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Phylogenetic Systematics of the Mesa Mints: *Pogogyne* (Lamiaceae)

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Abstract*—Pogogyne* (Lamiaceae) is a small genus of annual plants, occurring almost entirely in vernal pool habitats. To infer the phylogenetic relationships of this group, DNA sequence data from the *trnQ-rps16* chloroplast spacer and both ETS and ITS nuclear regions were obtained from all seven extant species and several outgroups and analyzed using both parsimony and Bayesian phylogeny reconstruction methods. Ancestral state reconstructions were performed for morphological characters that have been used to separate groups within *Pogogyne*. This study indicates that subgenus *Hedeomoides* is monophyletic with good support and can be diagnosed by two apomorphies: a reduction in stamen number and a reduced corolla size. Subgenus *Pogogyne* is paraphyletic, but with poor support. Several clades within the genus *Pogogyne* have discrete biogeographic distributions. The *Pogogyne* genus clade has a relatively long stem lineage, but its crown clade has quite short branches. Our estimate for the mean divergence time of the stem node is 5.1–7.7 million years ago and for that of the crown node is 0.9–1.9 million years ago, the latter overlapping with ages of vernal pool ecosystems. These results support the hypothesis that the members of *Pogogyne* underwent a rapid diversification in response to specialization to a periodically inundated habitat.

Keywords*—Acanthomintha,* divergence, Mentheae, Menthinae, *Monardella*, vernal pool.

Pogogyne Benth. is a small genus of eight (seven extant) currently recognized species of the plant family Lamiaceae (Table 1). Members of the genus are commonly known as mesa mints, from their common occurrence in vernal pool habitats (see below), which are typically found on mesas. *Pogogyne* is classified in subfamily Nepetoideae, tribe Mentheae, subtribe Menthinae of the Lamiaceae (Walker and Sytsma 2006; Drew and Sytsma 2012). Tribe Mentheae consists of 67 genera (according to Drew and Sytsma 2012) and contains many economically important plants, such as rosemary (*Rosmarinus officinalis* L.), mint (*Mentha* species), catnip (*Nepeta cataria* L.), and sage (*Salvia* species). Members of the genus *Pogogyne,* like most members of the family, have zygomorphic, bilabiate flowers (Fig. 1A–E) but are distinguished from other genera in the tribe in being annuals with trichomes on the style (Fig. 1E) and on the fruit (Jokerst, 1993; however, see Discussion). Species of *Pogogyne* occur mostly in California, with some populations in Oregon (and possibly Idaho; see Meinke 2006), and in northern Baja California, Mexico (Fig. 2). Different species of *Pogogyne* have been diagnosed based on several morphological features, including stem diameter and habit, inflorescence shape (including length and width), calyx and calyx lobe length, calyx lobe shape and apical process, calyx vestiture, corolla length and relative exsertion from bracts and calyces, and fertile stamen number (Silveira et al. 2012).

Pogogyne is often divided into two infrageneric groups, subgenus *Pogogyne* [subgen. *Eupogogyne* J. T. Howell], and subgenus *Hedeomoides* A. Gray (Table 1). The two subgenera differ in the number of fertile stamens (four in subgen. *Pogogyne* versus two in subgen. *Hedeomoides*) and exsertion of the corolla relative to the inflorescence bracts (larger and exserted in subgen. *Pogogyne* versus small and included in subgen. *Hedeomoides*; see Jokerst, 1993; Silveira et al. 2012). Subgenus *Pogogyne* has four species: *P. abramsii* J. T. Howell, *P. clareana* J. T. Howell, *P. douglasii* Benth. (the type for the genus), and *P. nudiuscula* A. Gray. (See Fig. 2A–C for distributions.) Three of these are listed as California endangered species, and two of these three are also listed as federally endangered species (Table 1). Two additional species and several subspecies or varieties have been described in the past, all of which we treat as synonyms of *P. douglasii* (Silveira et al. 2012; Table 1). Subgenus *Hedeomoides* (also spelled *Hediomoides* in Howell 1931 and referred to as section *Hediomoides* in Jokerst 1992) has four species: *P. floribunda* Jokerst, *P. serpylloides* A. Gray, *P. tenuiflora* A. Gray, and *P. zizyphoroides* Bentham (Jokerst 1993; Silveira et al. 2012; see Fig. 2D–F for distributions). *Pogogyne serpylloides* is documented from numerous vouchers collected in central and northern California plus a single, historical collection (*Orcutt 1361*, 21 Apr 1886, UC 25599) from San Quintin, Baja California, Mexico (Fig. 2E). This species has not been found in Mexico since and is presumed extirpated there. *Pogogyne tenuiflora* is known solely from one collection (*E. Palmer 65,* 1875, GH 00001496) from Guadalupe Island, Baja California, Mexico. This species was listed by Watson (1875), who cited the collection notes as "very rare, among sagebrush, on the eastern side." *Pogogyne tenuiflora* has not been found since, and the species is presumed extinct. Subgenus *Hedeomoides* has been treated as genus *Hedeomoides* Briquet (Table 1), but this classification has not been recognized in recent treatments (Jokerst 1993; Silveira et al. 2012; see Silveira 2010 for full taxonomic history). Two possible new species of subgenus *Hedeomoides* from Oregon and northern Baja California have been suggested, but are still in the process of being investigated (Meinke 2006).

Most *Pogogyne* species are found in vernal pool habitats (Fig. 1F). A "vernal pool" is generally defined as a shallow depression subjected to both an inundated period and a dry period in a year. Vernal pools typically have a water-impermeable layer of substrate (e.g. a "hard pan," "clay pan," or basalt flow) just below the ground surface, allowing for accumulation and retention of precipitation during the wet season. Some definitions restrict the term vernal pool to only include those within a Mediterranean climate region on the American west coast (Zedler 2003). A broader term, ephemeral wetland, is sometimes used to include both vernal pools and similar environments, such as seasonally wet meadows and intermittent streams, which would generally encompass all *Pogogyne* habitats. Plants and animals of these habitats possess specific adaptations to survive a period of inundation, followed by a long dry period (De Meester et al. 2005). In the historical past, vernal pools were much

TABLE 1. Currently recognized subgenera and species of *Pogogyne*, with synonyms in brackets and environmental listing shown (from CNPS 2012). Primary environmental listing sources are: **CNPS** = California Native Plant Society Inventory Listing; **CA** = California Endangered Species Act (CESA) listing; **Fed** = Federal Endangered Species Act (FESA) listing; see CNPS 2012). Symbols: **1B** = Rare, threatened, or endangered in California and elsewhere; **4** = Limited distribution (Watch List); **.1** = Seriously endangered in California (over 80% of occurrences threatened/high degree and immediacy of threat); **.2** = Fairly endangered in California (20–80% occurrences threatened); **CBR** = Considered but rejected; **CE** = California endangered; **FE** = federally endangered **†** = Type for genus.

Taxon	CNPS	CA	Fed
Subgenus *Pogogyne* [*Eupogogyne* J. T. Howell, Proc. Calif. Acad. Sci. 20:105–128. 1931]			
P. abramsii J. T. Howell, Proc. Calif. Acad. Sci. 20: 119. 1931	**1B.1**	**CE**	**FE**
P. clareana J. T. Howell, Four Seasons 4: 22. 1973	**1B.2**	**CE**	
†*P. douglasii* Benth., Labiat. Gen. Spec. 414. 1834	**CBR**		
[*P. d.* subsp. *minor* J. T. Howell, Proc. Calif. Acad. Sci. 20: 116. 1931]			
[*P. d.* var. *multiflora* Briquet, Nat. Pflanz. [Engler & Prantl] 4, Abt. 3a: 304. 1896]			
[*P. d.* var. *parviflora* J. T. Howell, Proc. Calif. Acad. Sci. 20: 117. 1931]			
[*P. d.* subsp. *ramosa* J. T. Howell, Proc. Calif. Acad. Sci. 20: 116. 1931]			
[*P. d.* var. *tricolor* Regel, Gartenflora 21: 226. 1872]			
[*P. multiflora* Benth., Labiat. Gen. Spec. 414. 1834]			
[*P. parviflora* Benth., Labiat. Gen. Spec. 414. 1834]			
P. nudiuscula A. Gray, Bot. California [W.H.Brewer] 1: 597. 1876	**1B.1**	**CE**	**FE**
Subgenus *Hedeomoides* A. Gray, Proc. Amer. Acad. Arts 11: 100. 1876			
[*Hedeomoides* Briquet, Nat. Pflanz. [Engler & Prantl] iv. III A. 295. 1896]			
P. floribunda (Standley) Jokerst, Aliso 13(2): 347. 1992	**4.2**		
P. serpylloides (Torrey) A. Gray, Proc. Amer. Acad. Arts 7: 386. 1868	**CBR**		
[*Hedeoma serpylloides* Torrey(?), in Pacif. Rail. Rep. 4: 123. 1856]			
[*Hedeomoides s.* (Torrey) Briquet, Nat. Pflanz. [Engler & Prantl] 4, Abt. 3a: 295. 1896]			
[*P. s.* subsp. *intermedia* J. T.Howell, Proc. Calif. Acad. Sci. 20: 125. 1931]			
P. tenuiflora A. Gray, Proc. Amer. Acad. Arts 11: 100. 1876 (**PRESUMED EXTINCT**)			
[*Hedeomoides* (A. Gray) *t.* Briquet, Nat. Pflanz. [Engler & Prantl] 4, Abt. 3a: 295. 1896]			
P. zizyphoroides Benth., Pl. Hartw. 330. 1849			
[*Hedeomoides* (Benth.) *z.* Briquet, Nat. Pflanz. [Engler & Prantl] 4, Abt. 3a: 295. 1896]			

more common in the California Floristic Province, but only about 10% are thought to still exist today (Keeley and Zedler 1998). These unique lands are continuing to vanish at a rapid pace due to direct habitat destruction (particularly for human development) and other impacts, such as pollution and invasive species (De Meester et al. 2005; Holland and Keil 1995).

