.uk/software/tracer/. (accessed 10 March 2010).

TURNER, B. L. 2011. A new gypsophilic *Phacelia* (Hydrophyllaceae) from Coahuila, México. Phytologia 93(1): 88–93.

U.S. DEPARTMENT OF THE INTERIOR, FISH AND WILDLIFE SERVICE. 1978. Endangered and threatened wildlife and plants; determination of five plants as endangered species, final rule. Federal Register 43:44810–44812.

———. 1982. Endangered and threatened wildlife and plants; determination that *Phacelia formosula* is an endangered species, final rule. Federal Register 59:38540–38543.

———. 1997. Endangered and threatened wildlife and plants; final rule for 13 plant taxa from the northern Channel Islands, California. Federal Register 62:40954–40974.

———. 2004. Endangered and threatened wildlife and plants; review of species that are candidates or proposed for listing as endangered or threatened; annual notice of findings on resubmitted petitions; annual description of progress on listing actions; notices of review; proposed rule. Federal Register 69:24876–24904.

———. 2011. Endangered and threatened wildlife and plants; determination of endangered status for *Ipomopsis polyantha* (Pagosa Skyrocket) and threatened status for *Penstemon debilis* (Parachute Beardtongue) and *Phacelia submutica* (DeBeque Phacelia); final rule. Federal Register 76:45054–45075.

URBATSCH, L. E., B. G. BALDWIN, AND M. J. DONOGHUE. 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chlorplast DNA restriction site data. Systematic Botany 25:539–565.

WALDEN, G. K. 2010. Phylogeny of infrageneric relationships within *Phacelia* (Boraginaceae) inferred from chloroplast sequence data. M.S. Thesis, Biology, San Francisco State University, San Francisco, CA.

——— AND R. PATTERSON. 2012. Nomenclature of subdivisions in *Phacelia* (Boraginaceae). Madroño 59:211–222.

WEEKS, A., K. E. BAIRD, AND C. K. MCMULLEN. 2010. Origin and evolution of endemic Galapagos *Varronia* species (Cordiaceae). Molecular Phylogenetics and Evolution 57:948–954.

WHITE, T. J., T. BRUNS, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. A. Innis, D. H. Gelfand, J. J. Sinisky, and T. J. White (eds.), PCR protocols: a guide to methods and applications. Academic Press, Inc., New York.

WINKWORTH, R. C., J. GRAU, A. W. ROBERTSON, AND P. J. LOCKHART. 2002. The origins and evolution of the genus *Myosotis* L. (Boraginaceae). Molecular Phylogenetics and Evolution 24:180–193.

WOOD, T. E., N. TAKEBAYASHI, M. S. BARKER, I. MAYROSE, P. B. GREENSPOON, AND L. H. RIESEBERG. 2009. The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences 106:13875–13879.

## APPENDIX 1

List of taxa sampled in this study: *named taxon* (presented in alphabetical order, with bolded type and botanical authority given for first instance of taxon), locality of collection, date of collection or n.d., name of collector(s) and collection number, acronym of herbarium where voucher specimen is deposited and herbarium accession number (if available), GenBank accession numbers for nrITS and *ndh*F (en dash [–] if information or sequence not available). Taxa names follow recent treatments in the second edition of *The Jepson Manual* (Baldwin et al. 2012) and treatments in preparation for FNANM, botanical authorities follow *Authors of Plant Names* edited by R. K. Brummitt and C. E. Powell (1992), and herbarium acronyms follow Index Herbariorum (http://sweetgum.nybg.org/ih/). Vouchers or sequences originally published under different identifiers are indicated with an asterisk (*) and discussed parenthetically following the information string. Voucher specimens not examined for this study are indicated (n.v.).

