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# Unravelling Species Relationships and Diversification within the Iconic California Floristic Province Sages (*Salvia* subgenus *Audibertia*, Lamiaceae)

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***Abstract***—In the California Floristic Province (CA-FP) and nearby deserts, 19 species of *Salvia* (Lamiaceae, Mentheae) form a small radiation but an important component of the chaparral and desert communities. Traditionally, two groups within these Californian *Salvia* have been recognized (usually treated as sections), but relationships within each, to each other, and to other *Salvia* are unclear. Phylogenetic relationships of all species, with multiple accessions for most, were obtained using chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) markers. Ancestral character state reconstruction of both vegetative and floral features was done on the resulting nrDNA tree. Biogeographical analysis of the groups within the CA-FP and adjacent floristic provinces was done in BioGeoBEARS and species diversification assessed with BAMM. Significant conclusions drawn from the study include: 1) California *Salvia* should be classified into two monophyletic sections, *Audibertia* (15 species) and *Echinosphace*, (four species) in the new subgenus *Audibertia*; 2) subg. *Audibertia* and the Neotropical subg. *Calosphace* are sister clades, most closely related to Asian groups, and are likely Asian in origin; 3) nrDNA provides a fairly resolved tree for subg. *Audibertia* with all species monophyletic; 4) cpDNA and nrDNA trees are strongly incongruent and provide evidence that hybridization and chloroplast capture have played an important role in the evolution of subg. *Audibertia*; 5) ancestral character reconstruction of states in habit, possession of spines, calyx lobing, and staminal features highlights a complex (sometimes convergent) evolutionary history of this iconic CA-FP lineage; 6) subg. *Audibertia* arose in desert areas and more recently diversified into the southwestern California region and adjacent regions with the formation of the Mediterranean-like climate; and 7) this diversification exhibits a slight decrease in speciation and an increase in extinction rates over the group's 11 million year history.

***Keywords***—BAMM, BioGeoBEARS, biogeography, *Calosphace*, chloroplast capture, *Echinosphace*, hybridization, staminal evolution.

The California Floristic Province (CA-FP; Raven and Axelrod, 1978) covers an area of about 300,000 km$^2$ and is one of five regions worldwide that feature the cool wet winters and hot dry summers that define the Mediterranean-type climate. Habitat diversity is rich within the region, but perhaps the most iconic habitat of the CA-FP is the chaparral community type. Within the CA-FP chaparral community, the genus *Salvia* L. (Salviinae; Mentheae; Nepetoideae; Lamiaceae), commonly known as sage, is a conspicuous and sometimes dominant component of the vegetation (Epling, 1938). The only native *Salvia* represented in the CA-FP are members of sect. *Audibertia* (ca. 15 species) and sect. *Echinosphace* (four species; Fig. 1). Though the distributions of *Salvia* sects. *Audibertia* and *Echinosphace* are clearly centered in the CA-FP, the species range from Baja California and adjacent deserts surrounding the CA-FP north to Washington, and from the Pacific Ocean east to central Utah. These sages are found primarily in two shrub formations: the *Larrea-Franseria* formation of the Colorado Desert, and the related *Artemisia californica-Salvia* formation of the coastal plain (Epling, 1938). Twelve *Salvia* species are important or dominant elements in one or both of the above formations, while the remaining seven are more broadly distributed or associated with other formations adjacent to these two shrub formations.

*Salvia* (sensu Walker and Sytsma, 2007) itself is a widespread assemblage of over 900 species with centers of diversity in Mexico/Central America (ca. 300 species), northern and central South America (ca. 150 and 60 species, respectively), the Mediterranean (ca. 250 species), and temperate Asia (ca. 90 species), with smaller radiations in western North America (19 species) and southern Africa (ca. 30 species). *Salvia* are distinguished from other members of tribe Mentheae by expressing only two stamens, with each having their anther sacs (thecae) separated by an elongation of the connective tissue (Fig. 2). The separation of sect. *Audibertia* from other species of *Salvia* has been based on chemical compounds, shrubby habit with strongly lignified stems (although not present in all species), and, most importantly, on the structure of its stamens (Epling, 1938; Neisess, 1983). Sect. *Audibertia* is unusual within *Salvia* in having the posterior branch of the staminal connective and the posterior theca entirely aborted (Fig. 2). The morphologically similar sect. *Echinosphace* does express the posterior theca, albeit somewhat reduced in size. Furthermore, members of both sects. *Audibertia* and *Echinosphace* do not employ the lever mechanism of pollination commonly associated with the genus *Salvia* (e.g. Figure 2A, E, N; Claßen-Bockhoff et al., 2003; Walker and Sytsma, 2007). Epling (1938) suggested that the species comprising this southwestern North American group were probably related to *Salvia* subg. *Calosphace* (500 species), distributed from Mexico to south-central South America, based on geography and morphology. He noted, however, that in contrast to the large and somewhat homogeneous subg. *Calosphace*, the southwestern North American species exhibited considerably more variation in habit and floral features, especially stamens.

*Salvia* sects. *Audibertia* and *Echinosphace* were originally described as their own genus, *Audibertia* (Bentham, 1833). A rather complicated and nonlinear series of group reorganizations ensued (Greene, 1892; Briquet, 1897; Jepson, 1925; Munz, 1927), ultimately resulting in Epling (1938) incorporating 18 species into *Salvia* sect. *Audibertia* (one species, *S. chionopeplica,* was added later by Epling (1940)), with five subsections therein (Table 1). It is unclear why Epling (1939) chose to treat *Calosphace* as a subgenus, while treating sect. *Audibertia*, a group he considered natural and most closely related to subg. *Calosphace*, as a section (Epling, 1938). Neisess (1983), using morphological and phytochemical data, chose to break *Salvia* sect. *Audibertia* into two unrelated sections, *Echinosphace* and *Audibertia*, and to suggest affinities of those sections to Bentham's (1876) *Salvia* subg. *Leonia* and the Old


FIG. 1. Species composition and floral diversity within the California *Salvia* sects. *Audibertia* and *Echinosphace*. Species are arranged according to the findings of this study based on nuclear ribosomal and chloroplast DNA. Photo credits: *S. apiana* (© 2013 Keir Morse), *S munzii* (© 2008 Stan Shebs), *S. vaseyi* (© 2009 Robert Steers), *S. eremostachya* (© 2006 Michael Charters), *S. clevelandii* (© 2009 Keir Morse), *S. mellifera* (© 2008 Gary McDonald), *S. brandegeei* (© 2005 Steve Matson), *S. dorrii* (© 2009 Thomas Stoughton), *S. pachyphylla* (© 2012 Robert Sikora), *S. mohavensis* (© 2009 Aaron Schusteff), *S. sonomensis* (© 2012 Steven Perry), *S. spathacea* (© 2013 John Doyen), *S. leucophylla* (© 2006 Steve Matson), *S. chionopeplica* (© 2010 Frank Sovich), *S. columbariae* (© 2009 Keir Morse), *S. funerea* (© 2005 Steve Matson), *S. californica* (© 2008 Phillip Ruttenbur), *S. greatae* (© 2009 Curtis Croulet), *S. carduacea* (© 2004 Hartmut Wisch).

World genus *Rosmarinus*, respectively (Table 1). The reader is referred to Neisess (1983)) for a complete discussion of the taxonomic history of the group.

The morphology and distribution of the species of sects. *Audibertia* and *Echinosphace* have been well documented through two comprehensive treatments (Epling, 1938; Neisess, 1983). Section *Audibertia*, especially, has been the focus of detailed studies examining issues dealing with biogeography (Epling, 1944), allelopathy (e.g. Muller, 1965, 1966; Muller and Muller 1964; Muller and Hauge 1967; Muller et al. 1968a, b), phytochemical evolution (e.g. Emboden and Lewis 1967; Neisess 1983; Neisess et al. 1987; Hashemi et al. 1993), myxocarpy (Whistler 1982), heterostyly (Neisess 1984), chromosome number evolution (Stewart 1939; Epling et al. 1962), and both hybridization and subsequent introgression (Jepson 1925; Munz 1935; Epling 1938, 1947; Epling et al. 1962; Grant