High-level phylogenetic studies that included *Pogogyne* have been done recently. Walker and Sytsma (2006), in a phylogenetic study of the tribe Mentheae, included one species of *Pogogyne* (*P. floribunda*) in their analysis. Their phylogeny placed this *Pogogyne* species as sister to *Acanthomintha lanceolata* Curran (bootstrap support value = 67); the clade containing these two genera was embedded in a polytomy of twelve other genera (Walker and Sytsma 2006). Drew and Sytsma (2011), in a more detailed analysis of the Mentheae, included a different species of *Pogogyne* (*P. douglasii*). In this analysis, *Pogogyne douglasii* was sister to a clade composed of *Acanthomintha lanceolata* and *Monardella villosa* Bentham, both clades being well-supported. Drew and Sytsma (2012) obtained similar results, with *P. douglasii* sister either to a clade of two *Acanthomintha* species [*A. duttonii* (Abrams) Jokerst and *A. lanceolata*] using ITS data alone, or to a clade consisting of these two *Acanthomintha* species plus *Monardella villosa*, using chloroplast DNA data alone. There are no previously published phylogenetic studies investigating relationships within the genus *Pogogyne*.

The primary objective of this study is to infer the phylogenetic relationships of the seven extant species of *Pogogyne* using ETS and ITS nuclear ribosomal DNA markers and the *trnQ-rps16* spacer region of chloroplast DNA. The resultant phylogenetic trees are used to test the monophyly of the two accepted subgenera of *Pogogyne*, to assess subgroupings within them, and to infer and evaluate aspects of character evolution, biogeographic history, species delimitation, and diversification times.

MATERIALS AND METHODS

Taxon Sampling—A total of 59 samples were collected in the field or obtained from vouchered herbarium specimens. Of these, 34 samples (of 16 verified species) yielded sequence data that could be used in our analysis (Appendix 1). At least two individuals of each of the seven, extant species of *Pogogyne* were included (Fig. 3). Six specimens of *P. douglasii* were included, from regions that corresponded to the range of infraspecies described in the past. In addition, one specimen of *Pogogyne* was included from each of the three field sites from a previous conservation study in Oregon (Meinke 2006), which were tentatively identified as *Pogogyne* aff. *serpylloides* (two populations) and *P.* aff. *zizyphoroides* (one population; see Appendix 1; Fig. 3). Also, one specimen from a possible new species of *Pogogyne* (labeled "*P.* sp.") from northern Baja California, Mexico, was included. In order to test the monophyly of *Pogogyne*, three species and samples of *Acanthomintha* [*A. lanceolata, A. ilicifolia* (A. Gray) A. Gray, and *A. obovata* Jepson], three of *Monardella* [*M. lanceolata* A. Gray, *M. macrantha* A. Gray, and *M. villosa* subsp. *franciscana* (Elmer) Jokerst], and one species and sample of the more distant *Hedeoma* [*H. nana* (Torrey) Briquet] were included. Finally, two species and samples of *Mentha* (*M. arvensis* L. and *M. pulegium* L.) were included as most distant outgroups to root the tree. All taxa studied are in tribe Mentheae of Drew and Sytsma (2011) and subtribe Menthinae of Drew and Sytsma (2012).

Extractions, Amplification, and Sequencing—DNA was extracted from leaf tissue using either a modified version of the CTAB protocol (Doyle and Doyle 1987) or DNeasy plant mini kits (Qiagen, Valencia, California). The manufacturer's protocol of the DNeasy plant mini protocol was followed with the exception of eluting with 50 l, instead of 100 l, of AE buffer in order to yield a higher concentration of DNA. Amplification was carried out in an Applied Biosystems 2720 Thermal Cycler (Life Technologies Corporation, Carlsbad, California). All reaction volumes were 25 l and consisted of 0.125 l TAQ polymerase, 1 l diluted DNA, 1.25 l forward primer, 1.25 l reverse primer, 1.25 l $MgCl_2$, 1.25 l DNTP, 2 l DMSO (8% of total reaction), 2.5 l Mg-free buffer, and 14.375 l H_20.

The ITS region of the nuclear ribosomal DNA was amplified using the plant specific primers, ITS5a and ITS241r, designed by Ken Wurdack

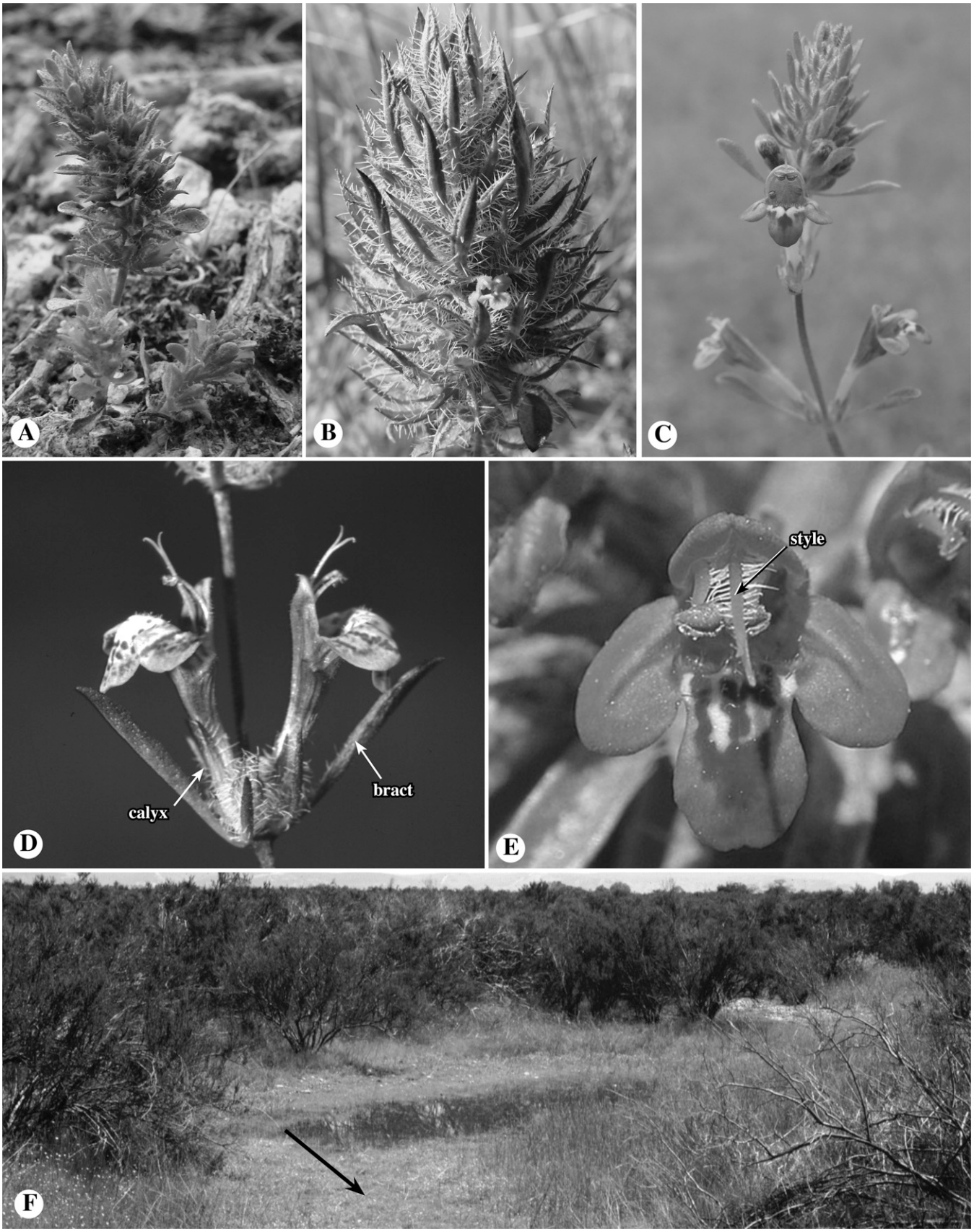

FIG. 1. Photographs of *Pogogyne* species and habitat. A–B. *Pogogyne* subgenus *Hedeomoides.* A. *Pogogyne serpylloides* inflorescence, showing flowers included within bracts and calyces. B. *Pogogyne zizyphoroides* inflorescence, also with included flowers. C–F. *Pogogyne* subgenus *Pogogyne.* C. *Pogogyne clareana,* having solitary flowers in axils of inflorescence bracts. D. *Pogogyne abramsii,* also with solitary flowers in bract axils. Note calyces and bracts. E. *Pogogyne nudiuscula,* showing two (of four) fertile stamens and pubescent style, the latter characteristic of genus. F. Vernal pool containing *P. abramsii* at periphery (arrow).