***Eriodictyon californicum*** (Hook. & Arn.) Torr., USA, CA, Contra Costa Co., 27 Apr 1994, *D. M. Ferguson 59* (GH00402724, n.v.), AF091159, AF047820; ***Euploca campestris*** (Griseb.) Diane & Hilger, –, n.d., *Nee & Wen 53873* (US, n.v.), EF688856, EF688908; ***Draperia systyla*** (A. Gray) Torr., USA, CA, Tulare Co., 4–5 Sep 1979, *R. Thorne 53719* (RSA341263, n.v.), AF091155, AF047770; ***Hesperochiron pumilus*** (Griseb.) Porter, –, n.d., *R. Olmstead and D. M. Ferguson 92* (GH, n.v.), AF091167, AF047783; ***Howellanthus dalesianus*** (J. T. Howell) Walden & R. Patt., USA, CA, Siskiyou Co., 21 Jun 2005, *R. Patterson and S. Santos 1982* (SFSU), –, JQ249933; ***Nama demissum*** A. Gray, USA, CA, San Bernardino Co., 12 April 1987, *M. O. Bagley and P. Athey 1932* (RSA395999, n.v.), AF091174, AF047767; ***Phacelia adenophora*** J. T. Howell, USA, CA, Lassen Co., 16 May 1993, *J. Dempcy 114-2** (SFSU), AY630260, – (*cited as *J. Dempcy 114* in Gilbert et al. 2005); *P. adenophora*, USA, NV, border of Storey Co. and Lyon Co., 11 May 1993, *J. Dempcy 117-1** (SFSU), AY630261, – (*cited as *J. Dempcy 117* in Gilbert et al. 2005, and a duplicate collection of *J. Dempcy 117-2*); *P. adenophora*, USA, NV, border of Storey Co. and Lyon Co., 11 May 1993, *J. Dempcy 117-2** (SFSU), AY630262, – (*cited as *J. Dempcy 117* in Gilbert et al. 2005, and a duplicate collection of *J. Dempcy 117-1*); *P. adenophora*, USA, NV, Washoe Co., 14 Jun 2008, *A. Tiehm 15643* (SFSU), –, JQ249940; ***Phacelia affinis*** A. Gray, USA, NV, Nye Co., 10 Jun 1995, *J. Dempcy 137-1* (SFSU), –, JQ249941; *P. affinis*, USA, NV, Nye Co., 10 Jun 1995, *J. Dempcy 137-2* (SFSU), AY630625, –; *P. affinis*, USA, NV, Nye Co., 10 Jun 1995, *J. Dempcy 138-1* (SFSU), AY630264, –; *P. affinis*, USA, AZ, Santa Cruz Co., 21 Mar 2005, *L. M. Garrison 31* (SFSU), JX233424, JQ249942; *P. affinis*, USA, AZ, Mohave Co., *E. McClintock 52-264* (CAS, n.v.), AY630263, –; ***Phacelia argentea*** A. Nelson & J. F. Macbr., USA, OR, n.d., *D. M. Ferguson 82* (WTU, n.v.), AF091185, AF047810; *P. argentea*, USA, OR, Curry Co., wild collected for cultivation 26 Aug 1984, *V. Stansell s.n.*, sourced from the Berry Botanic Garden, Portland, OR, 11 July 1986 (Berry BG SB84-103), living collection at UC Berkeley Botanic Garden (CA, Alameda Co.), UCBG collection for molecular research 24 Nov 2008, *H. Forbes s.n.* (UCBG 86.1064), –, JQ249944; *P. argentea*, USA, OR, Coos Co., 24 Jul 2008, *G. K. Walden 81* (SFSU), FJ814625, JQ249943; ***Phacelia arizonica*** A. Gray, USA, AZ, Santa Cruz Co., 21 Mar 2005, *L. M. Garrison 32* (SFSU), JX233426, –; ***Phacelia aff. artemisioides*** Griseb., CHILE, Antofagasta, El Loa Province, 11 Apr 1994, *C. M. Taylor and A. Pool 11569* (MO002977, n.v.), –, JQ250033; ***Phacelia bicolor*** Torr. ex S. Watson, USA, CA, Mono Co., 16 May 1993, *J. Dempcy 89-2* (SFSU), AY630266, –; *P. bicolor*, USA, CA, Lassen Co., 16 May 1993, *J. Dempcy 112-1* (SFSU), AY630267, –; *P. bicolor*, USA, NV, Humboldt Co., 13 May 1993, *J. Dempcy 118-2* (SFSU), AY630268, –; *P. bicolor*, USA, CA, Mono Co., 18 Jun 1993, *D. M. Ferguson 12* (GH), AF091186, AF047819; *P. bicolor*, USA, NV, Ormsby Co., 19 May 2008, *A. Tiehm 15523* (SFSU), –, JQ249945; ***Phacelia bolanderi*** A. Gray, –, n.d., *R. Olmstead 93-65* (WTU, n.v.), AF091187, AF047762; *P. bolanderi*, USA, CA, Humboldt Co., near Weott, wild collected for cultivation *s.d.*, *W. Roderick s.n.*, living collection at UC Berkeley Botanic Garden (CA, Alameda Co.), UCBG collection for molecular research 24 Nov 2008, *H. Forbes s.n.* (UCBG 61.0093), FJ814622, JQ249946; ***Phacelia bombycina*** Wooton & Standl., USA, AZ, Graham Co., 28 Mar 2004, *L. M. Garrison 15* (SFSU), JX233427, –; *P. bombycina*, USA, AZ, Pima Co., 28 Mar 2004, *L. M. Garrison 16* (SFSU), JX233434, –; *P. bombycina*, USA, AZ, Pima Co., 28 Mar 2004, *L. M. Garrison 17* (SFSU), JX233428, –; *P. bombycina*, USA, AZ, Pima Co., 22 Mar 2005, *L. M. Garrison 35* (SFSU), JX233429, –; *P. bombycina*, USA, AZ, Cochise Co., 25 Mar 2005, *L. M. Garrison 43* (SFSU), JX233430, JQ249947; ***Phacelia brachyloba*** (Benth.) A. Gray, USA, CA, Santa Barbara Co., 19 Jun 1965, *J. Ammirati 315* (SFSU08229), AY630271, –; *P. brachyloba*, USA, CA, Santa Barbara Co., 12 May 1994, *J. Dempcy 123-1* (SFSU), AY630272, –; *P. brachyloba*, USA, CA, Santa Barbara Co., 12 May 1994, *J. Dempcy 124-1* (SFSU), AY630273, –; *P. brachyloba*, USA, CA, San Diego Co., 28 May 2008, *J. P. Rebman and M. Mulligan 15294*