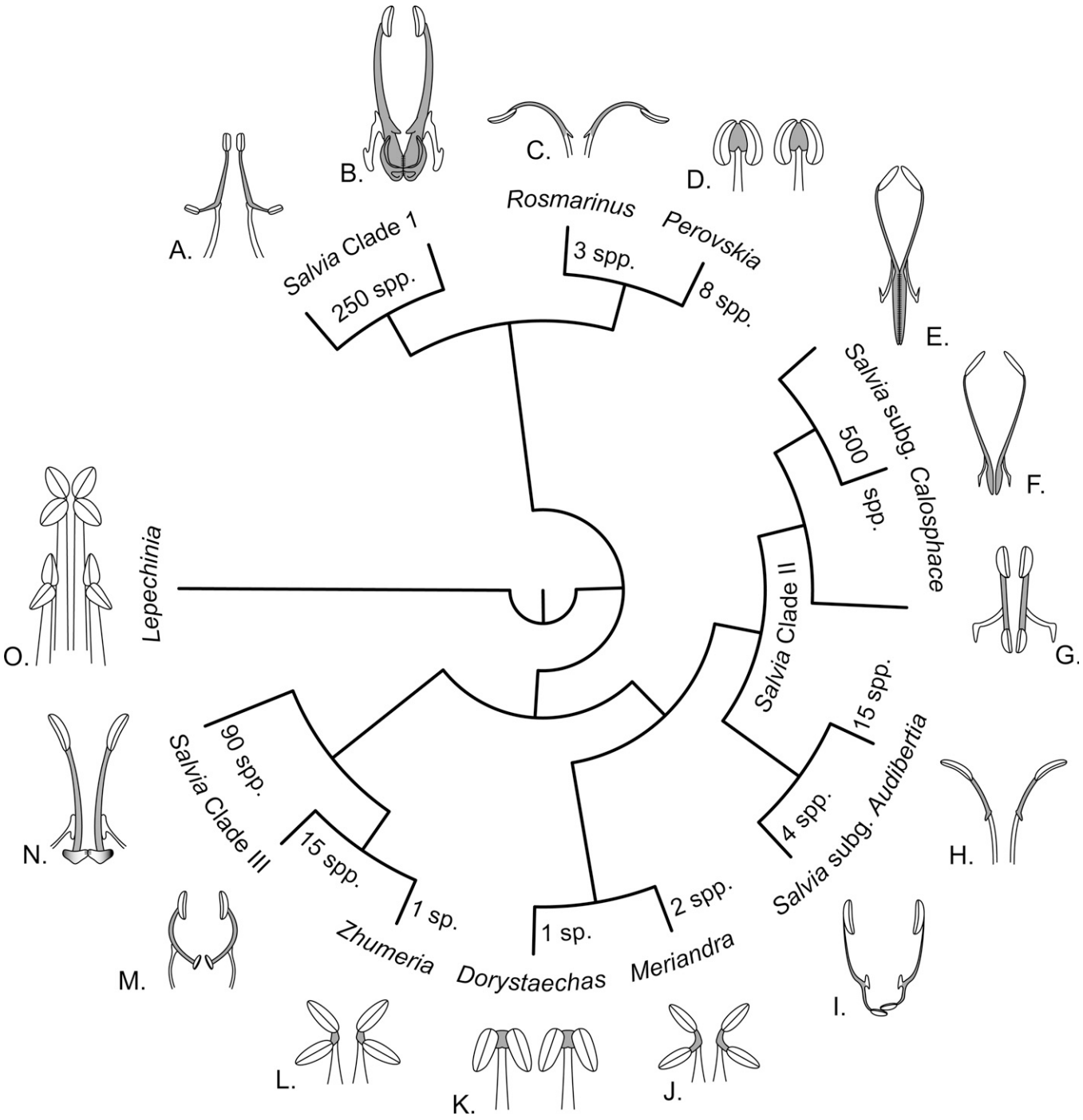


Fig. 2. A stylized representation of staminal evolution in the Lamiaceae tribe Mentheae subtribe Salviinae. The grey shaded areas within stamens represent connective tissue, with the filaments and thecae non-shaded. Four stamens with no elongated connective tissue (type O) is found in *Lepechinia* (and *Melissa*) comprising the sister to the "*Salvia*" clade; the latter all possessing only two stamens (types A–N). Within the latter, the three *Salvia* clades exhibit an elongated connective tissue separating the two thecae of each stamen (types A and B; E–I; M and N; respectively) and are each sister to other genera with less elongated connective tissue and more typical anther thecae (types C and D; J and K; L; respectively). Within each *Salvia* clade, loss of posterior thecae function and fusion of the posterior thecae to form a staminal lever arises in a convergent fashion (e.g. types B; E–F; N; respectively). Stamen forms found in California *Salvia* sections *Echinosphace* (type I) and *Audibertia* (type H) exhibit either functional but smaller posterior thecae or complete loss of the posterior thecae. Stamen types and species diversity for each clade are modified after Walker and Sytsma (2007) but updated. Topology is based on Walker and Sytsma (2007), as modified by Drew and Sytsma (2011, 2012, 2013).

and Grant 1964; Emboden and Lewis 1967; Emboden 1969, 1971; Neisess 1983). Hybridization between *Salvia apiana* and *S. mellifera* has been widely cited as a model system in plants (Epling 1947; Anderson and Anderson 1954; Grant and Grant 1964; Grant 1981, 1994; Meyn and Emboden 1987). Furthermore, based on distributional and morphological evidence, Epling (1938) argued that *Salvia vaseyi* was a diploid hybrid species that had arisen from a cross of *S. apiana* × *S. eremostachya*. Verification of its origin as a homoploid hybrid species has not been attempted prior to this study.

TABLE 1. Comparison between Epling's (1938, 1940) and Neisess' (1983) systems of classification of the western North American and Californian *Salvia* species.

| Epling | Neisess |
|---|---|
| **section *Audibertia*** | **section *Echinosphace*** |
| subsection *Echinosphace* | subsection *Douglasiana* |
| series *Douglasiana* | series *Eplingia* |
| *S. carduacea* | *S. carduacea* |
| series *Munzia* | series *Damonia* |
| *S. californica* | *S. californica* |
| | subsection *Munzia* |
| | series *Parishiella* |
| *S. funerea* | *S. funerea* |
| | series *Kobalya* |
| *S. greatae* | *S. greatae* |
| | **section *Audibertia*** |
| subsection *Parishiella* | subsection *Parishiella* |
| | series *Revoluta* |
| *S. brandegeei* | *S. brandegeei* |
| | series *Stachyoides* |
| *S. mellifera* | *S. mellifera* |
| *S. munzii* | *S. munzii* |
| subsection *Pycnosphace* | series *Pycnosphace* |
| *S. columbariae* | *S. columbariae* |
| subsection *Greeneostachya* | subsection *Greeneostachya* |
| *S. spathacea* | *S. spathacea* |
| subsection *Jepsonia* | subsection *Jepsonia* |
| | series *Incanum* |
| *S. dorrii* | *S. dorrii* |
| *S. pachyphylla* | *S. pachyphylla* |
| | series *Ramona* |
| *S. apiana* | *S. apiana* |
| *S. vaseyi* | *S. vaseyi* |
| | series *Wolfringia* |
| *S. eremostachya* | *S. eremostachya* |
| | series *Humilis* |
| *S. sonomensis* | *S. sonomensis* |
| | series *Nivea* |
| *S. leucophylla* | *S. leucophylla* |
| *S. chionopeplica* | *S. chionopeplica* |
| | series *Clevelandiana* |
| *S. clevelandii* | *S. clevelandii* |
| | series *Mohavia* |
| *S. mohavensis* | *S. mohavensis* |

Although the sages of *Salvia* sects. *Audibertia* and *Echinosphace* are conspicuous components of the CA-FP flora, are important ecologically, culturally, and ornamentally, and have been a focus group for many biosystematic studies, no comprehensive molecular phylogenetic analysis has been attempted for the sections. Furthermore, the relationship between *Salvia* sect. *Audibertia* and sect. *Echinosphace* has not been well-investigated using DNA evidence. Previous molecular phylogenetic studies focusing on *Salvia* or other Mentheae have sampled only a few representatives of these two sections and subg. *Calosphace*, used only one gene region, or have uncovered weakly supported relationships (Walker et al. 2004; Walker and Sytsma 2007; Drew 2011; Drew and Sytsma 2011, 2012; Jenks et al. 2012; Lancaster and Kay 2013). Although under-sampled, these studies do indicate that *Salvia* sects. *Audibertia* and *Echinosphace* are related to subg. *Calosphace*, as first suggested by Epling (1938). Here, we employ molecular phylogenetic approaches using both nuclear and cpDNA and thorough taxon sampling to investigate the origins and affinities of *Salvia* sections *Audibertia* and *Echinosphace*. We then use the resulting phylogenetic framework to examine vegetative and floral character evolution, reconstruct ancestral biogeographic areas, and assess rates of diversification in context of the rise of the Mediterranean-like climate. Specifically, we address the following questions: 1) Do sections *Audibertia* and *Echinosphace* form a clade? 2) Are the two sections monophyletic, and if so do they warrant treatment as separate taxonomic entities? 3) What are the relationships of species within *Salvia* sections *Audibertia* and *Echinosphace*? 4) Is there evidence for convergent evolution in key vegetative and floral features? 5) Is there evidence that hybridization and/or introgression have been important in both on-going and past evolutionary histories of these western North American *Salvia* species? 6) What is the ancestral biogeographical area for sections *Audibertia* and *Echinosphace*, and how have these species diversified in the CA-FP and adjacent areas?

## Materials and Methods

***Taxa and Gene Sampling***—A total of 99 accessions were sampled as part of this study, including all species from *Salvia* sections *Audibertia* (15) and *Echinosphace* (four). A total of 91 samples from sections *Audibertia* and *Echinosphace* were included in the molecular analyses. Multiple collections representing a wide geographical sampling were made from each species whenever possible. *Salvia chionopeplica* and *S. californica* were the only species not wild collected, but both were collected from cultivated plants grown from wild collected seed from known locations. We also included a morphologically distinctive population of *S. mohavensis* from the Cerro del Pinacate in northern Sonora, Mexico. All samples from *Salvia* sections *Audibertia* and *Echinosphace*, except this *S. mohavensis* accession and one collection of *S. carduacea*, were collected and identified by the first author. Hybridization is known or suspected to occur frequently within sect. *Audibertia* (e.g. Epling 1938; Emboden 1971; Neisess 1983; Meyn and Emboden 1987), and thus efforts were made to only collect individuals not exhibiting morphological evidence of introgression. One of the most taxonomically challenging and widespread species groups in sect. *Audibertia* is that comprising *S. pachyphylla* and *S. dorrii*. In addition to the work of Epling (1938) and Neisess (1983), Strachan (1982) completed a revision of these two species. A molecular analysis of the relationships between and among the varieties and subspecies of *S. dorrii* and *S. pachyphylla* is addressed in depth by Taylor and Ayers (2006). For the purposes of this study, two samples of *S. pachyphylla* and six samples of *S. dorrii* were included to help identify the placement of the *S. dorrii*/*S. pachyphylla* complex, rather than the relationships therein.

Based on earlier phylogenetic work within the subtribe Salviinae (Walker et al. 2004; Walker and Sytsma 2007; Drew and Sytsma 2011, 2012; Jenks et al. 2012), outgroup taxa included three samples of *Salvia* subg. *Calosphace* and one sample each of the Asian genera *Dorystaechas* and *Meriandra*. These groups have usually been found to be the closest lineages to the few California salvias included in these broader surveys. Also included as outgroups were two more distantly related species belonging to *Salvia* clades I (*Salvia roemeriana*) and III (*Salvia glutinosa*) (Fig. 2A, N; Walker and Sytsma 2007). *Lepechinia chamaedryoides*, a member of Mentheae subtribe Salviinae and sister (along with *Melissa*) to *Salvia* and related genera (Drew and Sytsma 2012), was thus used to root all trees. Specific information on collection locality, voucher information, and GenBank accession numbers are included in Appendix 1.

Analyses included two nuclear ribosomal DNA (nrDNA) regions, the internal and external transcribed spacers (nrITS and nrETS), and eight chloroplast regions, *trnL-trnF, trnG-trnS, psbA-trnH, atpB-rbcL, rps16,* 5′ and 3′ *trnK-matK, ycf1*, and the *ycf1-rps15* spacer. All 99 accessions in this study were sampled for nrITS, but we did not obtain nrETS sequences for eight taxa including three accessions of *Salvia apiana* (*JBW 3080, 3192, 3208*), two accessions of *S. carduacea* (*JBW 3091, 3176*) and one accession each of *S. munzii* (*JBW 3210*), *S. mohavensis* (*PW 504*), and *S. clevelandii* (*JBW 3216*). In the cpDNA data set only 27 accessions were sequenced for the large *ycf1* gene and *ycf1-rps15* spacer region. These two markers were added to improve support along the phylogenetic backbone, and at least one accession of each species was sampled for these markers. Additionally, we were only able to sample *Meriandra bengalensis* for the *trnL-F, ycf1*, and *ycf1-rps15* spacer regions. *Salvia patens* and *S. axillaris* only included data from the *trnL-trnF, psbA-trnH, ycf1*, and *ycf1-rps15* spacer regions. For the *atpB-rbcL* spacer, sequence data were not collected for *S. cedrocensis* (*JBW 2539*). For the *trnS-trnG* spacer, data were not obtained

for the following taxa: *S. sonomensis* (*JBW 3163*), *S. funerea* (*JBW 3131*), *S. clevelandii* (*JBW 3216* and *JBW 3079*), and *S. columbariae* (*JBW 3066*).