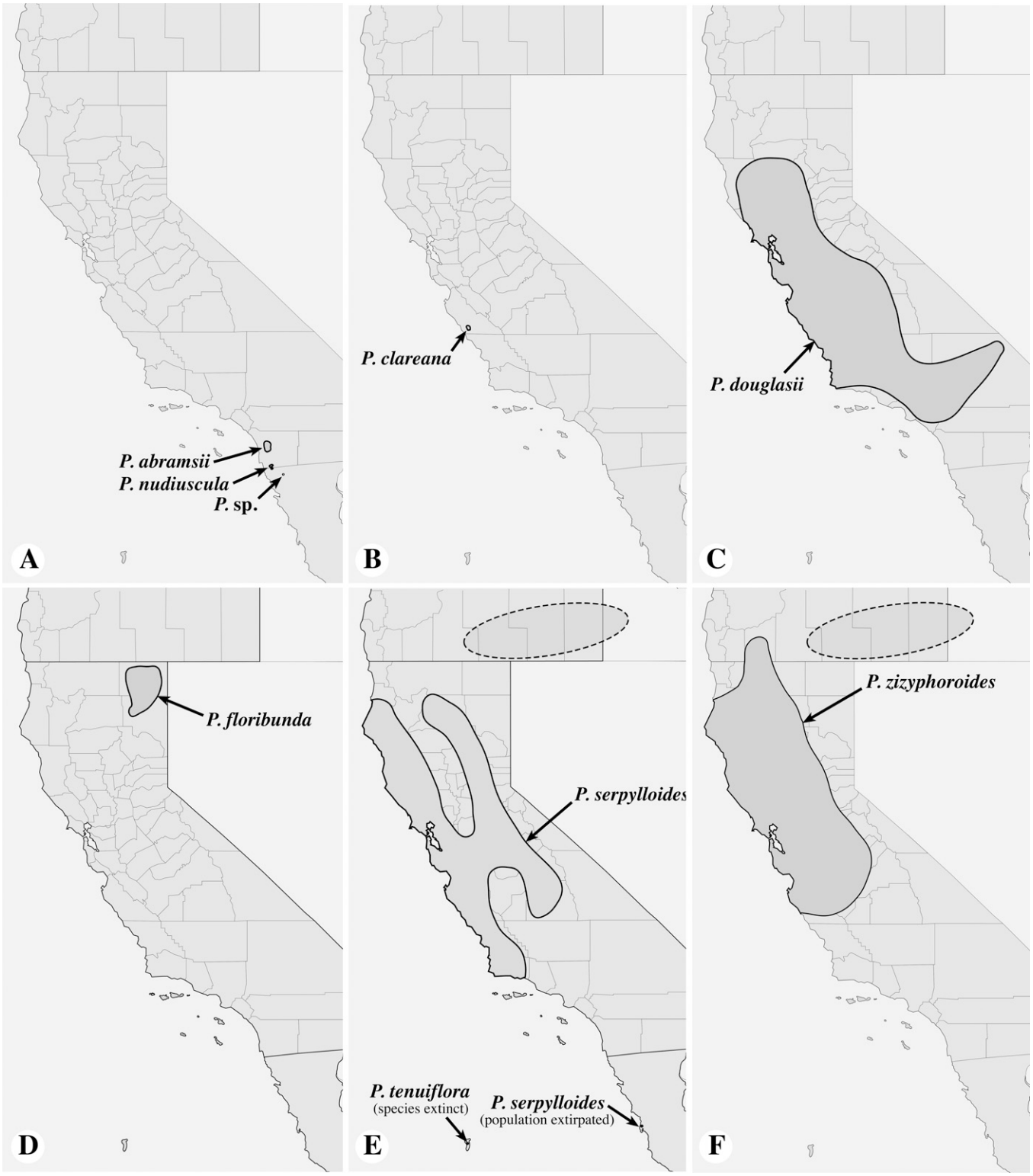

FIG. 2. Distribution range maps of the eight species of *Pogogyne*. A–C. Subgenus *Pogogyne*. A. *Pogogyne abramsii*, *P. nudiuscula*, and *Pogogyne* sp. B. *Pogogyne clareana*. C. *P. douglasii*. D–F. Subgenus *Hedeomoides*. D. *Pogogyne floribunda*. E. *Pogogyne serpylloides* and *P. tenuiflora* (presumed extinct). F. *Pogogyne zizyphoroides*. Range delimited by dashed line are *Pogogyne* taxa of uncertain identity (Meinke 2006), including "*P.* aff. *serpylloides*" and "*P.* aff. *zizyphoroides*" of this study.

of the Smithsonian (Prince and Kress 2006). While more widely-used ITS primers worked easily for taxa outside of the genus *Pogogyne*, they were of little or no utility within the genus. This gene region was amplified using the following polymerase chain reaction (PCR) conditions: 1) an initial heating step of 94˚C for 4 minutes; 2) 35 cycles of 94˚C for 45 sec, 58˚C for 45 sec, and 72˚C for 2 minutes; and 3) an extension of 72˚C for 6 minutes (Prince and Kress 2006).

The external transcribed spacer (ETS) region of the nuclear ribosomal DNA was amplified using the plant specific primer 18S-E, developed by Baldwin and Markos (1998). The other primer used was ETS-B, a 5′

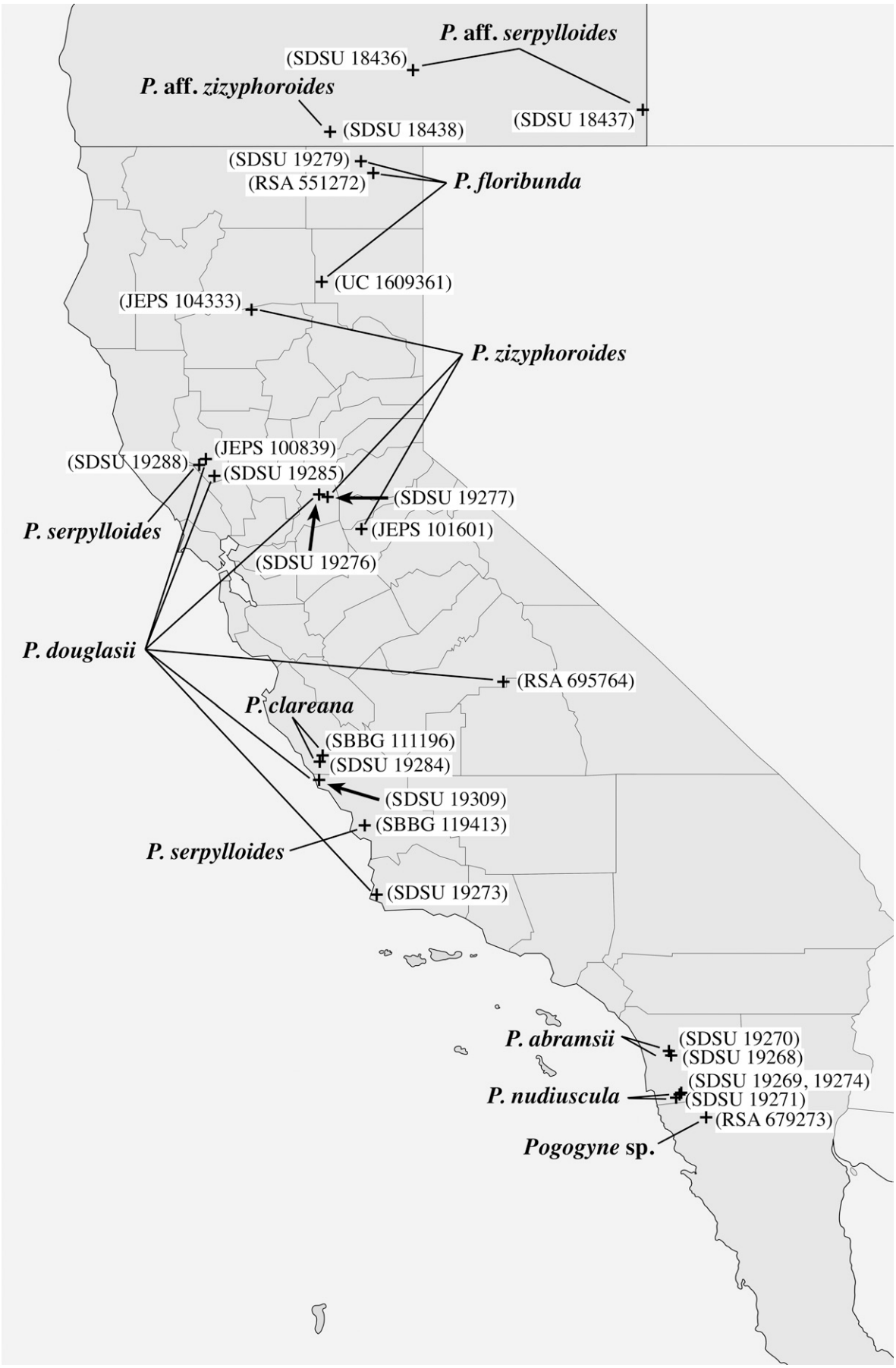

FIG. 3. Specific localities of samples of *Pogogyne* used in this analysis, with herbarium accession numbers (see Appendix 1) indicated.

primer used in Phrymaceae (Beardsley and Olmstead 2002). This gene region was amplified using the following PCR conditions: 1) an initial heating step of 94˚C for 5 minutes; 2) 35 cycles of 94˚C for 1 minute, 61˚C for 1 minute, and 72˚C for 1 minute; and 3) an extension of 72˚C for 5 minutes.

The *trnQ-rps16* spacer region of chloroplast DNA was amplified using primers *trn*$Q^{(UUG)}$ and *rps16*x1, cited by Shaw et al. (2007). This chloroplast spacer region was amplified using the following PCR conditions: 1) an initial heating step of 80˚C for 5 minutes, 2) 30 cycles of 95˚C for 1 minute, 50˚C for 1 minute, 53˚C for 12 sec, 56˚C for 12 sec, 59˚C for 12 sec, 62˚C for 12 sec, and 65˚C for 4 minutes; and 3) an extension of 65˚C for 5 minutes. The five annealing temperatures were used to simulate the annealing temperature ramping up to a temperature of 62˚C as previously published (Shaw et al. 2007).

All PCR products were visualized using agarose gel electrophoresis and successful ones were cleaned using QIAquick PCR purification kits (Qiagen, Valencia, California). In using the kit to purify PCR products, the microcentrifuge protocol was used with the exception of the final elution of the product being done with 30 l instead of 50 l in order to recover a higher concentration of product. Cleaned PCR products were cycle-sequenced on an ABI 377 automated sequencer (Applied Biosystems) at the San Diego State University MicroChemical Core Facility.