(SD186946, n.v.), –, JQ249949; *P. brachyloba*, USA, CA, Orange Co., 26 Jun 2008, *G. K. Walden 68* (SFSU), –, JQ249948; ***Phacelia aff. brannanii*** Kellogg*, USA, CA, Kern Co., 17 May 1981, *J. Shevock 8564* (CAS713422), AY630269, – (*cited as *P. bicolor* in Gilbert et al. 2005); *P. aff. brannanii**, Kern Co., CA, 26 Mar 2008, *G. K. Walden 33* (SFSU), FJ814624, JQ249967 (*cited as *P. fremontii* Torr. in Hansen et al. 2009); ***Phacelia breweri*** A. Gray, USA, CA, Stanislaus Co., 23 Apr 1993, *C. Condos 23* (SFSU), AY630274, –; ***Phacelia californica*** Cham., USA, CA, San Francisco Co., 8 Apr 1991, *M. Ely 40* (SFSU), AY630275, –; *P. californica*, USA, CA, Marin Co., wild collected for cultivation 12 Jun 1960, *W. Roderick s.n.*, living collection at UC Berkeley Botanic Garden (CA, Alameda Co.), UCBG collection for molecular research 24 Nov 2008, *H. Forbes s.n.* (UCB60.0723), FJ814626, JQ249950; ***Phacelia calthifolia*** Brand, USA, CA, Inyo Co., 29 Mar 1970, *D. Breedlove 17289A* (RSA217251, n.v.), AY630276, –; *P. calthifolia*, USA, CA, Inyo Co., 30 Mar 1995, *J. Dempcy 128-1* (SFSU), AY630278, –; *P. calthifolia*, USA, CA, Inyo Co., 8 Mar 1973, *J. Thorne 42503* (RSA239876, n.v.), AY630277, –; ***Phacelia campanularia*** A. Gray var. ***campanularia***, USA, CA, San Bernardino Co., 29 Mar 2004, *D. R. Hansen 16* (SFSU), FJ814643*, JQ249951 (*sequence identical to FJ814644); *P. campanularia* var. *campanularia*, USA, CA, San Bernardino Co., 22 Mar 2004, *D. R. Hansen 22* (SFSU), FJ814644*,JQ249952 (*sequence identical to FJ814643); *P. campanularia* var. *campanularia*, USA, CA, San Diego Co., 2005, *D. R. Hansen 45* (SFSU), FJ814640, –; *P. campanularia* A. Gray var. *campanularia*, USA, CA, San Bernardino Co., n.d., *C. R. Richards 45* (SFSU), FJ814642, –; ***Phacelia campanularia*** A. Gray var. ***vasiformis*** (G. W. Gillett) Walden & R. Patt., USA, CA, Los Angeles Co., in cultivation at RSABG, voucher harvested 19 Apr 1994, *D. M. Ferguson 56* (GH), AF091188, AF047786; *P. campanularia* A. Gray var. *vasiformis*, USA, CA, Riverside Co., 2005, *D. R. Hansen 47* (SFSU), FJ814646, –; *P. campanularia* A. Gray var. *vasiformis*, USA, CA, Riverside Co., 2005, *D. R. Hansen 49* (SFSU), FJ814647, –; *P. campanularia* A. Gray var. *vasiformis*, USA, CA, San Bernardino Co., 25 Mar 2008, *G. K. Walden 20* (SFSU), –, JQ249953; ***Phacelia capitata*** Kruckeb., USA, OR, Douglas Co., 25 May 2008, *R. R. Halse 7466* (SFSU), –, JQ249954; ***Phacelia cephalotes*** A. Gray, USA, UT, Washington Co., 11 May 2004, *L. C. Higgins 25490* (DAV172082), –, JQ249935; ***Phacelia cicutaria*** Greene var. ***cicutaria***, USA, CA, Kern Co., 22 Mar 2004, *L. M. Garrison 02* (SFSU), JX233431, JQ249955; *P. cicutaria* var. *cicutaria*, USA, CA, Kern Co., 6 May 2005, *L. M. Garrison 45* (SFSU), JX233432, –; ***Phacelia cicutaria*** Greene var. ***hispida*** (A. Gray) J. T. Howell, USA, CA, Los Angeles Co., 6 May 2004, *L. M. Garrison 24* (SFSU), JX233433, –; *P. cicutaria* Greene var. *hispida*, USA, CA, San Diego Co., 3 Apr 2008, *J. P. Rebman and M. Wall 14303* (SD184369, n.v.), –, JQ249936; ***Phacelia coerulea*** Greene, USA, AZ, Cochise Co., 21 Mar 2005, *L. M. Garrison 30* (SFSU), JX233435, JQ249956; ***Phacelia congesta*** Hook., USA, TX, Starr Co., 23 Feb 1992, *R. R. Halse 4436* (GH00288821), AF091189, AF047780; ***Phacelia cookei*** Constance & Heckard, USA, CA, Siskiyou Co., 29 May 2008, *K. Schierenbeck s.n.* (SFSU), –, JQ249957; ***Phacelia corrugata*** A. Nelson, USA, AZ, Coconino Co., 10 May 2005, *L. M. Garrison 53* (SFSU), JX233437, –; *P. corrugata*, USA, UT, Carbon Co., 12 May 2005, *L. M. Garrison 62* (SFSU), JX233438, –; ***Phacelia cottamii*** N. D. Atwood, USA, UT, Emery Co., 16 Jun 1999, *N. D. Atwood 24858* (DAV166215), –, JQ249958; ***Phacelia crenulata*** Torr. ex S. Watson var. ***ambigua*** (M. E. Jones) J. F. Macbr., USA, CA, Imperial Co., 26 Mar 2004, *L. M. Garrison 10* (SFSU), JX233440, –; *P. crenulata* var. *ambigua*, USA, CA, Imperial Co., 27 Mar 2004, *L. M. Garrison 11* (SFSU), JX233441, –; *P. crenulata* var. *ambigua*, USA, AZ, Maricopa Co., 27 Mar 2004, *L. M. Garrison 12* (SFSU), JX233442, –; *P. crenulata* var. *ambigua*, USA, AZ, Pima Co., 28 Mar 2004, *L. M. Garrison 18* (SFSU), JX233443, –; *P. crenulata* var. *ambigua*, USA, CA, Riverside Co., 29 Mar 2004, *L. M. Garrison 19* (SFSU), JX233444, –; *P. crenulata* var. *ambigua*, USA, CA, San Bernardino Co., 12 Apr 1992, *K. Whitney 28* (SFSU), AY630279, –; ***Phacelia crenulata*** Torr. ex S. Watson var. ***angustifolia*** N. D. Atwood, USA, AZ, Coconino Co., 10 May 2005, *L. M. Garrison 55* (SFSU), JX233446, –; ***Phacelia crenulata*** Torr. ex S. Watson var. ***crenulata***, USA, CA, Inyo Co., 23 Mar 2004, *L. M. Garrison 05* (SFSU), JX233447, –; *P. crenulata* var. *crenulata*, USA, CA, Inyo Co., 24 Mar 2004, *L. M. Garrison 06* (SFSU), JX233448, –; *P. crenulata* var. *crenulata*, USA, AZ, Pinal Co., 6 May 2005, *L. M. Garrison 44* (SFSU), FJ814630, JQ249959; *P. crenulata* var. *crenulata*, USA, CA, San Bernardino Co., 6 May 2005, *L. M. Garrison 46* (SFSU), JX233450, –; *P. crenulata* var. *crenulata*, USA, CA, Inyo Co., 8 May 2005, *L. M. Garrison 48* (SFSU), JX233451, –; ***Phacelia cryptantha*** Greene, USA, CA, Riverside Co., 29 Mar 2004, *L. M. Garrison 20* (SFSU), JX233453, –; ***Phacelia cumingii*** (Benth.) A. Gray, CHILE, *L. Constance 3502* (CAS, n.v.), AY630282, –; *P. cumingii*, CHILE, *Werdeman 1042* (CAS, n.v.), AY630283, –; ***Phacelia curvipes*** Torr. ex S. Watson, USA, CA, Mono Co., 10 Jun 2008, *G. K. Walden 55* (SFSU), –, JQ249961; ***Phacelia davidsonii*** A. Gray, USA, CA, Orange Co., 26 Jun 2008, *G. K. Walden 72* (SFSU), –, JQ249962; ***Phacelia demissa*** A. Gray, USA, NM, San Doval Co., 1 Jun 2004, *N. D. Atwood and A. Clifford 30342* (DAV175809), –, JQ249963; ***Phacelia distans*** Benth. ***sensu* lato***, USA, CA, Kern Co., 22 Mar 2004, *L. M. Garrison 03* (SFSU), FJ814654, – (*cited as *P. tanacetifolia* in Hansen et al. 2009); *P. distans* s.l., USA, AZ, Pima Co., *L. M. Garrison 14* (SFSU), JX233454, –; *P. distans* s.l., USA, CA, San Bernardino Co., 29 Mar 2004, *L. M. Garrison 21* (SFSU), JX233478, JQ250026; *P. distans* s.l., USA, CA, Los Angeles Co., 6 May 2004, *L. M. Garrison 25* (SFSU), JX233479, –; *P. distans* s.l., USA, AZ, Pima Co., 22 Mar 2005, *L. M. Garrison 34* (SFSU), JX233455, –; *P. distans* s.l.*, USA, CA, San Diego Co., n.d., *R. Peters 01* (SFSU), AY630280, – (*cited as *P. crenulata* var. *minutifolia* in Gilbert et al. 2005); *P. distans* s.l.,, USA, CA: Marin Co., 12 May 1991, *P. Wharton 24* (SFSU), AY630284, –; ***Phacelia divaricata*** A. Gray, USA, CA, San Mateo Co., 23 Apr 1997, *M. A. Hewlett 581mah* (SFSU3414), AY630285, –; *P. divaricata*, USA, CA, Colusa Co., 30 May 2008, *G. K. Walden 35* (SFSU), –, JQ249964; ***Phacelia douglasii*** (Benth.) Torr., USA, CA, Monterey Co., April 1969, *L. S. Rose 69018* (SFSU), AY630286, –; ***Phacelia dubia*** (L.) Trel. & Small, n.d., direct submission to GenBank, *P. Glass s.n.* (TENN, n.v.), JN619425, –; ***Phacelia egena*** (Brand) J. T. Howell, USA, CA, Tehama Co., 30 Mar 1980, *M. A. Showers 1679* (SFSU08308), AY630287, –; ***Phaceliaeisenii*** Brandegee, USA, CA, Sierra Co., 11 Jun 2008, *D. Kruse-*