***Extractions, Amplification, and Sequencing***—Total genomic DNA was extracted using DNeasy plant mini kits (Qiagen, Valencia, California). Leaves used for DNA extractions were usually from fresh, -80°C frozen, or silica dried material, but in a few cases we used herbarium specimens. The nrITS, nrETS, *trnL-F*, *ycf1*, and *ycf1-rps15* spacer markers were amplified and sequenced using the primers described in Drew and Sytsma (2011). The *psbA-trnH* region used primers described in Walker and Sytsma (2007). The *atpB-rbcL* spacer was amplified and sequenced using the atpBE (Hodges and Arnold 1994) and rbcL346R (Olmstead et al. 1993) primer pair. The *trnG-trnS* spacer region was obtained using primers detailed in Shaw et al. (2005), and the *rps16* region was amplified and sequenced with the rps16F and rps16R2 primer pair from Oxelman et al. (1997). Two portions (both ends) of the *trnK-matK* region were sequenced using the primer pairs 2-trnK-3914F/ Sat16–880R (Johnson and Soltis 1994; Bräuchler et al. 2010) and Sat2–1780F/16-trnK-2R (Johnson and Soltis 1994; Bräuchler et al. 2010).

Thermal cycler protocols for polymerase chain amplification (PCR) and cycle sequencing followed procedures described elsewhere (Sytsma et al. 2002). The PCR product was cleaned with either QIAquick PCR purification kit (Qiagen) or with AmPure PCR purification kit (Agencourt, Beverly, Massachusetts). Sequenced products were cleaned with either CleanSEQ sequencing reaction clean-up system (Agencourt, Beverly, Massachusetts) or the Agencourt magnetic bead protocol (Agencourt). Contiguous alignments were manually edited using Sequencher v. 4.0 (Gene Codes, Ann Arbor, Michigan). For nrDNA sequences, double peaks were scored as ambiguous characters.

***Phylogenetic Analyses***—Sequences were aligned in MacClade 4.08a (Maddison and Maddison 2005). Three regions of ambiguous alignment in the *psbA-trnH* data set, including an inversion 18 nucleotides in length, were excluded from all analyses (78 base pairs in total). Phylogenetic relationships were evaluated using three data sets. The first data set employed two nrDNA markers, nrITS and nrETS, and included 99 accessions (91 samples from *Salvia* sects. *Audibertia* and *Echinosphace*). The second data set was a 54-accession subset of the larger nrDNA data set that permitted direct comparison to the third data set, 54 accessions of cpDNA data. These 54-taxa subsets included 46 samples of sects. *Audibertia*/*Echinosphace*, including at least one sample of each species, as well as the eight outgroup species. Alignments for all three datasets are available on TreeBASE (study number TB2:S16712).

All data sets were analyzed using maximum likelihood (ML) in Garli v. 2.0 (Zwickl 2006) and Bayesian inference (BI) using Mr. Bayes v.3.1.2 (Huelsenbeck and Ronquist 2001) and implemented on the **Cyberinfrastructure for Phylogenetic Research (**CIPRES) cluster (Miller et al. 2010). Prior to conducting ML analyses, we used Modeltest version 3.07 (Posada and Crandall 1998) to determine a model of evolution for each gene partition as suggested by the Akaike information criterion (AIC). For both nrITS and nrETS, GTR + Γ + I was suggested as the appropriate model and thus nrDNA was analyzed in Garli without partitions. For the cpDNA data set we used Garli to analyze our data set in eight partitions. The K81uf + Γ model was suggested for *trnG-S*, the GTR + I model for *trnL-F*, the TIM + Γ + I model for *psbA-trnH*, the TVM + I model for *atpB*, the TVM + Γ model for *rps16*, the GTR + Γ model for the 5′ portion of *trnK-matK* intron and the *ycf1* and *ycf1-rps15* spacer (these latter two regions were included together), and the TIM + Γ model for the 3′ portion of the *trnK-matK* intron. Other than partitioning our data by gene according to model of evolution, we used the default values for the Garli configuration files and conducted three independent search replicates to find the best tree. ML bootstrap (Felsenstein 1985) values were obtained using the same settings as the initial best tree search except we conducted one search replicate per bootstrap replicate (100). These 100 ML bootstrap trees were saved for subsequent topology tests. For BI, we conducted runs for 3,000,000 generations for all three data sets using the GTR + Γ + I model of evolution. For the cpDNA BI analysis we set the temp to 0.1 (as opposed to the 0.2 default), but all other parameters were kept at default settings except we did not automatically terminate our runs based on a pre-defined threshold (e.g. when the standard deviation of the split frequencies fell below 0.01). In all BI analyses, the standard deviation of the split frequencies fell below 0.01 in less than 1.8 million generations (cpDNA, 415,000 generations; 54-taxa nrDNA, 711,000 generations; 99-taxa nrDNA alignment, 1,800,000 generations). In addition, potential scale reduction factor (PSRF) values were ~1 in all analyses. In all three analyses we discarded the initial 25% of trees as burn-in; at this point mixing had been achieved in all runs.

To assess congruence between the 54 accession nrDNA and cpDNA data sets, 1,000 replicates of the partition homogeneity test (Farris et al. 1995) were conducted, as implemented in the ILD test of PAUP* 4.0b10 (Swofford 2003). The ILD test can be a useful tool as an initial assessment of congruence between data sets (Hipp et al. 2004). Discordant relationships were further examined visually between the phylogenetic trees from nrDNA and cpDNA to find accessions placed strongly (as evident by high bootstrap or PP values) in different subclades of the trees. We explored the impact on the ILD statistic of the removal of subsets of accessions that appeared to be discordant. In cases where accessions of the same species did not form a monophyletic clade (only in cpDNA trees), we examined the degree of support for non-monophyly by constructing sets of topologies in MacClade enforcing monophyly of each species. Topologies of cpDNA trees were also constructed in which individual species, discordant in position within cpDNA and nrDNA trees, were placed in the alternative position evident in the nrDNA trees. We used the likelihood-based Shimodaira-Hasegawa (1999) method in PAUP* to test for significant differences in tree length within sets of trees each containing an enforced topology tree and the 100 ML bootstrap trees obtained from the Garli searches. We used 10,000 RELL bootstrap replicates for each Shimodaira-Hasegawa (SH) test following the methods of Hipp et al. (2004).

***Ancestral Character State Reconstruction***—Four floral or vegetative features were examined for character evolution within sects. *Audibertia* and *Echinosphace* using the inferred phylogenetic framework based on nrDNA: habit (annual vs. perennial (sub)shrub), leaves and calyx (spiny vs. not spiny), calyx lobing (obviously 3–5 lobed vs. no lobing), and functional thecae per stamen (one vs. two). The ML reconstructions were implemented in BayesTraits v.1.0 (Pagel and Meade 2007) using the MultiState function and sampling across the 100 ML bootstrap trees. Tips of the trees were pruned in R version 3.1 (R Development Core Team 2014) so that a single representative accession of each California *Salvia* species (chosen randomly) was left. We retained only the first two clades of related outgroups; the three species representing *Salvia* subg. *Calosphace* and the small, Old World genera *Dorystaechas* and *Meriandra*. To ensure capturing the best signal of character variation near the base of the species-rich subg. *Calosphace*, we sampled species representing the first two diverging lineages of subg. *Calosphace* (Jenks et al. 2012). *Salvia axillaris* is the sole member of one lineage (sect. *Axillares*); *Salvia patens* (sect. *Blakea*) is one of a few members of the second lineage; the "Hastatae clade" (sects. *Blakea* and *Hastatae*). We also added *S. cedrocensis* (sect. *Flocculosae*) as a representative of the remaining, diverse group of ca. 500 species of subg. *Calosphace*. We used the branch scaling parameter (k) to adjust the weight of branch lengths in the model and allow it to take its maximum likelihood (Pagel 1994) for each ML tree. Additionally, we explored ancestral character state reconstruction with the same set of ML trees but adjusted to be ultrametric. We used the semi-parametric penalized likelihood (PL) approach (Sanderson 2002), as implemented in "ape" (Paradis et al. 2004), and used the chronopl function (lambda = 0.1). Ancestral reconstruction of character states under ML was depicted with pie charts indicating state probabilities at each node in the nrDNA tree.

***Biogeographical Reconstruction and Diversification Analyses***—We conducted ancestral area estimation using the dispersal-extinction-cladogenesis (DEC) models as implemented in the recently developed program BioGeoBEARS (Matzke 2013, 2014). Similar to the program LaGrange (Ree et al. 2005; Ree and Smith 2008), BioGeoBEARS evaluates ML parameters for anagenetic events involving range expansion and extinction, and for cladogenetic events involving sympatry and vicariance. Unlike LaGrange, BioGeoBEARS also parameterizes cladogenetic "founder-events" (Templeton 1980) by incorporating the J parameter for "jump-dispersals". The DECj models have been shown to be significantly better than DEC models for island groups (Matzke 2014) and for intercontinental distributions (Spalink et al. in press), but they have not been evaluated for more localized species distributions such as within the CA-FP and associated regions.

Geographical distributions of species from *Salvia* sections *Audibertia* and *Echinosphace* were obtained from the Jepson Online Interchange California Floristics database (http://ucjeps.berkeley.edu/interchange/), using only the verified distributional records. These data were augmented as needed (e.g. California Baja species) with the information provided by Epling (1938, 1940) and Neisess (1983). The *S. mohavensis* accession from the Cerro del Pinacate in Northern Sonora, Mexico was included, as were the two species restricted to Baja, Mexico (*S. californica, S. chionopeplica*). We utilized the biogeographical subdivisions described in *The Jepson manual: Vascular plants of California* (Hickman 1993) as updated in The Jepson Flora Project (2014). Eight broad regions were used: northwestern California (NW), Cascade Ranges (CaR), Sierra Nevada (SN), Great Central Valley (GV), central western California (CW), southwestern California (SW), Great Basin (GB), and desert (D). As one species of *Salvia*

(*S. californica*) occurs in both the Sonoran and Baja Deserts of Baja California, we combined distributions of the Mojave, Sonoran, and Baja Deserts.