Sequences were aligned and edited using Sequencher v. 4.7 (Gene Codes, Ann Arbor, Michigan). Because of the similarity among sequences, a final alignment could be performed manually in MacClade 4.08 (Maddison and Maddison 2005). All sequences were uploaded to GenBank (Benson et al. 2010). Five data matrices were constructed: 1) ITS only, 2) ETS only, 3) *trnQ-rps16* only, 4) ITS and ETS, and 5) ITS, ETS, and *trnQ-rps16*. The final, aligned data were submitted to TreeBASE (study number 12775). A total of 23.6% of data matrix cells were scored as missing data in the combined data matrix because they were either absent, ambiguous, or gaps.

Phylogenies were inferred from each data set using parsimony and Bayesian analyses. A heuristic parsimony search was performed on each dataset in PAUP* 4.0b10 (Swofford 2002), with 1,000 random addition replicates and TBR branch swapping. A total of 500 bootstrap replicates were performed with 10 random additions per replicate to assess branch support (Felsenstein 1985). Indels were treated as missing data. MrModeltest version 2.3 was used to determine the model of evolution that best fit the data for Bayesian inference (Nylander 2004). Bayesian analyses were performed using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003). All Bayesian analyses were performed for 10,000,000 generations, sampling every 100 generations. The first 30% were discarded as burn-in, and only those reaching stationarity were retained. Congruence between the ITS, ETS, and *trnQ-rps16* data sets was assessed using the incongruence length difference (ILD) test (Farris et al. 1995) as implemented in PAUP* v4.0b10 (Swofford 2002).

The tree topology resulting from the Bayesian analysis of the concatenated data (ITS, ETS, and *trnQ-rps16*) was used for assessing character evolution in *Pogogyne* because it had the best clade support (see below). Morphological characters (Table 2) used to differentiate taxa within the genus were examined. Character states for each taxon were obtained from personal observation of specimens used in this analysis (Table 2). Characters traced were relative corolla length, fertile stamen number, and inflorescence width, all of which have been used as diagnostic features in the group. Character states for relative corolla length and fertile stamen number data were coded as discrete and binary. For corolla relative length, a species was coded as "exserted" if the corolla protruded well beyond the bracts and calyces and as "included" if the corolla was did not protrude beyond bracts and calyces. The number of fertile stamens present was coded as two stamens versus four stamens. Inflorescence width included bracts, but not corollas, for the specimens used in the study; measurements ranged from 6.0–26.4 mm and were treated as continuous data. All character evolution analyses were performed in Mesquite, version 4.08 (Maddison and Maddison 2010). Both parsimony optimizations using the unordered states assumption and likelihood reconstructions were implemented for the corolla relative length and fertile stamen number data. Only parsimony optimization was used for the inflorescence width data because Mesquite does not support likelihood reconstruction for continuous data.

TABLE 2. Characters and character states used in character evolution study for specimens of all taxa included in the molecular analysis.

Taxon	Accession #	Corolla relative length	Fertile stamens	Inflorescence width (mm)
Acanthomintha ilicifolia	SDSU 12198	Exserted	2	22.6
Acanthomintha lanceolata	SDSU 17310	Exserted	4	22.0
Acanthomintha obovata	RSA 593773	Exserted	4	7.2
Hedeoma nana	RSA 701934	Exserted	2	12.2
Mentha arvensis	UC 1862058	Exserted	4	13.3
Mentha pulegium	JEPS 108833	Exserted	4	13.8
Monardella lanceolata	SDSU 16814	Exserted	4	14.0
Monardella macrantha	SDSU 16912	Exserted	4	17.1
Monardella villosa subsp. *franciscana*	SDSU 17279	Exserted	4	14.5
Pogogyne abramsii	SDSU 19268	Exserted	4	12.5
Pogogyne abramsii	SDSU 19270	Exserted	4	9.4
Pogogyne aff. *serpylloides*	SDSU 18436	Included	2	8.8
Pogogyne aff. *serpylloides*	SDSU 18437	Included	2	9.3
Pogogyne aff. *zizyphoroides*	SDSU 18438	Included	2	11.3
Pogogyne clareana	SBBG 111196	Exserted	4	7.9
Pogogyne clareana	SDSU 19284	Exserted	4	6.0
Pogogyne douglasii	JEPS 100839	Exserted	4	6.6
Pogogyne douglasii	RSA 695764	Exserted	4	18.2
Pogogyne douglasii	SDSU 19273	Exserted	4	18.3
Pogogyne douglasii	SDSU 19276	Exserted	4	14.7
Pogogyne douglasii	SDSU 19285	Exserted	4	22.0
Pogogyne douglasii	SDSU 19290	Exserted	4	26.4
Pogogyne floribunda	SDSU 19279	Included	2	11.8
Pogogyne floribunda	UC 1609361	Included	2	9.1
Pogogyne floribunda	RSA 551272	Included	2	8.4
Pogogyne nudiuscula	SDSU 19269	Exserted	4	12.1
Pogogyne nudiuscula	SDSU 19274	Exserted	4	11.8
Pogogyne nudiuscula	SDSU 19271	Exserted	4	13.8
Pogogyne serpylloides	SBBG 119413	Included	2	8.5
Pogogyne serpylloides	SDSU 19288	Included	2	10.2
Pogogyne sp.	RSA 679273	Exserted	4	7.7
Pogogyne zizyphoroides	JEPS 101601	Included	2	17.4
Pogogyne zizyphoroides	JEPS 104333	Included	2	17.0
Pogogyne zizyphoroides	SDSU 19277	Included	2	16.4

The geographic distribution of each taxon was determined from the primary literature and from collections included in this study. The ranges were superposed on the concatenated data Bayesian tree, and clades corresponding with biogeographic ranges were noted.

A phylogram was generated from the concatenated Bayesian analysis to visualize the time of lineage divergence in the complex. The data and tree were input into the software BEAST version 1.7.1 (and associated software; Drummond et al. 2012) to estimate times of divergence at the stem and crown node of extant *Pogogyne* species. All *Pogogyne* taxa were set as monophyletic with the stem excluded or included, respectively, as priors. Tip dates were set at zero. A GTR+G model was used, as determined from MrModeltest (see RESULTS). Because we used a standardized rate, a strict clock was used, with a mean rate of 0.2% for the *trnQ-rsS16* data and 0.45% for the ITS data, these based on average divergence rates in angiosperms of 0.1–0.3% per million years for non-coding cpDNA (Wolfe et al. 1987; Muse 2000) and 0.1–0.8% per million years for non-coding nrDNA (Kay et al. 2006). The analysis was run for 10 million generations.

Results

From the 34 samples for which useful data were derived, 28 chloroplast DNA haplotypes, 21 unique ITS sequences, and 28 unique ETS sequences were obtained. For the chloroplast region *trnQ-rps16* (990 bp in length), 870 sites were constant, 48 were variable but parsimony uninformative, and 72 were both variable and parsimony informative (six parsimony informative characters within *Pogogyne*). For the ITS region (845 bp in length), 778 sites were constant, 35 were variable but parsimony uninformative, and 32 were both variable and parsimony informative (eight parsimony informative characters within *Pogogyne*). For the ETS region (456 bp in length), 300 sites were constant, 75 were variable but parsimony uninformative, and 81 were both variable and parsimony informative (12 parsimony informative characters within *Pogogyne*). We did not check for repeats in the ETS region.

Parsimony analyses of the *trnQ-rps16* chloroplast data produced the maximum of 50,000 resolved trees that were 134 steps long. The parsimony analysis of the ITS data resulted in 50,000 resolved trees that were 76 steps long. A total of 50,000 ETS data trees, each 232 steps long, were resolved from the heuristic parsimony search. The incongruence length difference (ILD) test confirmed congruence among the three data sets. The analyses using ITS and ETS together sequences also resolved the maximum of 50,000 equally parsimonious trees that were 312 steps long. The parsimony analysis that used the data set containing ITS, ETS, and *trnQ-rps16* sequences resulted in 32,075 equally parsimonious trees that were 453 steps long. Parsimony analyses of all datasets resolved a strongly-supported, monophyletic *Pogogyne*, but support for clades within the genus was generally weak.

MrModeltest (using AIC) determined that GTR + G was the appropriate model of evolution for each set of data (ETS, ITS, and *trnQ-rps16* evaluated independently). This model of evolution was used in the Bayesian analyses, with each marker in a separate data partition. Bayesian analyses of each dataset resolved a strongly-supported, monophyletic *Pogogyne*. For the trees derived from these datasets, the following numbers of nodes were well-supported (which we define as a posterior probability of 90%) within the *Pogogyne* clade: 1) *trnQ-rps16* tree (0 nodes); 2) ITS tree (1 node); 3) ETS tree (3 nodes); 4) ETS plus ITS tree (4 nodes); 5) ETS, ITS, and *trnQ-rps16* tree (9 nodes). Given the much greater support values for the total evidence tree, in which ITS, ETS, and *trnQ-rps16* were concatenated, only these data are reported here (Fig. 4).