*Pickler 21* (SFSU),–, JQ249965; ***Phacelia formosula*** Osterh., USA, CO, Jackson Co., 11 Aug 1973, *N. D. Atwood and L. C. Higgins 5830* (DAV67851), –, JQ249966; ***Phacelia franklinii*** (R. Br.) A. Gray, CANADA, Saskatchewan, Saskatoon, 11 Jul 1973, *V. L. Harms 20025* (GH00288820), –, AF047772; ***Phacelia fremontii*** Torr., USA, NV, Nye Co., 15 Apr 1992, *J. Dempcy 101** (SFSU), AY630289, – (*cited as *J. Dempcy 101-1* in Gilbert et al. 2005); *P. fremontii*, USA, CA, San Bernardino Co., 17 Apr 1992, *J. Dempcy 102** (SFSU), AY630288, – (*cited as *J. Dempcy 102-2* in Gilbert et al. 2005); *P. fremontii* Torr., USA, CA, Inyo Co., 9 May 1992, *J. Dempcy 105** (SFSU), AY630290, – (*cited as *J. Dempcy 105-13* in Gilbert et al. 2005); ***Phacelia glaberrima*** (Torr.) J. T. Howell, USA, NV, Pershing Co., n.d., *A. Tiehm 11666* (OSC, n.v.), AY630291, –; ***Phacelia glandulifera*** Piper, USA, OR, Lake Co., 15 Jun 1993, *J. Dempcy 119-2* (SFSU), –, JQ249968; *P. glandulifera*, USA, OR, 15 Jun 1993, *J. Dempcy 119-3* (SFSU), AY630293, –; *P. glandulifera*, USA, OR, Harney Co., 16 Jun 1993, *J. Dempcy 120** (SFSU), AY630294, – (*cited as *J. Dempcy 120-2* in Gilbert et al. 2005); *P. glandulifera*, USA, OR, Harney Co., n.d., *A. Tiehm 11063* (CAS, n.v.), AY630292, –; ***Phacelia glandulosa*** Nutt., USA, CO, Mineral Co., 1984, *Weber and Randolph 17444* (TEX, n.v.), JX233456, –; ***Phacelia glechomifolia*** A. Gray, USA, AZ, Mohave Co., 12 May 2004, *N. D. Atwood 30196* (DAV174936), –, JQ249969; ***Phacelia grandiflora*** (Benth.) A. Gray, USA, CA, Orange Co., 2008, *B. Allen s.n.* (SFSU), –, JQ249970; *P. grandiflora*, USA, CA, Los Angeles Co., 23 Jun 1993, *D. M. Ferguson 26* (GH), AF091190, AF047818; *P. grandiflora*, USA, CA, San Diego Co., 7 May 2004, *D. R. Hansen 23* (SFSU), FJ814648, JQ249971; *P. grandiflora*, USA, CA, Los Angeles Co., 8 May 2004, *D. R. Hansen 29* (SFSU), FJ814649, JQ249972; ***Phacelia greenei*** J. T. Howell, USA, CA, Siskiyou Co., 21 Jun 2005, *R. Patterson and S. Santos 1981* (SFSU), –, JQ249973; ***Phacelia grisea*** A. Gray, USA, CA, Monterey Co., 3 Jun 2009, *G. K. Walden 151* (SFSU), –, JQ249974; ***Phacelia gymnoclada*** Torr. ex S. Watson, USA, NV, Washoe Co., 18 May 1993, *J. Dempcy 115-1* (SFSU), AY630295, –; *P. gymnoclada*, USA, NV, Nye Co., 9 Jun 1995, *J. Dempcy 136-1* (SFSU), AY630297, –; *P. gymnoclada*, USA, NV, Esmeraldo Co., n.d., *Holmgren 11352* (CAS, n.v.), AY630296, –; *P. gymnoclada*, USA, OR, Malheur Co., 26 May 1995, *P. F. Zika 12351* (GH, n.v.), AF091191, AF047793; ***Phacelia gypsogenia*** I. M. Johnst., MÉXICO, Nuevo Leon, 1992, *Aramberri 00248757* (TEX, n.v.), JX233457, –; ***Phacelia hastata*** Douglas ex Lehm. var. ***compacta*** (Brand) Cronquist, USA, NV, Humboldt Co., 5 Jun 2008, *A. Tiehm 15577* (SFSU), –, JQ249975; ***Phacelia heterophylla*** Pursh., USA, OR, Harney Co., 10 Jun 1996, *D. M. Ferguson 126* (GH), AF091192, AF047805; ***Phacelia heterophylla*** Pursh var. ***virgata*** (Greene) R. D. Dorn, USA, CA, Modoc Co., 12 Jun 2008, *F. Gauna s.n.* (SFSU), –, JQ249976; *P. heterophylla* var. *virgata*, USA, OR, 2008, *R. R. Halse 7464* (SFSU), –, JQ249978; *P. heterophylla* var. *virgata*, USA, NV, Douglas Co., 29 May 2008, *A. Tiehm 15548* (SFSU), –, JQ249977; ***Phacelia hirsuta*** Nutt., USA, AR, Montgomery Co., 10 May 1991, *D. E. Boufford, V. M. Bates and E. W. Wood 25539* (A), AF091193, AF047777; ***Phacelia howelliana*** N. D. Atwood, USA, UT, Grand Co., 11 May 2005, *L. M. Garrison 59* (SFSU), JX233458, JQ249979; ***Phacelia hubbyi*** (J. F. Macbr.) L. M. Garrison, USA, CA, Ventura Co., 30 Mar 2004, *L. M. Garrison 23* (SFSU), –, JQ249980; ***Phacelia humilis*** Torr. & A. Gray, USA, CA, Mono Co., Jun 1993, *D. M. Ferguson 21* (GH), AF091194, AF047817; *P. humilis*, USA, CA, Sierra Co., 10 Jun 2008, *D. Kruse-Pickler 20* (SFSU), –, JQ249981; *P. humilis*, USA, CA, Sierra Co., 4 Jul 1982, *R. Patterson 1795* (SFSU13836), AY630302, –; ***Phacelia hydrophylloides*** Torr. ex A. Gray, USA, CA, Sierra Co., Jun 2008, *P. Hankamp s.n.* (SFSU), –, JQ249982; *P. hydrophylloides*, USA, CA, Sierra Co., *J. Shevock 5140* (CAS, n.v.), AY630304, –; ***Phacelia imbricata*** Greene var. ***imbricata***, USA, CA, Lake Co., 9 Jun 1974, *D. Toren 3582* (SFSU08372), AY630305, –; ***Phacelia infundibuliformis*** Torr., USA, TX, Presidio Co., 24 Mar 2005, *L. M. Garrison 40* (SFSU), JX233436, –; ***Phacelia insularis*** Munz var. ***insularis***, USA, CA, Santa Barbara Co., 2005, *D. R. Hansen 55* (SFSU), FJ814627, JQ249937; ***Phacelia integrifolia*** Torr. var. ***integrifolia***, USA, NM, Eddy Co., 24 Mar 2005, *L. M. Garrison 37* (SFSU), JX233460, –; *P. integrifolia* var. *integrifolia*, USA, TX, Culberson Co., 24 Mar 2005, *L. M. Garrison 38* (SFSU), JX233461, –; *P. integrifolia* var. *integrifolia*, USA, NM, Dona Ana