The nuclear DNA ML tree was used for ancestral area reconstruction. As described above, tips of the trees were pruned in R version 3.1 (R Development Core Team 2014) so that a single representative accession of each of the 19 *Salvia* species was left. As the outgroup taxa sampled all occur outside of these 10 regions and thus provide no resolution of ancestral area for sects. *Audibertia* and *Echinosphace*, we restricted the analyses to only these two sections. We implemented the PL approach (Sanderson 2002) in "ape" (Paradis et al. 2004) using the function chronopl (lambda = 0.1) to generate an ultrametric tree as required by BioGeo BEARS. In chronopl, the stem and crown of the clade containing sects. *Audibertia* and *Echinosphace* were dated at 15.5 my (19.1–11.9, 95% confidence interval) and 11.2 my (15.6–6.6, 95% confidence interval), respectively, based on a BEAST analysis of the tribe Mentheae (Drew and Sytsma 2012). To obtain confidence intervals for dates obtained for each node, we ran chronopl separately with maximum and minimum values from the 95% confidence intervals. We implemented conservative settings in BioGeoBEARS: a single time interval, dispersal probabilities of 1.0 for all areas, maximum range size at eight areas.

We measured diversification rates in *Salvia* sections *Audibertia* and *Echinosphace* using BAMM (Bayesian analysis of macroevolutionary mixtures) v2.0 (Rabosky et al. 2014). We utilized the chronogram from chronopl with complete species sampling, four independent chains of 300,000,000 generations each, and assessed convergence and effective samples sizes using the R package CODA (Plummer et al. 2006). The diversification model with the highest Bayes factor score was used as the overall best model. Rates of speciation, extinction, and net diversification were evaluated in BAMMtools and compared to rates from a recent analysis of CA-FP *Salvia* (and 15 other clades) but with limited taxa and only nrITS sampling (Lancaster and Kay 2013).

## Results

***nrDNA Analyses***—The total aligned length of the 99-taxa nrDNA data set was 1,184 base pairs; the nrITS alignment was 717 characters and the nrETS alignment accounted for 467 characters. Further information is shown in Table 2. Overall, the number of polymorphic sites (clear double sequence peaks) was low, ranging from 0% up to 1.3% (*Salvia sonomensis JBW 2519*). For one accession, *S. clevelandii* (*JBW 2508*), 1.8% of the nucleotide positions were scored as ambiguous, but this was due to poor ETS sequence quality as opposed to clear polymorphic sites. The observed polymorphic sites were generally random and did not appear related to putative hybridization events. All character scoring and polymorphic sites in our alignments can be seen on TreeBASE (study number S16712). The 91 accessions from *Salvia* sects. *Audibertia* and *Echinosphace* formed a clade with 55% bootstrap support (BS) in the ML analysis and 0.67 posterior probability (PP) in the Bayesian analysis (Fig. 3). The seven accessions of *Salvia* sect. *Echinosphace* formed a clade (81% BS; 0.99 PP) that was sister to a clade containing all 84 accessions of *Salvia* sect. *Audibertia* (100% BS; 1.00 PP). These two western North American *Salvia* sections were sister to the large American *Salvia* subg. *Calosphace* (100% BS; 1.00 PP). The Eurasian genera *Dorystaechas* and *Meriandra* then formed the sister clade to *Salvia* sects. *Audibertia* and *Echinosphace* and subg. *Calosphace* (99% BS; 1.00 PP). Representatives of other Eurasian lineages within *Salvia* were more distantly related.

Within *Salvia* sect. *Echinosphace*, *Salvia carduacea* was monophyletic (100% BS; 1.00 PP) and sister to a clade (100% BS; 1.00 PP) consisting of two subclades. The first contained the two accessions of monophyletic *S. funerea* (100% BS; 1.00 PP) and the second contained an accession each of two narrowly distributed taxa, *S. californica* and *S. greatae* (90% BS; 0.97 PP).

Within *Salvia* sect. *Audibertia*, all but two species for which we included multiple accessions proved monophyletic with BS support and PP above 85% and 0.95, respectively. *Salvia pachyphylla* and *S. dorrii* formed a moderately well supported clade (85% BS; 1.00 PP), but neither species was reciprocally monophyletic. The 13 accessions of *S. columbariae* were monophyletic (100% BS; 1.00 PP) and sister to all remaining taxa ("core *Audibertia*") of sect. *Audibertia* (100% BS; 1.00 PP). The one accession of *S. chionopeplica* formed a strong clade (100% BS; 1.00 PP) sister to five accessions comprising a strongly monophyletic *S. leucophylla* (100% BS; 1.00 PP). These two species were in turn sister to the remainder of sect. *Audibertia*. Within the remaining subclade of sect. *Audibertia*, the distinctive accession of *S. mohavensis* from Sonora, Mexico formed a clade (100% BS; 1.00 PP) sister to the other three accessions of *S. mohavensis*. The three sampled accessions of *S. apiana* var. *compacta* were polyphyletic within a strongly monophyletic *S. apiana* (16 accessions). The reduced nrDNA phylogeny (54 accessions) is depicted in Fig. 4A (and Fig. S1 as online supplementary data). Relationships in the reduced tree were similar to that derived from the expanded data set (Fig. 3), differing only in a few weakly supported areas of both trees.

***Chloroplast DNA Analyses***—The cpDNA data alignment totaled 11,465 characters, with the *ycf1* gene and *ycf1-rps15* spacer region accounting for 5,184 positions of the alignment. The alignment partitions and variability are summarized in Table 2. In the cpDNA analyses (54 accessions), relationships among the major groups (*Salvia* sects. *Audibertia* and *Echinosphace*, *Salvia* subg. *Calosphace*, other genera and *Salvia* subclades) are well supported and mirror those found with nrDNA (Fig. 4B; and Fig. S2 as online supplementary

Table 2. Comparison of DNA sequence length, variation, and phylogenetic information content for different regions of nuclear ribosomal DNA (91 taxa) and chloroplast DNA (46 taxa) among Californian *Salvia* species (ingroup taxa only).

| Gene region | Total Characters | Variable characters | Parsimony informative |
|---|---|---|---|
| nrITS | 717 | 128 | 107 (14.9%) |
| nrETS | 467 | 139 | 117 (25.0%) |
| nrDNA | 1,184 | 267 | 224 (18.9%) |
| *trnL-F* | 877 | 10 | 5 (0.6%) |
| *trnG-trnS* | 1,535 | 49 | 35 (2.3%) |
| *psbA-trnH* | 407 | 30 | 18 (4.4%) |
| *atpB-rbcl* | 1,091 | 23 | 16 (1.5%) |
| *rps16* | 889 | 19 | 17 (1.9%) |
| *trnK* 1 | 816 | 13 | 12 (1.5%) |
| *trnK* 2 | 663 | 16 | 15 (2.3%) |
| *ycf1*, *ycf1-rps15* (19 taxa) | 5,184 | 251 | 89 (1.7%) |
| all cpDNA | 11,462 | 411 | 207 (1.8%) |
| all regions | 12,646 | 678 | 431 (3.4%) |

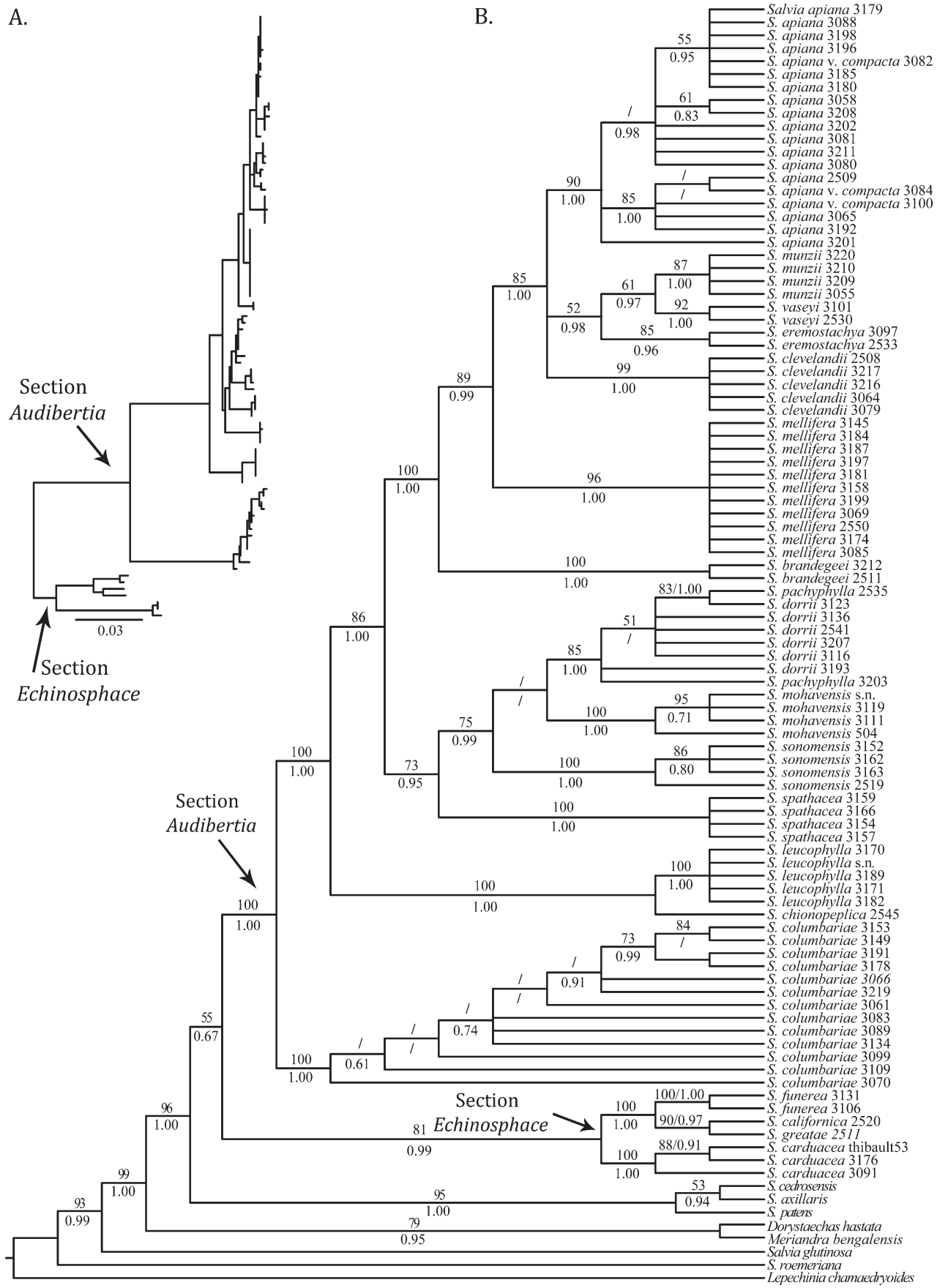


FIG. 3. Maximum likelihood (ML) trees showing relationships among 91 accessions of *Salvia* sects. *Audibertia* and *Echinosphace* as inferred from nuclear ribosomal ITS and ETS. A. ML phylogram showing branch lengths. B. ML cladogram with ML bootstrap values > 50% ) and Bayesian inference posterior probability (> 0.6) support values shown on branches. A slash (/) is used for all values lower than these minima.