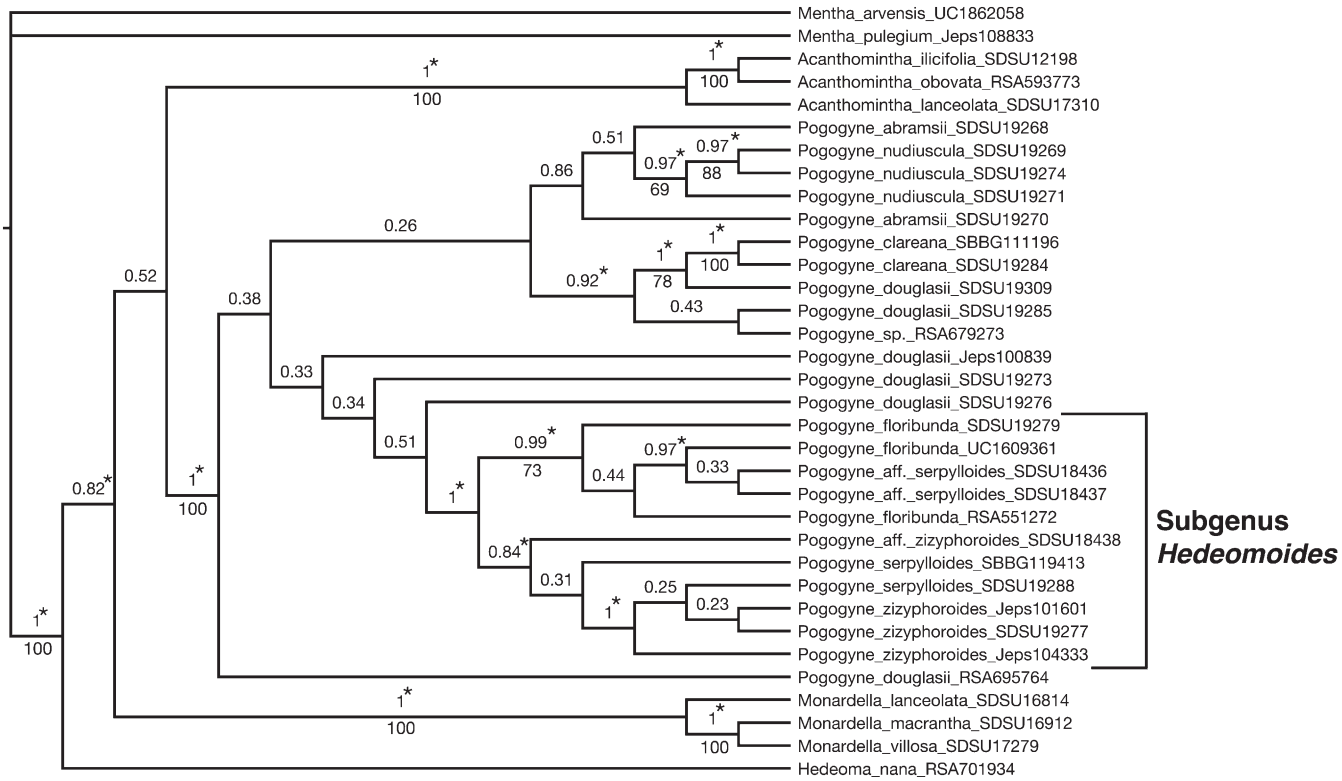

Fig. 4. Tree from Bayesian analysis of concatenated data (ITS, ETS, and *trnQ-rps16*). Values above branches correspond to Bayesian posterior probabilities and values below correspond to bootstrap values above 65% obtained from separate heuristic parsimony analysis. Nodes distinguished with * correspond to those that were also found in the strict consensus tree of the heuristic parsimony search.

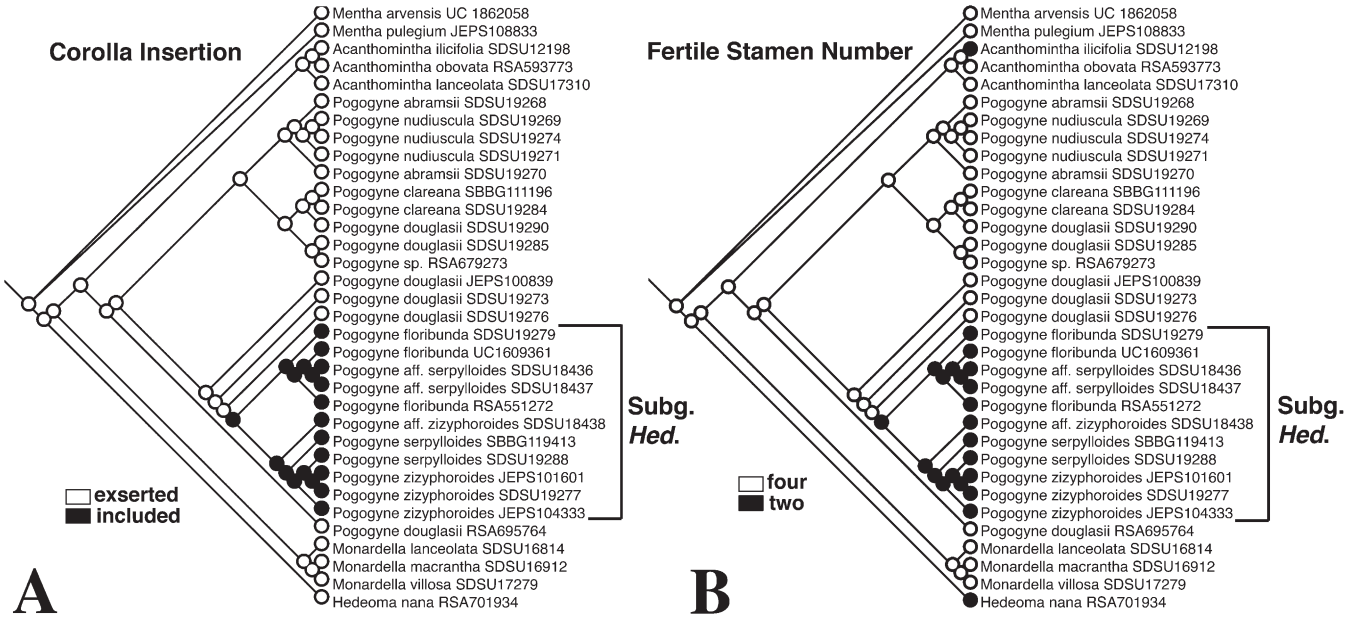

FIG. 5. Parsimony and likelihood optimization of characters on tree from concatenated Bayesian analysis. A. Relative corolla length: conspicuous corollas exserted from bracts and calyces (white) versus inconspicuous corollas included within bracts and calyces (black). B. Fertile stamen number: four (white) versus two (black). Subg. *Hed*. = Subgenus *Hedeomoides*.

The consensus Bayesian inference tree from the concatenated ITS, ETS, and *trnQ-rps16* data, was used in ancestral state reconstruction. Character analysis of relative corolla length, whether exserted from or included within adjacent bracts and calyces, is shown in Fig. 5A. Parsimony optimization determined that a change from exserted to included corollas occurred once on the tree. The likelihood analysis derived a 100% probability that the change happened on the

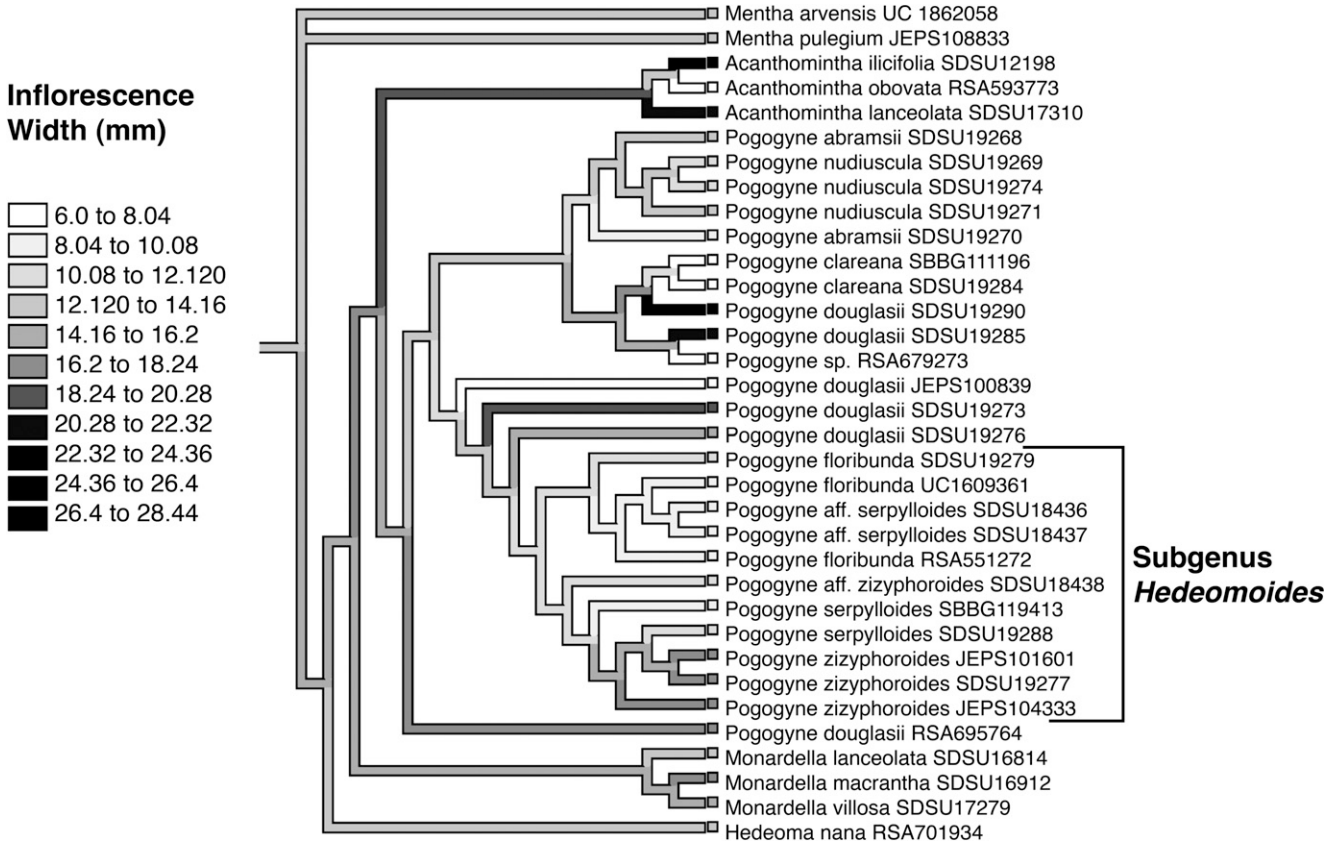

FIG. 6. Parsimony optimization of inflorescence width continuous data based on tree from concatenated Bayesian analysis. Continuous data from inflorescence width measurements were divided into discrete categories and mapped on the tree. Darker shades indicate wider inflorescences.