Co., 25 Mar 2005, *L. M. Garrison 42* (SFSU), JX233462, –; *P. integrifolia* var. *integrifolia**, USA, TX, Mason Co., 2004, *J. McDill 2004-20* (SFSU), JX233459, – (*cited as *P. robusta* in Garrison 2007); ***Phacelia integrifolia*** Torr. var. ***texana*** (J. W. Voss) N. D. Atwood, USA, TX, Reeves Co., n.d., – (TEX-00300216, n.v.), JX233463, –; ***Phacelia inyoensis*** (J. F. Macbr.) J. T. Howell, USA, CA, Inyo Co., 10 Apr 1995, *M. DeDecker 6444* (RSA627326, n.v.), AY630307, –; ***Phacelia inundata*** J. T. Howell, USA, CA, Lassen Co., 13 May 1993, *B. Bartholomew, M. Gilbert, and L. Skog 6559* (CAS889055, n.v.), AY630306, –; *P. inundata*, USA, NV, Washoe Co., 2008, *A. Tiehm 15553* (SFSU), –, JQ249983; ***Phacelia ivesiana*** Torr. var. ***pediculoides*** J. T. Howell, USA, CA, San Bernardino Co., 10 May 1978, *B. A. Prigge and R. F. Thorne 2952** (RSA278650, n.v.), AY630308, – (*cited as *Priggle 2952*in Gilbert et al. 2005); *P. ivesiana* var. *pediculoides*, USA, CA, San Bernardino Co., Mar 2008, *G. K. Walden 22* (SFSU), FJ814623, JQ249984; ***Phacelia keckii*** Munz & I. M. Johnst., USA, CA, Orange Co., 26 Jun 2008, *G. K. Walden 71* (SFSU), –, JQ249985; ***Phacelia laxiflora*** J. T. Howell, USA, AZ, Coconino Co., 23 May 1950, *J. T. Howell 26440* (DAV19059), –, JQ249986; ***Phacelia leibergii*** Brand, USA, OR, Deschutes Co., 6 Jul 1991, *J. Dempcy 100-2* (SFSU), AY630311*, – (*sequence identical to AY630312); *P. leibergii*, USA, OR, Deschutes Co., 16 Jun 1993, *J. Dempcy 121** (SFSU), AY630312*, JQ249987 (*cited as *J. Dempcy 121-2* in Gilbert et al 2005, *sequence identical to AY630311); ***Phacelia linearis*** (Pursh) Holz., USA, OR, Lake Co., 9 Jun 1996, *D. M. Ferguson 123* (WTU, n.v.), AF091195, AF047806; *P. linearis*, USA, CA, Modoc Co., 12 Jun 2008, *F. Gauna s.n.* (SFSU), FJ814629, JQ249988; *P. linearis*, USA, CA, Siskiyou Co., 21 Jun 2005, *R. Patterson and S. Santos 1978* (SFSU), –, JQ249989; ***Phacelia longipes*** Torr. ex A. Gray, USA, CA, Santa Barbara Co., 2005, *D. Hansen 57* (SFSU), FJ814645, –; *P. longipes*, USA, CA, Santa Barbara Co., 2005, *D. Hansen 59* (SFSU), FJ814641, –; ***Phacelia lutea*** (Hook. & Arn.) J. T. Howell, USA, OR, Lake Co., n.d., *D. M. Ferguson 122* (WTU, n.v.), AF091196, AF047807; ***Phacelia lutea*** (Hook. & Arn.) J. T. Howell var. ***lutea***, USA, NV, Washoe Co., n.d., *A. Tiehm 10617* (CAS, n.v.), AY630314, –; ***Phacelia lutea*** (Hook. & Arn.) J. T. Howell var. ***calva*** Cronquist, USA, NV, Humboldt Co., n.d., *A. Tiehm 12085* (CAS, n.v.), AY630313, –; ***Phacelia mammarillensis*** N. D. Atwood, USA, UT, Kane Co., 11 May 2005, *L. M. Garrison 63* (SFSU), JX233464, –; ***Phacelia marshal-johnstonii*** N. D. Atwood & D. J. Pinkava, MÉXICO, 1973, – (LL00248820, n.v.), JX233465, –; ***Phacelia minor*** (Harvey) Thell. ex F. Zimm., USA, CA, Los Angeles Co., 22 Jun 1993, *D. M. Ferguson 24* (GH), AF091197, AF047802; *P. minor*, USA, CA, San Bernardino Co., 2004, *D. R. Hansen 14* (SFSU), FJ814633*, JQ249990 (*identical sequence to FJ814632, FJ814634); *P. minor*, USA, CA, Los Angeles Co., 2004, *D. R. Hansen 15* (SFSU), FJ814632*, JQ249991 (*identical sequence to FJ814633, FJ814634); ***Phacelia minor*** × ***Phacelia parryi*** (putative $F_1$ hybrid, maternal and paternal identity unknown), USA, CA: Los Angeles Co., 2005, *D. R. Hansen 30* (SFSU), FJ814634*, JQ249992 (*identical sequence to FJ814632, FJ814633); *P. minor* × *P. parryi* (putative $F_1$ hybrid, maternal and paternal identity unknown), USA, CA: San Diego Co., 17 Apr 2003, *J. P. Rebman and J. Gregory 8526* (SD159431, n.v.), FJ814631, –; ***Phacelia monoensis*** Halse, USA, NV, Lyon Co., 19 May 1993, *J. Dempcy 116-1** (SFSU), AY630315, – (duplicate collection of *Dempcy 116-2*); *P. monoensis*, USA, NV, Lyon Co., 19 May 1993, *J. Dempcy 116-2** (SFSU), AY630316, – (*duplicate collection of *Dempcy 116-1*); *P. monoensis*, USA, CA, Sierra Co., 10 Jun 2008, *D. Kruse-Pickler 16* (SFSU), –, JQ249993; *P. monoensis*, USA, NV, *A. Tiehm 15516* (SFSU), –, JQ249938; ***Phacelia mutabilis*** Greene, USA, CA, Mono Co., 11 Jun 2008, *G. K. Walden 63* (SFSU), –, JQ249994; ***Phacelia nashiana*** Jeps., USA, CA, Kern Co., 19 Mar 2003, *D. R. Hansen 04* (SFSU), FJ814637, JQ249995; *P. nashiana*, USA, CA, Kern Co., 13 Mar 2004, *D. R. Hansen 13* (SFSU), FJ814638, JQ249996; *P. nashiana*, USA, CA, Kern Co., 2005, *D. R. Hansen 41* (SFSU), FJ814639, –; *P. nashiana*, USA, CA, Kern Co., 26 Mar 2008, *G. K. Walden 28* (SFSU), –, JQ249997; ***Phacelia neglecta*** M. E. Jones, USA, CA, Inyo Co., n.d., *Castagnoli et al. 124* (CAS, n.v.), AY630319, –; *P. neglecta*, USA, CA, San Bernardino Co., 2 Apr 1978, *J. Hendrickson 16473* (RSA278404, n.v.), AY630318, –; *P. neglecta*, USA, CA, Riverside Co., 4 Apr 1992, *A. C. Sanders and E. J. Lawlor 12090* (RSA554652, n.v.), AY630317, –; ***Phacelia nemoralis*** Greene var. ***nemoralis***, USA, CA, Marin Co., n.d., *H. Leschke s.n.