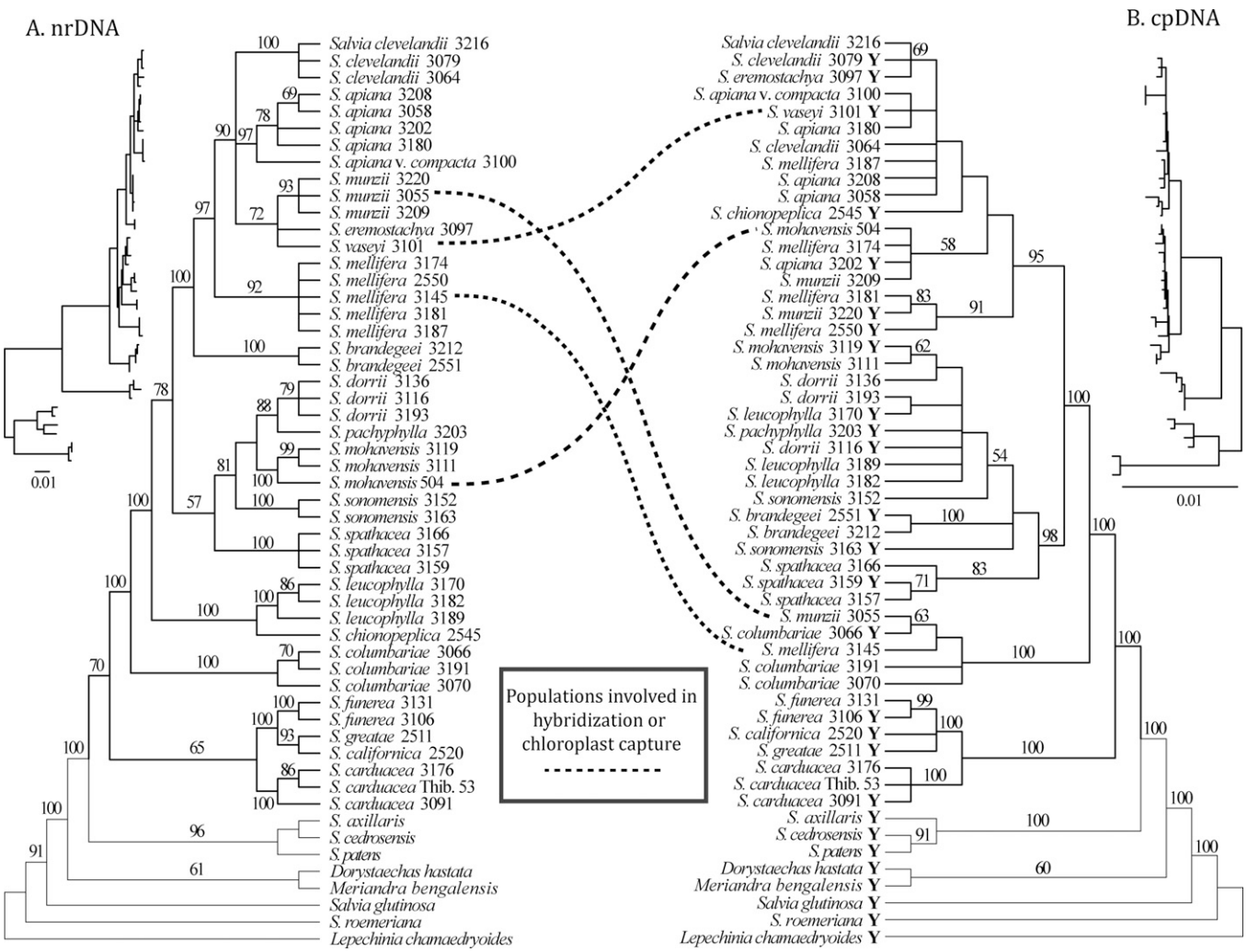


FIG. 4. Maximum likelihood (ML) trees for 54 accessions of *Salvia* sects. *Audibertia* and *Echinosphace* and outgroups illustrating incongruence of the (A) nuclear ribosomal DNA and (B) chloroplast DNA phylogenies. ML trees are shown as cladograms with thinner branches among the outgroups. Smaller offset topologies illustrate branch lengths in ML phylograms; the offset phylograms have outgroups removed and depict only *Salvia* sects. *Audibertia* and *Echinosphace*. Support values are ML bootstrap. Y indicates that *ycf1/ycf1-rps-15* was sequenced for the accession. Hybridization and possible chloroplast capture events demonstrated in individual samples of *S. mellifera*, *S. mohavensis*, *S. munzii*, and *S. vaseyi* are highlighted.

data). *Salvia* sects. *Audibertia* and *Echinosphace* together formed a clade with high support (100% BS; 1.00 PP). In turn, the respective sections were both recovered as monophyletic (100% BS; 1.00 PP). Within *Salvia* sect. *Echinosphace*, a monophyletic *S. carduacea* (100% BS; 1.00 PP) was sister to a clade (100% BS; 1.00 PP) consisting of *S. greatae*, *S. californica*, and *S. funerea*. Relationships among the latter three taxa were unresolved, but the two accessions of *S. funerea* formed a clade (99% BS; 1.00 PP). Within *Salvia* sect. *Audibertia*, a clade (100% BS; 1.00 PP) of three accessions of *S. columbariae*, one accession of *S. munzii*, and one accession of *S. mellifera* were sister to the remainder of the section (100% BS; 1.00 PP). Within the remainder of the section, two main clades were recovered with high statistical support (99–100% BS; 1.00 PP), but relationships within each of those two clades were mostly poorly supported (BS < 70; PP < 0.8). Of the 15 species in *Salvia* sect. *Audibertia*, only three were recovered as monophyletic, *S. spathacea* (83% BS; 1.00 PP), *S. brandegeei* (100% BS; 1.00 PP), and *S. mohavensis* (62% BS; 0.99 PP).

***Incongruence Between nrDNA and cpDNA Analyses***— The partition homogeneity test of the nrDNA and cpDNA data sets suggested significant incongruity between the data sets ($p < 0.001$). The significant topological incongruity is immediately obvious in a comparison of the nrDNA and cpDNA trees (Figs. 4, S1, S2) and suggests hybridization events, chloroplast capture, or other events leading to non-dichotomously branching molecular evolution. For these reasons, an analysis combining the cpDNA data with the nrDNA data was not performed.

Almost all of the incongruence between the nrDNA and cpDNA data sets resides in sect. *Audibertia* and exhibits three patterns. First, some topological differences seen between the nrDNA and cpDNA trees (Fig. 4) within sect. *Echinosphace* (and subg. *Calosphace*) can be attributed to branches with low support values, generally in the cpDNA tree. Of the 11 (out of 15) species represented by more than one accession, only *Salvia spathacea* and *S. brandegeei* are monophyletic in the cpDNA tree (12 of 14 are monophyletic in the nrDNA tree; Fig. 4). *Salvia columbariae* perhaps could be added as a third because it is narrowly paraphyletic with accessions of two other species imbedded within its cpDNA lineage. Of the remaining eight species appearing non-monophyletic in the cpDNA tree,

the SH likelihood topology test is unable to support the non-monophyly of four (e.g. *p* values for *S. clevelandii*, *S. dorrii*, and *S. sonomensis* are > 0.484, > 0.148, and > 0.087, respectively).

A second pattern of incongruence is seen in which accessions of a species are not monophyletic in the cpDNA trees and the incongruence is statistically supported. The SH likelihood topology tests of cpDNA trees provide significant evidence for non-monophyly of four species (*S. munzii*, $p < 0.001$; *S. mohavensis*, $p < 0.008$; *S. mellifera*, $p < 0.01$; and *S. apiana*, $p < 0.037$). In each of these cases, incongruence

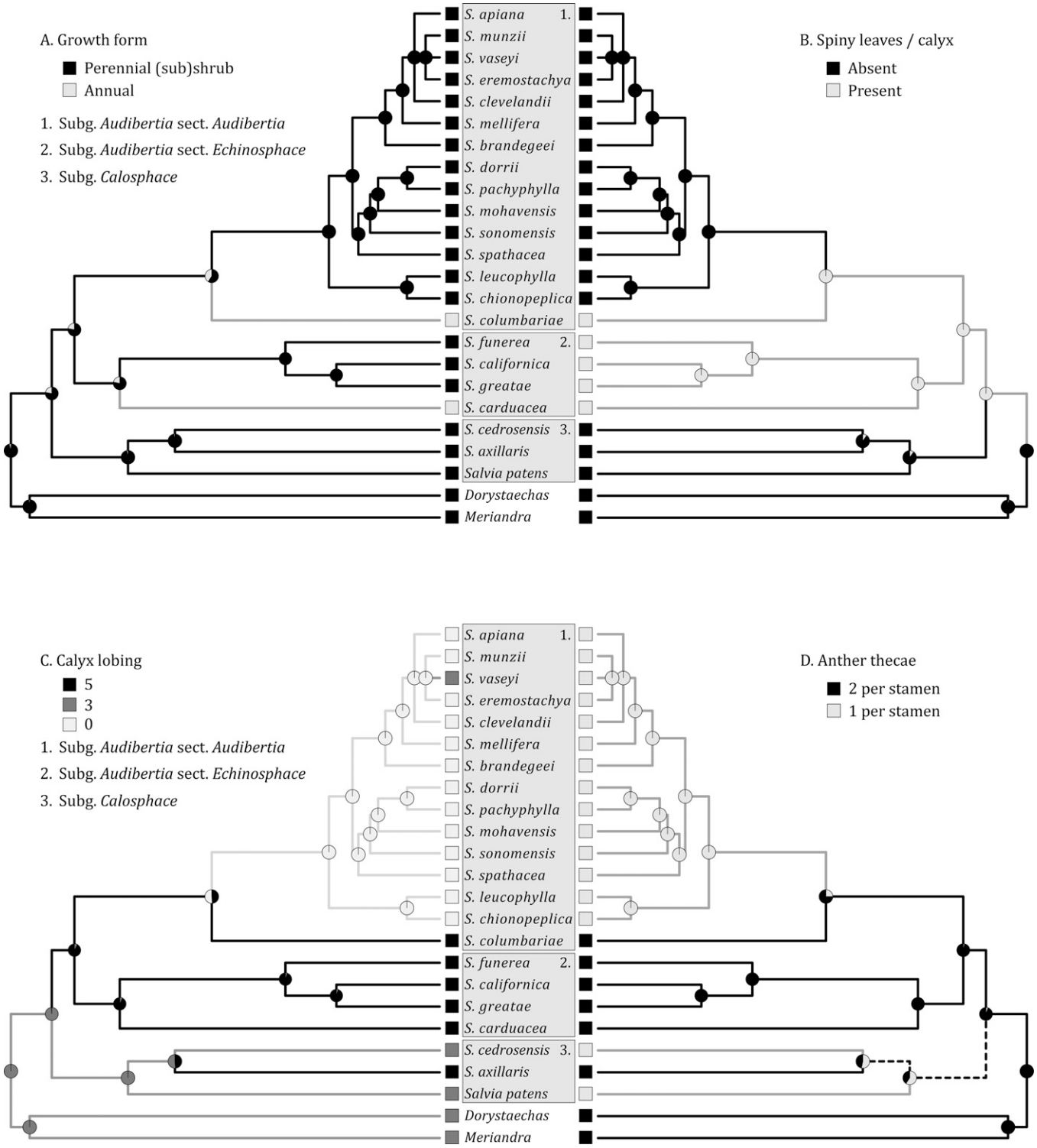


FIG. 5. Ancestral character state reconstruction within the California *Salvia* sects. *Audibertia* and *Echinosphace* and their two closest relatives, the New World Salvia subg. *Calosphace* and Old World genera *Dorystaechas* and *Meriandra*. A. Growth form. B. Presence of spines on leaves and/or calyx. C. Calyx lobing (unordered). D. Number of thecae per stamen. The topology is the ML tree converted to ultrametric form. Pies at each node depict the proportion of each state based on ML estimation in BayesTraits. For clarity in viewing character reconstruction, branches are color-coded by the most likely state using ML. Dashed lines indicate equivocal reconstruction.