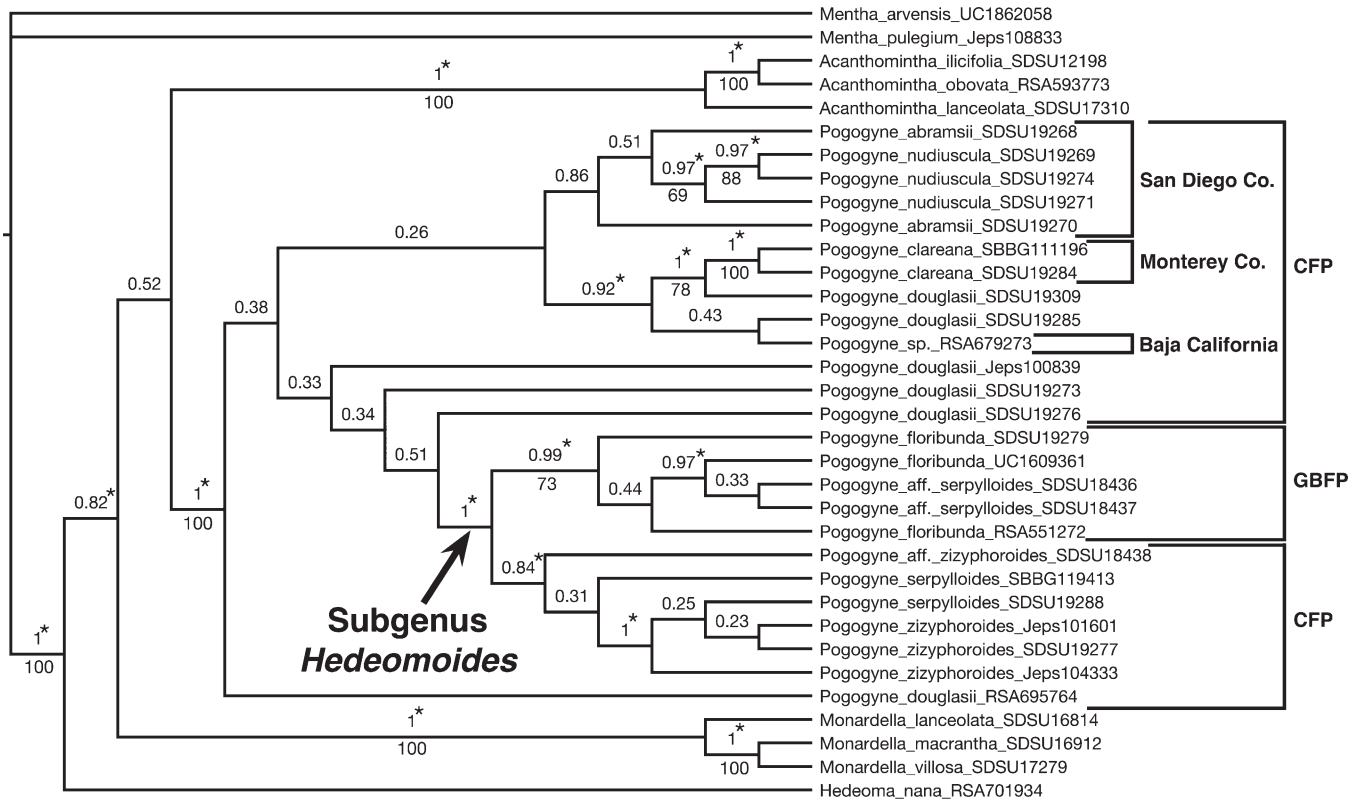

FIG. 7. Localities of select clades superposed on tree from concatenated Bayesian analysis. GBFP = Great Basin Floristic Province; CFP = California Floristic Province.

internal branch leading to the node containing all samples used with subgenus *Hedeomoides* (Fig. 5A). Character tracing of fertile stamen number, whether two or four, is shown in Fig. 5B. Both parsimony optimization and likelihood analysis support a single reduction in stamen number, from four to two, in the *Hedeomoides* clade of *Pogogyne* (likelihood 96.7%), as well as independent reductions in the lineages to *Acanthomintha ilicifolia* and to *Hedeoma nana* (Fig. 5B). Inflorescence width was only assessed by parsimony optimization, as the data were continuous and divided into 11 bins (Fig. 6); no clear trends were noted.

Superpositions of some county, state, or floristic region ranges on the concatenated Bayesian inference tree are shown in Fig. 7. A phylogram of the same analysis is seen in Fig. 8. Estimates of the times of divergence of nodes from the BEAST analysis using the *trnQ-rps16* data were 7.7 million years ago (Ma) (standard error ±0.05; 95% CI = 5.1–10.5 Ma) for the *Pogogyne* stem node and 1.9 Ma (standard error ±0.03; 95% CI = 1.1–3.0 Ma) for the *Pogogyne* crown node. Comparable times of divergence estimates from the ITS data were 5.1 Ma (standard error ± 0.1; 95% CI = 1.0–7.1 Ma) for the *Pogogyne* stem node and 0.9 (standard error ± 0.02; 95% CI = 0.4–1.4 Ma) for the *Pogogyne* crown node (Fig. 8).

Discussion

Phylogenetic Analyses and Classification—Overall, the phylogenetic study presented here has contributed to our understanding of the phylogenetic relationships of *Pogogyne*. This is the first molecular analysis of the entire genus and creates a baseline for future genetic work. The analysis using the concatenated ITS, ETS, and *trnQ-rps16* data shows a total of 15 nodes with a posterior probability (PP) 0.90, 17 nodes that were also found in the strict consensus tree of the heuristic parsimony search, and 10 nodes that had bootstrap (BS) values 70% (Fig. 4). The *Acanthomintha-Monardella-Pogogyne* clade, recovered in both the Bayesian tree and the strict consensus parsimony tree, had moderate to low support (PP = 0.82, BS = 50). Within this clade, *Acanthomintha* and *Pogogyne* form a group sister to *Monardella* with low support in the Bayesian analysis (PP = 0.52). However, the parsimony analysis (not shown) differs in grouping *Acanthomintha* and *Monardella* together with a BS value of 57%. These two different results are similar to those of Drew and Sytsma (2012), who used different molecular markers. All of these analyses indicate that *Acanthomintha* and *Monardella* are likely close relatives to the genus *Pogogyne,* but more information will be needed to resolve the relationships among these three genera.

Results from the concatenated Bayesian analysis indicate *Pogogyne* as a strongly-supported monophyletic group (PP = 1; BS = 100; Fig. 4). Subgenus *Hedeomoides* is monophyletic with high to moderate support (PP = 1; BS = 62) and was also recovered in the strict consensus tree of the heuristic parsimony search. Within *Hedeomoides*, the two *P.* aff. *serpylloides* samples and one *P. floribunda* specimen form a well-supported clade (PP = 0.97, this clade also found in the strict consensus tree of the parsimony analysis); these three samples and the two other *P. floribunda* specimens also comprise a well-supported clade (PP = 0.99; BS = 73; Fig. 4). The only other well-supported clade within *Hedeomoides* are the three *P. zizyphoroides* samples and one relatively nearby sample

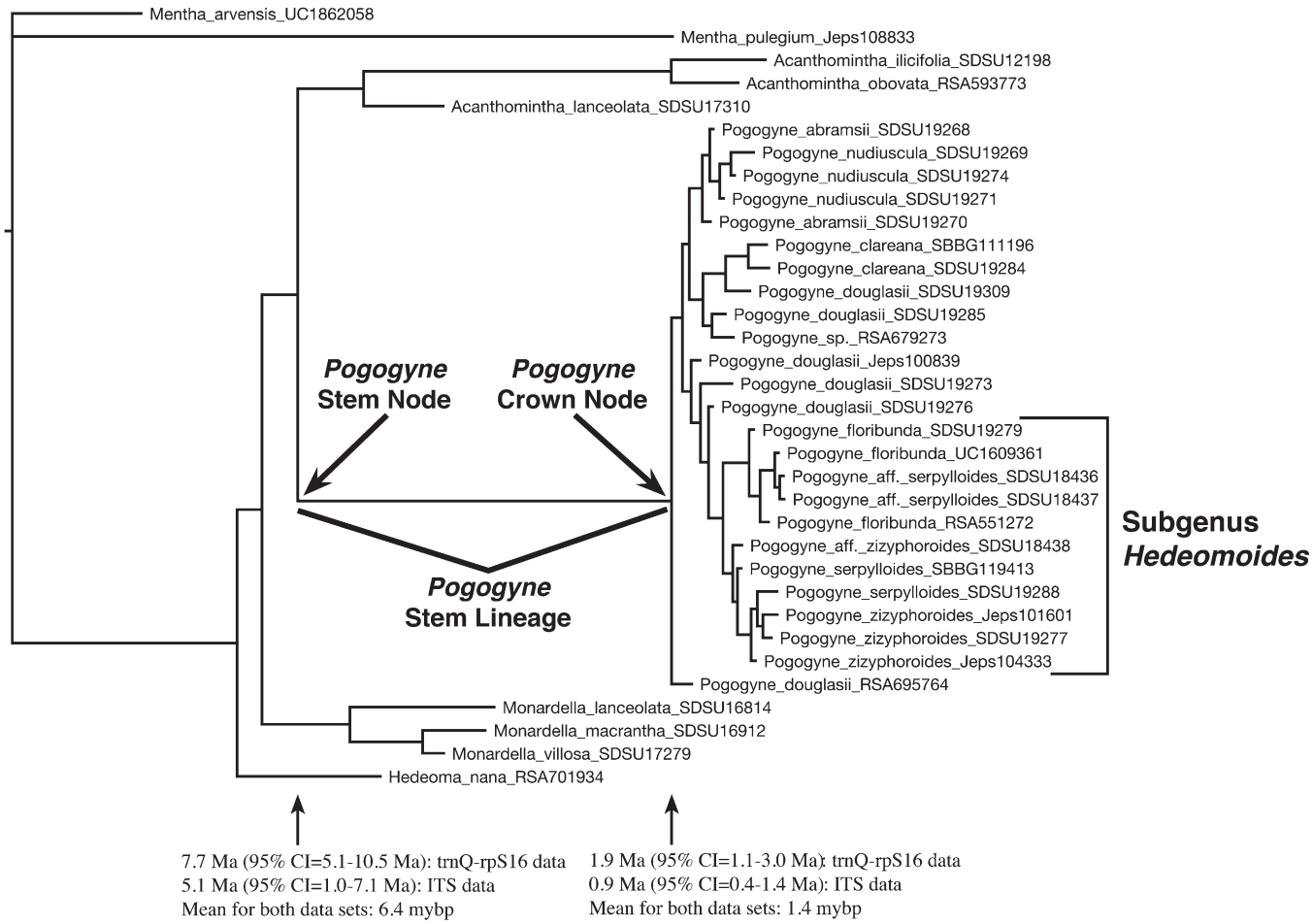

FIG. 8. Phylogram from concatenated Bayesian analysis. Shown below are BEAST estimates of the time of origin of the *Pogogyne* stem and crown nodes, the latter representing the time of divergence of extant *Pogogyne* taxa.