* (SFSU), AY630320, –; ***Phacelia nemoralis*** Greene var. ***oregonensis*** (Heckard) Walden & R. Patt., USA, OR, Lane Co., 29 Jul 2008, *R. R. Halse 7486* (SFSU), –, JQ249998; ***Phacelia neomexicana*** Thurb. ex Torr., USA, NM, 1997, –, (TEX00255260, n.v.), JX233466, –; ***Phacelia novenmillensis*** Munz, USA, CA, Kern Co., 26 May 1987, *B. Ertter, J. Shevock, and T. Sholars 7005* (DAV112895), –, JQ249999; ***Phacelia pachyphylla*** A. Gray, USA, CA, San Bernardino Co., 2 Apr 1995, *J. Dempcy 130-1** (SFSU), AY630323, – (*duplicate collection of *Dempcy 130-2, 130-3*); *P. pachyphylla*, USA, CA, San Bernardino Co., 2 Apr 1995, *J. Dempcy 130-2** (SFSU), AY630324, – (*duplicate collection of *Dempcy 130-1, 130-3*); *P. pachyphylla*, USA, CA, San Bernardino Co., 2 Apr 1995, *J. Dempcy 130-3** (SFSU), –, JQ250000 (*duplicate collection of *Dempcy 130-1, 130-2*); *P. pachyphylla*, USA, CA, Kern Co., 26 May 1977, *R. Gustavson 487* (RSA428130, n.v.), AY630321, –; *P. pachyphylla*, USA, CA, Kern Co., *Sanders 227* (CAS, n.v.), AY630322, –; ***Phacelia palmeri*** Torr. ex S. Watson, USA, UT, Washington Co., 9 May 2005, *L. M. Garrison 51* (SFSU), JX233467, –; ***Phacelia parryi*** Torr., USA, CA, Orange Co., 2008, *B. Allen s.n.* (SFSU), –, JQ250001; *P. parryi*, USA, CA, San Diego Co., 8 Apr 1990, *J. Dempcy 03* (SFSU), AY653742, –; *P. parryi*, USA, CA, San Diego Co., 7 May 2004, *D. R. Hansen 24* (SFSU), FJ814635, –; *P. parryi*, USA, CA, San Diego Co., 7 May 2004, *D. R. Hansen 26* (SFSU), FJ814636, JQ250002; *P. parryi*, USA, CA, San Diego Co., 03 Apr 2008, *J. P. Rebman 14277 and M. Wall* (SD184376, n.v.), –, JQ250003; ***Phacelia patuliflora*** (Engelm. & A. Gray) A. Gray, USA, TX, Hidalgo Co., 21 Feb 1992, *R. R. Halse 4425* (GH, n.v.), AF091198,AF047781; *P. patuliflora*, USA, TX, n.d., direct submission to GenBank (WTU, n.v.), –, AF130179; ***Phacelia pauciflora*** S. Watson, MÉXICO, Baja California Norte, 26 Mar 2009, *G. K. Walden 128* (SFSU), –, JQ250005; *P. pauciflora*, MÉXICO, Baja California Norte, 29 Mar 1989, *G. L. Webster 26155* (DAV133615), –, JQ250004; ***Phacelia pedicellata*** A. Gray, USA, CA, Inyo Co., 25 Mar 2004, *L. M. Garrison 07* (SFSU), JX233468, –; *P. pedicellata*, USA, AZ, Pima Co., 27 Mar 2004, *L. M. Garrison 13* (SFSU), JX233469, –; *P. pedicellata*, MÉXICO, Baja California Norte, 26 Mar 2009, *G. K. Walden 127* (SFSU), –, JQ250006; ***Phacelia perityloides*** Coville, USA, CA, Inyo Co., 7 May 2005, *L. M. Garrison 47* (SFSU), JX233470, JQ250007; ***Phacelia petrosa*** N. D. Atwood, F.J. Sm. & T. A. Knight, USA, AZ, Mohave Co., 27 Apr 2004, *N. D. Atwood and L. C. Higgins 30142* (DAV175105), –, JQ250008; ***Phacelia popei*** Torr. & A. Gray, USA, NM, Eddy Co., 23 Mar 2005, *L. M. Garrison 36* (SFSU), JX233471, JQ250009; *P. popei*, USA, TX, Culberson Co., 24 Mar 2005, *L. M. Garrison 39* (SFSU), JX233472, –; ***Phacelia pringlei*** A. Gray, USA, CA, Siskiyou Co., 21 Jun 2005, *R. Patterson and S. Santos 1980* (SFSU), –, JQ250010; ***Phacelia procera*** A. Gray, USA, CA, Sierra Co., Jun 2008, *P. Hankamp s.n.* (SFSU), –, JQ250011; *P. procera*, USA, CA, Shasta Co., 8 Jun 1969, *H. Thiers 23458* (SFSU08424), –, JQ250012; *P. procera*, USA, CA, Lake Co., 20 Jul 1975, *D. Toren 1986* (SFSU08423), AY630325, –; ***Phacelia pulchella*** A. Gray, USA, UT, Washington Co., 9 May 2005, *L. M. Garrison 52* (SFSU), JX233473, JQ250013; ***Phacelia purpusii*** Brandegee, USA, CA, El Dorado Co., 12 Jun 1990, *Barron s.n.* (DAV152217), –, JQ250014; ***Phacelia rafaelensis*** N. D. Atwood, USA, AZ, Coconino Co., 10 May 2005, *L. M. Garrison 54* (SFSU), JX233474, JQ250015; ***Phacelia ramosissima*** Douglas ex Lehm., USA, CA, Mono Co., 18 Jun 1993, *D. M. Ferguson 10* (GH), AF091199, AF047821; ***Phacelia ramosissima*** Douglas ex Lehm. var. ***austrolitoralis*** Munz, USA, CA, Santa Barbara Co., 24 Jun 2008, *G. K. Walden 67* (SFSU), –, JQ250016; ***Phacelia ramosissima*** Douglas ex Lehm. var. ***eremophila*** (Greene) J. F. Macbr., USA, CA, Mono Co., n.d., *H. D. Thiers 17121* (SFSU), AY630327, –; *P. ramosissima* var. *eremophila*, USA, CA, Mono Co., 11 Aug 2008, *G. K. Walden 83* (SFSU), –, JQ250024; ***Phacelia ramosissima*** Douglas ex Lehm. var. ***latifolia*** (Torr.) Cronquist, USA, CA, Orange Co., 26 Jun 2008, *G. K. Walden 76* (SFSU), –, JQ250017; ***Phacelia ramosissima*** Douglas ex Lehm. var. ***subglabra*** M. Peck, USA, CA, Inyo Co., 13 Jun 2009, *G. K. Walden 200a* (SFSU), –, JQ250018; ***Phacelia rotundifolia*** Torr. ex S. Watson, USA, CA, San Bernardino Co., 9 Apr 1993, *C. Condos 09* (SFSU), AY630328, –; *P. rotundifolia*, USA, CA, Inyo Co., 18 Mar 1986, *J. Morefield and McCarty 3274* (GH), AF091200, AF047779; *P. rotundifolia*, USA, CA, Inyo