contributing to the discordance between nrDNA and cpDNA relationships is likely due to hybridization and perhaps subsequent chloroplast capture (Fig. 4; see Discussion for more details). An accession each of *S. mellifera* and *S. munzii* appear to have captured the cpDNA of *S. columbariae*. *Salvia vaseyi* has its maternal (cpDNA) contribution from within *S. apiana*. The Mexican Sonoran accession of *S. mohavensis* shares a chloroplast genome with one accession each of *S. apiana*, *S. mellifera*, and *S. munzii*. A third pattern of incongruence is the placement of species such as *S. brandegeei*, monophyletic

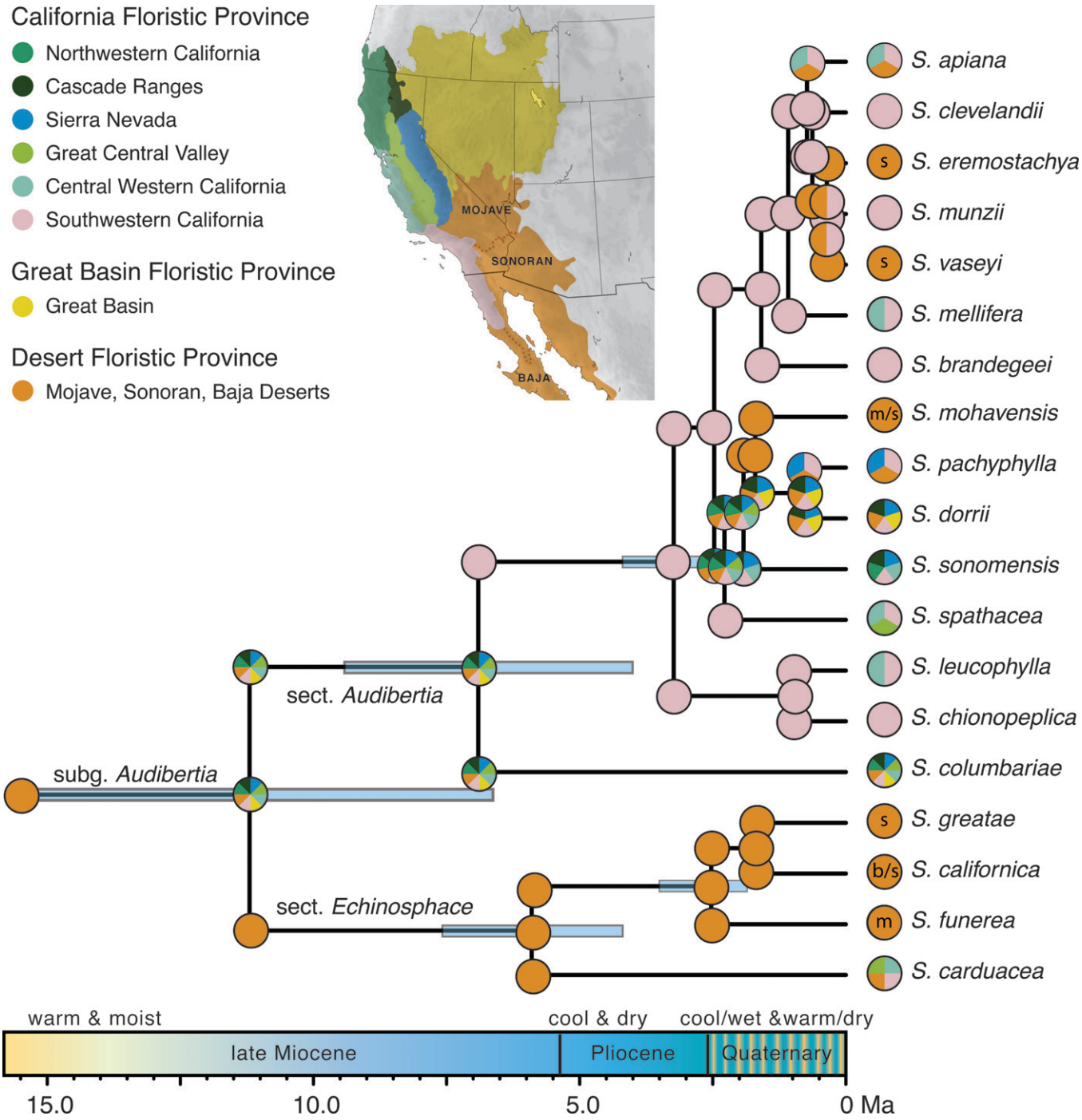


FIG. 6. Ancestral area reconstruction within the California *Salvia* sects. *Audibertia* and *Echinosphace*. Outgroup species from subg. *Calosphace*, inhabiting other biogeographical areas (central Mexico or South America) have been removed for clarity. Depicted is the DEC ML reconstruction using BioGeoBEARS with six CA-FP regions, a combined desert region province, and the Great Basin Floristic Province. Areas occupied by extant species are shown in the circles next to names. Ancestral area reconstructions (single or combined areas) are shown with circles at nodes and the inherited areas for both daughter lineages are depicted with circles at corners. The chronogram is derived using secondary dates from a fossil-calibrated chronogram of Mentheae (Drew and Sytsma 2012). Blue bars represent 95% confidence time intervals around the mean date at each node (only the five oldest nodes portrayed). Climatic reconstruction of California is based on Burge et al. (2011) and the map of biogeographic provinces modified after The Jepson Flora Project (2014).

in both analyses, in different clades of the nrDNA and cpDNA trees. The SH test of cpDNA topologies, to mirror the placement of *S. brandegeei* as seen in the nrDNA tree, gives significant $p$ values (< 0.034) for rejecting the null hypothesis of no difference between trees.

***Ancestral Character State Reconstruction***—The ancestor of the California sages appears to have been a woody perennial with spiny leaves and/or calices, a distinctively lobed calyx, and two anther thecae per each of its two stamens (Fig. 5). The shift to annual habit is seen in only two species, *Salvia columbariae* and *S. carduacea*, both sister to the remainder of their respective sections (sects. *Audibertia* and *Echinosphace*). The ML character reconstruction indicates two separate origins of the annual habit, although simple parsimony reconstruction would be equivocal (Fig. 5A), with either separate origins within each section, or a single origin at the crown of sects. *Audibertia* and *Echinosphace*. The presence of pronounced spines (leaf or calyx edges, or both) is plesiomorphic for the California sects. *Audibertia* and *Echinosphace* (Fig. 5B). A loss of these spines characterizes all species of sect. *Audibertia,* except the early diverging *S. columbariae*. Calyx lobing is variable within *Salvia*, but generally a bilabiate calyx with either three or five pronounced lobes is seen. The plesiomorphic condition in sects. *Audibertia* and *Echinosphace* is the 5-lobed calyx, which is seen in all members of sect. *Echinosphace* and in early diverging *S. columbariae* of sect. *Audibertia* (and in some members of closely related subg. *Calosphace*) (Fig. 5C). The remainder of sect. *Audibertia* have lost all calyx lobing, with the exception of *S. vaseyi* that has a slightly 3-lobed calyx. Similar to the distribution of calyx lobing, loss of theca functionality occurs in all species of sect. *Audibertia* except *S. columbariae* (Fig. 5D). Shifts from two to one functional anther theca are seen in other *Salvia* clades (e.g. subg. *Calosphace*; Fig. 5D).

***Biogeographical Reconstruction***—Ten of the 19 species are restricted to one biogeographic region, six to the desert region and four to the southwestern California (SW) region (Fig. 6). Five species are confined to only two or three regions, with the SW and central western California (CW) regions involved in five and four of these species, respectively. Four species are relatively widespread and occur in four or more of the eight regions, with *Salvia columbariae* found in all eight regions. In BioGeoBEARS, no significant improvement in the likelihood score of the model was seen when the "jump dispersal" parameter was added (DECj) vs. without (DEC), as indicated by a likelihood ratio test (DEC LnL -87.53138, DECj LnL = - 87. 53197, df = 1, $p$ = 0.441). Ancestral area reconstruction under the DEC model, illustrating the ML most probable ancestral area for each node and corner, is portrayed in Fig. 6. The desert region is the most probable ancestral area for the stem node for the clade comprising sects. *Audibertia* and *Echinosphace*, whereas the ancestral area for the crown is widespread. The desert region is the dominant biogeographic region for sect. *Echinosphace*, but within the diversification of sect. *Audibertia* it is important only within the last two my. The SW region is the dominant biogeographic region for sect. *Audibertia* during the last five my of crown group diversification. The BAMM analysis of diversification through time indicated no significant shifts in diversification over both sects. *Echinosphace* and *Audibertia*. An overall decrease in speciation rate, but a slight increase in extinction rate is seen over the approximately 11 million year history of the group (Fig. 7).