of *P. serpylloides* from Lake County, California (SDSU 19288; see locality at Fig. 3). These four samples plus the other sample of *P. serpylloides* and that of *P.* aff. *zizyphoroides* form a moderately well-supported clade (PP = 0.84, this clade also found in the strict consensus of the parsimony analysis; Fig. 4).

Subgenus *Pogogyne*, in contrast, is not monophyletic in our analyses, but the paraphyly of the subgenus is not conclusive given that support for basal branches is poor (PP 0.51, BS < 50; see Fig. 4). Within subgenus *Pogogyne* only a few clades are well-supported. *Pogogyne nudiuscula* (from three samples) is monophyletic with somewhat strong support (PP = 0.97; BS = 69). The three samples of *P. nudiuscula* are part of a clade including two samples of *P. abramsii,* (all from San Diego County, California; see below), this clade having moderate (PP = 0.86) to low (BS < 50) support (Fig. 4). *Pogogyne abramsii* is paraphyletic in all analyses, but this pattern lacks strong support (Fig. 4). *Pogogyne clareana* (two samples) is monophyletic with very strong support (PP = 1; BS = 100) and is sister with strong support (PP = 1; BS = 78) to one specimen of *P. douglasii* (SDSU 19309) collected from adjacent San Luis Obispo County (see localities at Fig. 3). The samples of this clade, along with a *P. douglasii* specimen (SDSU 19285) collected in Lake County and a possibly new *Pogogyne* sp. (RSA 679273) collected in northern Baja California, Mexico, form a clade that has strong support in the Bayesian analysis (PP = 0.92, also found in the parsimony analysis), but a low bootstrap value (BS < 50; see Fig. 4).

***Character Evolution*—**The common ancestor of *Pogogyne* clearly had exserted flowers and four fertile stamens, based on both parsimony optimization and likelihood analysis. These analyses suggest that the change from a conspicuous corolla to an inconspicuous corolla and from four to two fertile stamens happened on the same internal branch and are clear apomorphies for subgenus *Hedeomoides* (Fig. 5). The higher-level phylogenetic analysis of the Lamiaceae by Drew and Sytsma (2012), in which only one species of *Pogogyne* (*P. douglasii*, with 4 stamens) was included, supported three reductions in stamen number (along with 2–3 reversals) in their subtribe Menthinae. This study provides evidence for three additional reductions in stamen number in the complex, one in *Pogogyne* and independent reductions in *Acanthomintha ilicifolia* and *Hedeoma nana*.

Reduction in both corolla size and stamen number may be correlated because of a possible shift from outcrossing to self-pollination in subgenus *Hedeomoides*. Pollination studies in *Pogogyne* have only been conducted on *P. abramsii,* which has been found to be predominately outcrossing (Schiller et al. 2000). However, studies of other vernal pool plant species have noted that several are self-pollinating (Spencer and Riesberg 1998; Thorp and Leong 1998). None of the species within subgenus *Hedeomoides* have been studied with regard

to pollination mechanism, but it is possible that corollas inserted within the bracts and calyces of the inflorescence make them less visible to insect visitors; this, along with a reduction in fertile stamen number, could coincide with a reliance on self-pollination. Future reproductive studies, including measures of pollen:ovule ratio, pollinator exclusion, and pollen transfer manipulations, will be needed to verify reproductive mechanisms within the genus.

The measured inflorescence width data varied and were not always consistent within a described species. Analysis of the continuous data (Fig. 6) showed no obvious trends in this feature. Members of subgenus *Hedeomoides* tended to have small to moderate inflorescence widths, but with some variation and overlap with members of subgenus *Pogogyne* (Fig. 6). Some individual species had consistent measures of inflorescence width (e.g. *P. clareana* with a small width, and *P. zizyphoroides,* with a moderate width) while others had considerable variability among different samples, e.g. *P. douglasii* (Fig. 6). Inflorescence width is a feature that has been used to describe different infraspecies of *P. douglasii* (Howell 1931), but these analyses suggest that there is likely no signal or trend in the variability of inflorescence width that correlates with known phylogeny. This inference agrees with Jepson (1943), who lumped the subspecies of *P. douglasii* in his treatment of the group, a treatment similarly followed by later authors (e.g. Silveira et al. 2012). Inflorescence width is still used in combination with others to separate species in keys (e.g. Jokerst 1993; Silveira et al. 2012), but this feature may well be environmentally plastic and influenced by factors such as the amount of precipitation and length of vernal pool inundation, with wider inflorescences correlated with wetter years. (Inflorescence width for this study was measured from herbarium specimens, not in the field, although we would not expect there to be an appreciable difference.)

Other features of character evolution can be noted from our phylogenetic analysis. Correlated with the monophyly of the genus *Pogogyne* is a major ecological apomorphy: the ability to survive and reproduce in a wet (mostly vernal pool) habitat, a feature that is presumed to have a genetic basis. Within *Pogogyne*, a slight evolutionary shift may have occurred in *P. clareana*, which grows in periodically inundated streams as opposed to vernal pools. However, this species can occasionally be found in vernal pools or vernal pools with an intermittent stream running through (Elizabeth Painter, pers. comm.). Regardless, the habitat of *P. clareana* is aquatic. In addition, *P. serpylloides* is cited as occurring in grasslands. However, personal observation by one of us (Silveira) confirms that the habitat of this species can consist of periodically inundated swales within grasslands, very similar to a miniature vernal pool.

Finally, we note that a pubescent style, which has traditionally been used to characterize *Pogogyne*, is also found in *Acanthomintha lanceolata*. This is the only other known example of a pubescent style among genera closely related to *Pogogyne*. Because *Pogogyne* is strongly-supported as monophyletic, a pubsescent style in the two taxa occurred either by convergence in the two groups or was present in the common ancestor of *Pogogyne* and *Acanthomintha* and later lost in the *Acanthomintha ilicifolia-A. obovata* clade. Given the relatively poor resolution of the relationships among *Acanthomintha, Monardella,* and *Pogogyne,* further studies may be necessary to resolve this issue.

Biogeography—Given our relatively small sample size and difficulty with delimiting biogeographic regions, no detailed biogeography analysis was performed, but some correspondence can be made between various clades and geographic ranges. Within subgenus *Pogogyne,* both samples of *P. clareana* collected in Monterey County were found to be each other's closest relative with good support (Fig. 7); this result is not surprising given that *P. clareana* is restricted to this narrow region. As discussed earlier, the sister taxon of *P. clareana* of Monterey County was a *P. douglasii* specimen (SDSU 19309) collected from a nearby region of adjacent San Luis Obispo County. Specimens of *Pogogyne abramsii* and *P. nudiuscula*, all from San Diego County, California, form a clade (Fig. 7). These two geographically proximate species have long been thought to be close relatives, differing primarily in pubescence and number of flowers per leaf axil (Silveira et al. 2012). The single specimen of *Pogogyne* species collected in northern Baja California, Mexico (RSA 679273) was not recovered in the clade with San Diego County specimens but within a sister clade along with *P. clareana* and two specimens of *P. douglasii* (Fig. 7). However, we were unable to obtain ITS data for this sample (Appendix 1); thus, the analysis is based on *trnQ-rps16* and ETS data alone. This Mexican specimen, formerly identified as *P. nudiuscula* (represented as *P.* sp. in this study), could represent a new taxon and needs further investigation.

Subgenus *Hedeomoides* exhibited two biogeographically correlated clades, each moderately- to well-supported. One clade that is well-supported (P = 0.99, BS = 73) includes all specimens from the Great Basin Floristic Province (GBFP; Fig. 7). In this clade, specimens of *P. floribunda* are found in the GBFP of California, and the two specimens identified as *P.* aff. *serpylloides* are from the GBFP of Oregon. All members of the other clade of subgenus *Hedeomoides* are found (like all other *Pogogyne* samples used in this analysis) in the California Floristic Province (CFP; Fig. 7). This latter, moderately-supported clade (PP = 0.84, BS<50) includes all samples of *P. serpylloides*, *P. zizyphoroides*, and specimens identified as *P.* aff. *zizyphoroides* (Fig. 7). Interestingly, within this clade, a group consisting of three specimens of *P. zizyphoroides* and one of *P. serpylloides* (SDSU 19288) is strongly-supported as monophyletic (PS = 1.0); this single sample of *P. serpylloides* from Lake County, California is much more geographically proximate to the *P. zizyphoroides* specimens than is the other *P. serpylloides* sample (SBBG 119413) from San Luis Obispo County (Fig. 7). Lastly, a single specimen from Oregon, tentatively identified as *P.* aff. *zizyphoroides* (SDSU 18438), is sister to the rest of the subgenus *Hedeomoides* clade from the CFP.