Co., 17 Apr 1973, *B. Trowbridge 3180* (SFSU08433), –, JQ250019; ***Phacelia rupestris*** Greene, USA, TX, Jeff Davis Co., 31 Aug 1997, *W. R. Carr 16928* (TEX00041271, n.v.), JX233475, –; ***Phacelia salina*** (A. Nelson) J. T. Howell, USA, WY: Sweetwater Co., n.d., *B. E. Nelson 36344* (RM, n.v.), AY630329, –; ***Phacelia scariosa*** Brandegee, MÉXICO, Baja California Sur, Sierra de Guadalupe, Mulegé, *Miguel Dominguez Leon 3274* (SDNHM, n.v.), JX233476, JQ250020; ***Phacelia scopulina*** (A. Nelson) J. T. Howell, USA, NV, Elko Co., n.d., *A. Tiehm 10573* (OSC, n.v.), AY630330, –; ***Phacelia sericea*** (Graham) A. Gray, USA, UT, Grand Co., 22 Aug 1985, *B. Franklin 2301* (GH), AF091201, AF047778; *P. sericea*, USA, CA, Modoc Co., n.d., *M. A. Showers s.n.* (SFSU), AY630331, –; ***Phacelia splendens*** Eastwood, USA, UT, Grand Co., 1997, *N. D. Atwood and S. Welsh 22060* (TEX, n.v.), JX233477, JQ250021; ***Phacelia stebbinsii*** Constance & Heckard, USA, CA, Placer Co., 8 Jul 1977, *G. L. Stebbins 7761* (DAV79856), –, JQ250022; ***Phacelia stellaris*** Brand, USA, CA, San Diego Co., Mar 2008, *C. Burrascano s.n.* (SFSU), –, JQ250023; ***Phacelia suaveolens*** Greene, USA, CA, Santa Cruz Co., 10 Jul 2009, *V. T. Parker s.n.* (SFSU), –, JQ250025; ***Phacelia tetramera*** J. T. Howell, USA, NV, Humboldt Co., n.d., *A. Tiehm 12133* (CAS, n.v.), AY630333, –; *P. tetramera*, USA, NV, Humboldt Co., 11 Jun 2008, *A. Tiehm 15641* (SFSU), –, JQ250027; ***Phacelia thermalis*** Greene, USA, OR, Lake Co., 10 Jun 1996, *D. M. Ferguson 125* (GH), AF091202, AF047795; ***Phacelia vallis-mortae*** J. W. Voss, USA, CA, Inyo Co., 23 Mar 2004, *L. M. Garrison 04* (SFSU), –, JQ250028; ***Phacelia viscida*** (Benth.) Torr. var. ***albiflora*** A. Gray, USA, CA: Santa Barbara Co., 2005, *D. R. Hansen 50* (SFSU), FJ814653, –; *P. viscida* var. *albiflora*, USA, CA, Santa Barbara Co., Channel Islands, 28 Mar 2005, *D. R. Hansen 54* (SFSU), FJ814650, JQ250031; ***Phacelia viscida*** (Benth.) Torr. var. ***viscida***, USA, CA, Ventura Co., 9 May 2004, *D. R. Hansen 28* (SFSU), FJ814651*, JQ250029 (*sequence identical to FJ814652); *P. viscida* var. *viscida*, USA, CA, Santa Barbara Co., 2005, *D. R. Hansen 56* (SFSU), FJ814652*, JQ250030 (*sequence identical to FJ814651); ***Phacelia vossii*** N. D. Atwood, MÉXICO, Nuevo Leon, 1990, – (TEX00224027, n.v.), JX233480, JQ250032; ***Phacelia welshii*** N. D. Atwood, USA, AZ, Coconino Co., 10 May 2005, *L. M. Garrison 56* (SFSU), JX233481, –; ***Romanzoffia californica*** Greene, USA, CA, *Romanzoffia californica*, n.d., *D. M. Ferguson 128* (GH, n.v.), AF091205, AF047804; ***Romanzoffia thompsonii*** Marttala, *D. M. Ferguson 134* (GH, n.v.), AF091206, AF047784; ***Tricardia watsonii*** Torr. ex S. Watson, Inyo Co., 10 Apr 1986, *J. Morefield & McCarty 3375* (GH, n.v.), AF091209, AF047775.