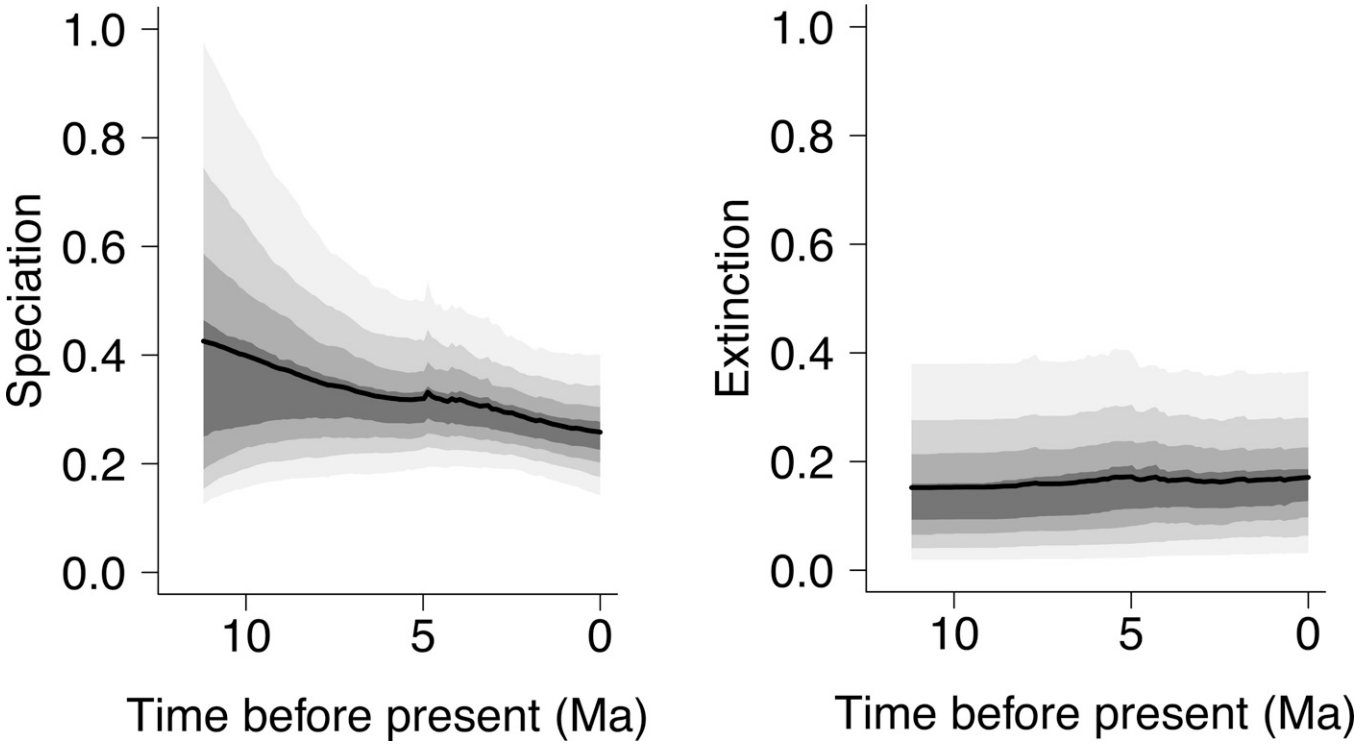


FIG. 7. Temporal dynamics in rates of speciation and extinction for California *Salvia* sects. *Audibertia* and *Echinosphace* based on BAMM analysis. Gray polygons indicate the 0.45–0.90 quantiles on the distribution of rates (increments of 0.15). Median values are shown in black.

## Discussion

The phylogenetic tree (Figs. 1, 3, 4A) generated by the combined nuclear ribosomal DNA data sets provides the most detailed and well supported view of phylogenetic relationships within California *Salvia* and their relationships to other clades within *Salvia*. These results are congruent with (but greatly expanded with more comprehensive species sampling) the relationships of California *Salvia* evident in wider taxonomic studies involving *Salvia* and other genera of tribe Mentheae (Walker et al. 2004; Walker and Sytsma 2007; Drew 2011; Drew and Sytsma 2011, 2012, 2013) as well as morphology. The cpDNA tree (Fig. 4B), although similar in many respects to the nrDNA tree, shows a clear history of chloroplast capture and/or introgression. Thus, most of the subsequent discussion dealing with species relationships, taxonomic considerations, biogeography, and character evolution will be based primarily on the nrDNA phylogeny but with support from the cpDNA phylogeny when possible.

## Taxonomic Treatment

The results of these analyses, along with other lines of evidence, support the subgeneric status of the Californian clade of *Salvia*.

***Status of sects. Audibertia and Echinosphace***—Importantly, both data sets support a single California *Salvia* clade of two monophyletic and sister subclades that comprise the sects. *Audibertia* and *Echinosphace*, respectively. These findings support Epling's (1938) inclusion of both as sect. *Audibertia* and disagree with Neisess' (1983) claim that the two were each related to other *Salvia* groups. This California clade is sister to the large *Salvia* subg. *Calosphace* that ranges from Central to South America, again confirming Epling's (1938) proposition of their close relationship.

Two different nomenclatural changes could be warranted for the California *Salvia* clade based on our phylogenetic results: the elevation of sect. *Audibertia* (sensu Epling) 1) to subgeneric rank within *Salvia* (becoming *Salvia* subg. *Audibertia*), or 2) to generic level, at which point the name *Audibertia* would be invalid and priority would be given to the name *Ramona* Greene (1892). When Bentham (1833) described the genus *Audibertia* in his monograph of the Labiatae, he reused the name from another genus that he had created earlier, subsequently subsumed into the genus *Mentha*, and thus created an improper synonomy (Neisess 1983). Based on our results above, and to simplify the nomenclatural changes, we formally recognize all 19 species of sects. *Audibertia* and *Echinosphace* (sect. *Audibertia* sensu Epling, 1938) as subgenus *Audibertia*, a designation not previously proposed in the literature. This decision is based first on the strong evidence provided here of the monophyly of sects. *Audibertia* and *Echinosphace* and of the sister relationship of these two sections combined to subgenus *Calosphace* (Walker and Sytsma 2007). It is also based on our view that the genus *Salvia* should not be fragmented into many smaller genera, but should be expanded by including the five small genera sister to subclades within *Salvia* (Walker et al. 2004; Walker and Sytsma 2007). This approach provides the opportunity to leave unchanged Neisess' (1983) designations and circumscriptions of sect. *Echinosphace* and sect. *Audibertia* (both now sections within the subgenus *Audibertia*; Table 1). However, his named subsections and series within each are not supported.

**Salvia subgenus Audibertia** (subg. nov.) J. B. Walker, B. T. Drew, & K. J. Sytsma

*Audibertia* Benth., in Bot. Reg. 17: 1469. 1831. Based upon *A. incana* (not Benth., op. Cit. 15: 1282. 1829)

*Ramona* Greene in Pittonia 2: 235, 301. 1892. Based upon *Audibertia polystachya* Benth., Lab. Gen. Et Sp. 314. 1833.

*Aubertiella* Briq., Bull. Herb. Boiss. 2: 73. 1894. Based upon *Audibertia* Benth.

***Relationships and Origin of Salvia Subgenus Audibertia***—Previous molecular studies, although with limited taxon sampling, have supported a clade within Mentheae subtribe Salviinae consisting of *Dorystaechas*, *Meriandra*, *Salvia* subg. *Calosphace*, and *Salvia* subg. *Audibertia* (Fig. 2; Walker et al. 2004; Walker and Sytsma 2007; Drew and Sytsma 2011, 2012). However, within that clade, it has been unclear as to the relationships among these four lineages. The results presented here support the Asian genera *Meriandra* and *Dorystaechas* as sister taxa, and together sister to a clade consisting of the New World *Salvia* subg. *Audibertia* and subg. *Calosphace*. The nrDNA and cpDNA analyses independently support these relationships.

Previous researchers have suggested various affinities of *Salvia* subgenus *Audibertia*. Epling (1938) primarily used staminal characters as a basis for his assertions that sects. *Audibertia* and *Echinosphace* were monophyletic and that they were most closely related to *S. axillaris* of subg. *Calosphace*. In contrast, Neisess (1983) used morphological and phytochemical data to argue that sect. *Echinosphace* was most closely related to *S. roemeriana* and other Old World members of *Salvia* subg. *Leonia* (*Salvia* clade III; Fig. 2N). Likewise, he argued that sect. *Audibertia* was most closely related to the Old World genus *Rosmarinu*s (Salvia clade I; Fig. 2C). The results from this study are in agreement with previous molecular work (Walker et al. 2004; Walker and Sytsma 2007) that supports Epling's (1938) assertions of a monophyletic subg. *Audibertia* sister to subg. *Calosphace* (*Salvia* clade II; Fig. 2); these results do not support the views of Neisess (1983).

A further testament to Epling's remarkable insight into this difficult group of plants is the fact that molecular investigations into subg. *Calosphace* (Walker 2006; Walker and Sytsma 2007; Jenks et al. 2012; Drew and Sytsma 2011, 2012) indicate that the Mexican *S. axillaris*, which Epling (1938) saw as a link to California subg. *Audibertia*, occupies a critical position in early diverging clades of subg. *Calosphace*. Chloroplast DNA analyses (Figs. 4B, S2; Jenks et al. 2012) place *Salvia axillaris*, the lone member of sect. *Axillares*, sister to all other species in subg. *Calosphace*, and then followed by sections of the small, largely Mexican "hastate clade" (*S. patens* in this study). Nuclear DNA analyses (Figs. 3, 4A, S1; Drew and Sytsma 2011, 2012) place *S. patens* and other members of the "hastate clade" as first diverging, then with *S. axillaris* diverging. *Salvia axillaris* may be the single most likely species within subg. *Calosphace* to share plesiomorphic characters with subg. *Audibertia*, as exemplified by its staminal form (Figs. 2G, 5D). Staminal evolution within the broadly defined *Salvia* exhibits recurrence of the unique, elongated anther connective and modifications of the posterior anther thecae (Walker et al. 2004; Walker and Sytsma 2007), presumably in co-evolutionary response with both insect and bird pollinators (Grant and Grant 1964; Faegri and Van Der Pijl 1979; Huck 1992; Claßen-Bockhoff et al.

2003; Reith et al. 2007; Wester and Claßen-Bockhoff 2006, 2007). Despite this recurrence, it is clear that staminal form provides strong signal for the close relationships of all California *Salvia*, and they to the Neotropical subg. *Calosphace* (Figs. 2, 5D), as first proposed by Epling (1938).

***Biogeographical Diversification*—**The molecular phylogenies of *Salvia* and relatives presented here and in earlier papers (Walker et al. 2004; Walker and Sytsma 2007; Drew and Sytsma 2012, 2013) strongly suggest an Asian origin of the subgenera *Calosphace* and *Audibertia*. Together, these two subgenera are sister to the central Asian genera *Dorystaechas* and *Meriandra*. The clade consisting of all four lineages (*Dorystaechas*, *Meriandra*, *Salvia* subg. *Calosphace* and subg. *Audibertia*) is in turn sister to a group of predominantly Asian members of the genus *Salvia* and the central Asian genus *Zhumeria* (*Salvia* clade III; Fig. 2) (Walker and Sytsma 2007). That larger clade is then sister to a group of European and Asian *Salvia*, the Asian genus *Perovskia*, and the European genus *Rosmarinus* (*Salvia* clade I; Fig. 2) (Walker and Sytsma 2007).