Species Delimitation—Our analysis constitutes a baseline study for elucidation of species boundaries within the genus *Pogogyne.* Some inferences on species types can be made from these analyses. A morphological species concept (Cronquist 1978, 1988) has traditionally been used to describe the different species of *Pogogyne*, using one or more morphological features. New populations have been discovered that do not fit the current species delimitations, so an in-depth morphometric study may be needed to redefine morphological species boundaries of *Pogogyne*. Because there have been virtually no studies of the reproduction biology of several *Pogogyne* species, a biological species concept (Mayr, 1963) cannot be evaluated. If some type of phylogenetic species concept were used (e.g. see De Queiroz 1998), there would

be support for both *P. clareana* and *P. nudiuscula* being phylogenetic species based on well-supported molecular apomorphies; however, no other clade has sufficient support to be deemed a phylogenetic species. *Pogogyne douglasii* could qualify as a paraphyletic species (plesiospecies of Olmstead 1995), given that some samples are more closely related to other species, with good support (Fig. 4). By this hypothesis, populations of the wide-ranging *P. douglasii* could have led to separate lineages that become isolated and subsequently diverged from each other, an example of peripheral isolate speciation. This could possibly explain why some samples of *P. douglasii* are more closely related to *P. clareana* than to other specimens of *P. douglasii*. The various species, subspecies, and varieties of *P. douglasii* that have been described in the past, treated as synonyms by us (Table 1), may represent a paraphyletic assemblage of lineages, some of which have undergone slight morphological changes. Other explanations for the observed paraphyly of *P. douglasii* include hybridization with other taxa or incomplete lineage sorting.

None of the individual species within *Hedeomoides* were recovered as monophyletic with strong support. *Pogogyne zizyphoroides* can be determined as neither monophyletic or paraphyletic, but *P. serpylloides* is definitively non-monophyletic in our analysis. In any case, it is clear that the data used may be unable to fully resolve relationships within subgenus *Hedeomoides*, and/or that this complex of species represents a recently evolved clade, the lineages of which are not clearly genetically distinct, perhaps because of incomplete lineage sorting or genetic introgression. This notion may be supported by the existence of samples from Oregon that are difficult to identify relative to described species. Although the taxa of subgenus *Hedeomoides* are generally identifiable, they could represent the products of incomplete speciation.

Although the molecular markers used in this study showed some variation and resolved the evolutionary framework of the group, further studies are needed to better understand the evolution of *Pogogyne*. Use of additional genetic data, such as single copy nuclear markers, microsatellite data (as in Edwards et al. 2009), or perhaps whole genome sequences, and potential use of species tree reconstruction methods (see Edwards 2008) may be needed to better understand species delimitations and their interrelationships in the genus.

***Divergence Times*—**The phylogram of the combined data set (Fig. 8) illustrates that *Pogogyne* (with a long stem lineage) is well separated from its close relatives in terms of patristic distance, i.e. the total number of changes occurring along lineages from one taxon to another (see also Drew and Sytsma 2012). The *Pogogyne* stem node is estimated (from chloroplast and ITS nrDNA data, respectively) to have arisen an average of 5.1–7.7 million years ago, although with considerably wide confidence intervals (Fig. 8). This date corresponds well with the *Pogogyne* stem node age of approximately 6 million years ago (95% CI = ca. 4–7 Ma) from the analyses of Drew and Sytsma (2012), who analyzed only a single *Pogogyne* species (*P. douglasii*) but studied numerous representatives of the Lamiaceae, using some fossil calibrations. In contrast to the relatively long *Pogogyne* stem lineage, there are very small branch lengths within the *Pogogyne* crown group (Fig. 8), especially when compared to *Acanthomintha* and *Monardella* (although the sample size for the latter is limited). *Acanthomintha* is of special note because three of the four *Acanthomintha* species were included in our study, and internal branch lengths are considerably longer in that genus than in *Pogogyne*, suggesting that *Acanthomintha* species have been separated for a longer period of time (Fig. 8).

Dating of the *Pogogyne* crown node was estimated (from chloroplast and ITS nrDNA data, respectively) to be an average of 0.9–1.9 million years ago. This compares with an estimated age of 0.6–4 million years before present for many vernal pool soils (see Harden 1987). Although these dates are quite rough estimates, we hypothesize that *Pogogyne* underwent a relatively rapid diversification in vernal pool ecosystems following their adaptation to a vernal pool habitat. This was likely correlated with the evolution of an apomorphic physiological adaptation to vernal pool or "temporary wetland" conditions in members of the genus.

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Appendix 1. Taxon, collector, collection number, sample number (if more than one of a given taxon), herbarium accession, and GenBank accession numbers for ETS, ITS, and trnQ-rps16 sequences, respectively. * = absence of ITS sequence data. Herbarium acronyms: JEPS = Jepson Herbarium (at University of California, Berkeley); RSA = Rancho Santa Ana Botanic Garden; SBBG = Santa Barbara Botanic Garden; SDSU = San Diego State University; UC = University of California, Berkeley.

Acanthomintha ilicifolia (A. Gray) A. Gray. *Reiser 30IV94* (SDSU 12198), KC591629, KC591662, KC591692. ***Acanthomintha lanceolata*** Curran. *Simpson 2866* (SDSU 17310), KC591630, KC591663, KC591693. ***Acanthomintha obovata*** Jepson. *Boyd 8558* (RSA 593773), KC591631, KC591664, KC591694. ***Hedeoma nana*** (Torrey) Briquet. *Boyd 11450* (RSA 701934), KC591632, KC591665, KC591695. ***Mentha arvensis*** L. *Colwell AC 4–110* (UC 1862058), KC591627, KC591661, KC591690. ***Mentha pulegium*** L. *Ahart 12913* (JEPS 108833), KC591628, *, KC591691. ***Monardella lanceolata*** A. Gray. *Lauri 295* (SDSU 16814), KC591633, *, KC591696. ***Monardella macrantha*** A. Gray. *Lauri 273* (SDSU 16912), KC591634, *, KC591697. ***Monardella villosa*** Bentham subsp. ***franciscana*** (Elmer) Jokerst. *Simpson 2842* (SDSU 17279), KC591635, *, KC591698. ***Pogogyne abramsii*** J. T. Howell 1. *Silveira 4* (SDSU 19268), KC591636, KC591666, KC591699; ***Pogogyne abramsii*** J. T. Howell 2. *Silveira 5* (SDSU 19270), KC591637, KC591667, KC591700. ***Pogogyne clareana*** J. T. Howell 1. *Painter HL-2753* (SBBG 111196), KC591638, KC591668, KC591701; ***Pogogyne clareana*** J. T. Howell 2. *McMillan 9IV93B* (SDSU 19284), KC591639, KC591669, KC591702. ***Pogogyne douglasii*** Bentham 1. *Taylor 17212* (JEPS 100839), KC591640, KC591670, KC591703; ***Pogogyne douglasii*** Bentham 2. *York 2159* (RSA 695764), KC591643, KC591673, KC591706; ***Pogogyne douglasii*** Bentham 3. *Silveira 18* (SDSU 19273), KC591641, KC591671, KC591704; ***Pogogyne douglasii*** Bentham 4. *Silveira 9* (SDSU 19276), KC591642, KC591672, KC591705; ***Pogogyne douglasii*** Bentham 5. *McMillan 10VI93B* (SDSU 19285), KC591644, KC591674, KC591707; ***Pogogyne douglasii*** Bentham 6, *McMillan 8VI93* (SDSU 19309), KC591645, KC591675, KC591708. ***Pogogyne floribunda*** (Standley) Jokerst 1. *Bartholomew 5932* (RSA 551272), KC591655, KC591684, KC591718; ***Pogogyne floribunda*** (Standley) Jokerst 2. *Silveira 20* (SDSU 19279), KC591646, KC591676, KC591709; ***Pogogyne floribunda*** (Standley) Jokerst 3. *Oswald 5669* (UC 1609361), KC591647, KC591677, KC591710. ***Pogogyne nudiuscula*** A. Gray 1. *Silveira 6* (SDSU 19269), KC591648, KC591678, KC591711; ***Pogogyne nudiuscula*** A. Gray 2. *Silveira 8* (SDSU 19271), KC591650, KC591680, KC591713; ***Pogogyne nudiuscula*** A. Gray 3. *Silveira 7* (SDSU 19274), KC591649, KC591679, KC591712. ***Pogogyne serpylloides*** (Torrey) A. Gray 1. *Painter SLO-80* (SBBG 119413), KC591656, KC591685, KC591719; ***Pogogyne serpylloides*** (Torrey) A. Gray 2. *McMillan 10VI93F* (SDSU 19288), KC591657, KC591686, KC591720. ***Pogogyne*** aff. ***serpylloides*** (Torrey) A. Gray 1. *Meinke 15VI05A* (SDSU 18436), KC591652, KC591681, KC591715; ***Pogogyne*** aff. ***serpylloides*** (Torrey) A. Gray 2. *Meinke 15VII05* (SDSU 18437), KC591654, KC591683, KC591717. ***Pogogyne*** *sp. Elvin 191* (RSA 679273), KC591651, *, KC591714. ***Pogogyne zizyphoroides*** Bentham 1. *Taylor 17136* (JEPS 101601), KC591658, KC591687, KC591721; ***Pogogyne zizyphoroides*** Bentham 2. *Ahart 10157* (JEPS 104333), KC591659, KC591688, KC591722; ***Pogogyne zizyphoroides*** Bentham 3. *Silveira 10* (SDSU 19277), KC591660, KC591689, KC591723. ***Pogogyne*** aff. ***zizyphoroides*** Bentham. *Meinke 15VI05B* (SDSU 18438), KC591653, KC591682, KC591716.