## Appendix 2

List of sequences excluded from analyses.

*Voucher specimens missing.* The herbarium at SFSU was renovated as part of a seismic retrofit of Hensill Hall (2000-2005). The vascular plant collection was stored offsite during a portion of the renovation, prior to installation into the current facility (H.D. Thiers Herbarium, SFSU). Some voucher collections previously cited in the literature have not been located in the current collections (including unmounted research material) at SFSU or located in personal collections of the researchers. These vouchers are presumed lost, missing, or destroyed, and GenBank sequences derived from those vouchers were excluded from this analysis and listed here: ***Phacelia adenophora*** J. T. Howell, USA, CA, Lassen Co., *J. Dempcy 116* (SFSU), AY630259 (nrITS); ***Phacelia bolanderi*** A. Gray, USA, CA, Del Norte Co., *C. Gilbert 54* (SFSU), AY630270 (nrITS); ***Phacelia humilis*** Torr. & A. Gray, USA, CA, Sonoma Co., *C. Gilbert 3A* (SFSU), AY630301 (nrITS) (*P. humilis* as currently circumscribed is not known to occur in Sonoma Co., and without the voucher specimen it is impossible to speculate on the putative identity of this specimen); ***Phacelia hydrophylloides*** Torr. ex A. Gray, USA, CA, Sierra Co., *J. Dempcy 126* (SFSU), AY630303 (nrITS); *P. ivesiana* Torr., USA, CA, San Bernardino Co., *J. Dempcy 8* (SFSU), AY630309 (nrITS); ***Phacelia leibergii*** Brand, USA, OR, Deschutes Co., *J. Dempcy 99-2* (SFSU), AY630310 (nrITS) (although *J. Dempcy 99-2* was not located, a duplicate sheet is deposited at SFSU as *J. Dempcy 99-1*).

*Voucher specimens destroyed.* The following voucher specimens previously cited in studies were consumed entirely during genomic extraction. No duplicate voucher material is deposited at SFSU for corroboration, and GenBank sequences derived from these vouchers were excluded from analyses and listed here: ***Phacelia rotundifolia*** Torr. ex S. Watson, USA, CA, Inyo Co., 2005, *L. M. Garrison and D. Hansen* ROT (SFSU), FJ814628 (nrITS), FJ814681 (*rpl16* intron). ***Romanzoffia californica***, USA, CA, in private cultivation in San Mateo Co., horticultural material from Annie's Annuals (Richmond, Contra Costa Co., CA), *R. Patterson s.n.*, no voucher, harvested for genomic extraction in 2008, FJ814619 (nrITS), FJ814691 (*rpl16* intron).

*Voucher specimens deposited at SFSU without label information.* Voucher specimens previously cited in studies were located at SFSU as unmounted research material without label information. GenBank sequences derived from these vouchers were also excluded from these analyses and listed here: ***Phacelia hastata*** Douglas ex Lehm., USA, CA, Inyo Co., *C. Gilbert 106* (SFSU), AY630298 (nrITS); *P. hastata* Douglas ex Lehm., USA, CA, Mono Co., *C. Gilbert 109* (SFSU), AY630299 (nrITS); ***Phacelia heterophylla*** Pursh var. ***virgata*** (Greene) R. D. Dorn, USA, CA, Alpine Co., *C. Gilbert 101* (SFSU), AY630300 (nrITS); ***Phacelia ramosissima*** Douglas ex Lehm., USA, CA, Alpine Co., *Gilbert 100* (SFSU), AY630326 (nrITS); ***Phacelia vallis-mortae*** J. W. Voss, USA, CA, Inyo Co., *C. Gilbert 108* (SFSU), AY630332 (nrITS) (cited as *P. tanacetifolia* Benth. in Gilbert et al. 2005).