Because all members of *Salvia* subg. *Audibertia* and subg. *Calosphace* are native to the New World and their successive sister groups are Asian, the clade comprising subg. *Calosphace* and *Audibertia* is likely the product of a single dispersal event from Asia to the New World. Three lines of evidence suggest that the dispersal event was likely to the west coast of North America or Mexico. First, subg. *Audibertia* is restricted to the CA-FP and adjacent deserts and Great Basin. Second, *S. axillaris, S. patens,* and other early diverging lineages of subg. *Calosphace* (sects. *Standleyana* and *Blakea*) are native to western parts of central Mexico (Walker et al. 2004; Walker and Sytsma 2007). Third, this scenario of a dispersal event from Asia to the west coast of North America is supported by the presence of Miocene pollen grains collected on the west coast of North America that are assignable to subg. *Audibertia* (Emboden 1964; Barnett 1989).

Biogeographic area reconstruction for subg. *Audibertia* suggests an origin of the group during the late Miocene (Fig. 6). The separation of subg. *Audibertia* from subg. *Calosphace* is dated at about 15.2 Ma (19.1–11.9, 95% confidence interval). During this time interval in the mid-late Miocene, prior to the widespread Mediterranean-type and desert climates now seen in western North America (see below), the Madro-Tertiary Geoflora was already the dominant vegetation over much of southwestern U. S. A. and adjacent Mexico and probably even reached west-central California by the early Pliocene (Axelrod 1958). The Madro-Tertiary Geoflora consisted largely of semiarid, sclerophyllous trees and shrubs. During the middle Pliocene, these live-oak and conifer woodlands diminished and disappeared over areas that would later become the southwestern North American deserts in response to sharp decreases in rainfall as the Sierra Nevada Peninsular ranges were uplifted (Axelrod 1958). Thus, the early histories of both subg. *Audibertia* and *Calosphace*, with its first diversifying lineages restricted to central Mexico (see above, Walker et al. 2004; Walker and Sytsma 2007), are undoubtedly linked with the Madro-Tertiary Geoflora.

All four species of sect. *Echinosphace* occur in the Desert Floristic Province, although *Salvia carduacea*, sister to the other species, is more widespread in the CA-FP. Similarly, the widespread *S. columbariae*, sister to remaining species of sect. *Audibertia*, also occurs in deserts. Both of these sections appear to have crown diversified around 6–5 Ma near the Miocene/Pliocene border. This timing is consistent with the 5 Ma date when the first true desert conditions in western North America are thought to have originated (Axelrod 1973, 1989). The rise of the western North American deserts are linked to worldwide cool and dry climatic conditions (Graham 1999), active mountain building in western North America (Mix et al. 2011), and the development of the Sierra Nevada rain shadow over the eastern borders of the CA-FP (Wernicke et al. 1996; Mulch et al. 2008; Mix et al. 2011). The other desert species within sect. *Audibertia*, including three desert endemics and three more widespread species, all arose within the Quaternary (Fig. 6). These recent origins in *Salvia* are consistent with the findings of a meta-analysis of 337 putative neoendemics that the Desert and Great Basin provinces are composed of the youngest neoendemics on average (Kraft et al. 2010).

Extensive radiation into coastal sage or chaparral communities of southwestern California and, to a lesser extent, central western California floristic regions is only seen within *Salvia* sect. *Audibertia* (Fig. 6). The diversification of *Salvia* into these two regions began at around 3 Ma near the end of the Pliocene, and after the split with the widespread *S. columbariae*. This diversification included subsequent movements into other regions of the CA-FP and a number of shifts back into the Mojave and Sonoran Desert region. The transition to the Mediterranean-type climate and its associated vegetation communities was already occurring by the Early Quaternary in the west-facing coastal regions of the CA-FP (Axelrod 1973, 1975, 1977; Raven 1973; Raven and Axelrod 1978; Ackerly 2009). The diversification of *Salvia* sect. *Audibertia* and the development of Mediterranean-like communities thus appear correlated, as has been documented with other large radiations in the CA-FP (e.g. *Ceanothus*, Burge et al. 2011).

Our results contrast with those of Lancaster and Kay (2013) who sampled incompletely within *Salvia* subg. *Audibertia* and only with nrITS, used an older date for the crown of subg. *Audibertia* (14.5 vs. 11.1 Ma) but a younger date for the crown of sect. *Audibertia* (5.0 vs. 7.6 Ma), found that diversification of California *Salvia* occurred prior to 5 Ma, and thus argued that it was not linked to the rise of the Mediterranean-like communities (Axelrod 1989). The BAMM results presented here for all 19 species of *Salvia* subg. *Audibertia* indicate a fairly constant rate of net diversification over the group's 11 Ma history of shifts to both desert and mediterranean-like areas. The entire group exhibits a decreasing rate of speciation (average $\lambda = 0.310$ lineages/million years) and a slightly increasing rate of extinction (average $\mu = 0.178$ extinctions/million years). This pattern of diversification, slightly decreasing or constant speciation, is consistent with 12 of the other 15 angiosperm clades from the CA-FP examined by Lancaster and Kay (2013), although their analysis of *Salvia* was one of four exceptions.

***Phylogenetics and Character Evolution within Salvia Subgenus Audibertia*—**Within the two well-supported sections identified within *Salvia* subg. *Audibertia*, an annual species (*S. carduacea* and *S. columbariae*, respectively) is sister to the remaining perennial species. It is unclear whether subg. *Audibertia* is primitively annual with two subsequent shifts to the perennial habit, or, as ML character reconstruction suggests (Fig. 5A), there were two independent but early shifts to the annual habit within the California sages. The

annual *Salvia carduacea* is sister to the other three species in sect. *Echinosphace* in both nuclear and cpDNA analyses. Nuclear DNA supports a closer relationship of *S. californica* and *S. greatae* relative to *S. funerea*, but support for this relationship is lost with cpDNA.

Within sect. *Audibertia*, *Salvia columbariae* is recovered as sister to the remaining 14 species of the "core *Audibertia*". The chronogram (Fig. 6) indicates that the branch leading to the crown diversification of the "core *Audibertia*" occurred considerably later. In this regard, *S. columbariae* could be viewed as a "transitional" species between the two sections of subg. *Audibertia*. Besides the sharing of the annual habit with *S. carduacea*, *S. columbariae* retains the plesiomorphic features of the subgenus seen in all members of sect. *Echinosphace*; spiny leaves or calyx, the 5-lobed calyx, and two anther thecae per stamen (Fig. 5). As such, the remaining and much younger diversifying "core *Audibertia*" of sect. *Audibertia* is distinctive among the California sages with their lack of spines, almost complete loss of calyx lobing, reduction to one functional theca per stamen, and the propensity to hybridize. Even though almost all species within sect. *Audibertia* form monophyletic lineages in the nrDNA analyses, the "backbone" of the section is not fully resolved (Figs. 3, 4, S1). *Salvia mohavensis* and *S. sonomensis* are closely allied in both cpDNA and nrDNA analyses, and although not supported by strong support values, *S. mellifera*, *S. clevelandii*, *S. munzii*, *S. vaseyi*, *S. eremostachya*, and *S. apiana* form a monophyletic lineage in both the nrDNA and cpDNA analyses (excluding the two accessions putatively involved in chloroplast capture).

***Staminal Evolution in Subgenus Audibertia***—Within the tribe Mentheae, at least four independent shifts from four to two stamens have occurred (Drew and Sytsma 2012). The one shift within subtribe Salviinae is placed along the stem leading to the *Salvia* clade following separation of *Lepechinia* and *Melissa* (Fig. 2). As discussed in detail by Walker and Sytsma (2007), within this large (ca. 1,000 species) *Salvia* clade multiple origins have occurred of the distinctive and defining morphological character of the genus *Salvia*: the elongate connective tissue separating the two thecae of the stamen (Figs. 2, 5D). With each of the three independent origins of this character, a remarkably similar (convergent) progression in staminal form is seen (Fig. 2; Walker and Sytsma 2007). This progression includes shifts from the plesiomorphic state of the two thecae not separated or separated only slightly by connective tissue (*Perovskia* (Fig. 2D), *Meriandra* (Fig. 2J), *Dorystaechas* (Fig. 2K), and *Zhumeria* (Fig. 2L)), to the significant elongation of the connective with two fertile thecae produced (e.g. sect. *Salvia* (Fig. 2A), sect. *Axillares* (Fig. 2G), sect. *Echinosphace* (Fig. 2I), and sect. *Heterosphace* (Fig. 2M)), and then to the entire abortion of the posterior theca. In separate lineages (e.g. subg. *Sclarea* (Fig. 2B), subg. *Calosphace* (Fig. 2E), and *S. glutinosa* (Fig. 2N)), the aborted posterior thecae or elongate connective tissue fuse and help form the lever mechanism traditionally associated with the genus *Salvia*. Thus, California sect. *Echinosphace* and related New World subg. *Calosphace* represent two of the convergent shifts in staminal evolution seen more widely across the *Salvia* clade.

The Californian sages of sect. *Audibertia* depict another striking example of convergent recurrence of a similar staminal type that involves complete abortion of the posterior theca *and* the posterior connective branch. This stamen type has been derived independently in *Salvia* sect. *Audibertia* (Fig. 2H), the genus *Rosmarinus* (Fig. 2C), and in some individuals of *Salvia verticillata* (Fig. 2A) (Himmelbaur and Stibal 1933–1935; Claßen-Bockhoff et al. 2004a, b; Walker et al. 2004; Walker and Sytsma 2007). We assigned *S. verticillata* to stamen type "A" because the staminal structure in *S. verticillata* is variable, but frequently expresses the stamen type "A" (see Mivart 1871; Hedge 1982; Baikova 1998). In each of these three examples, the stamens have undergone a complicated evolutionary progression only to end up with a stamen that in superficial appearance is scarcely distinguishable from the original plesiomorphic state of the *Salvia* lineage. However, in each case the stamen possesses one theca instead of two (Figs. 2, 5D). The anterior branch of the connective is still elongate, functionally acts like a simple filament, although it possesses only a single theca at its end (Bentham 1876; Epling 1938; Neisess 1983). This progression is particularly evident in subg. *Audibertia* (Figs. 2I, 5D) in which the four species of sect. *Echinosphace* and *S. columbariae*, the latter sister to the "core *Audibertia*" of sect. *Audibertia*, express two fertile thecae separated by an elongated connective. All other members of sect. *Audibertia* have the derived staminal form of complete abortion of the posterior theca and posterior connective tissue (Figs. 2H, 5D). Support for the independent (convergent) origin of this peculiar stamen type in *Salvia* sect. *Audibertia* and in *Rosmarinus* rests in a subtle although important distinction in staminal morphology. Whereas the "joint" between the filament and connective is indicated by a notch on the top of the stamen in *Rosmarinus* (Fig. 2C), an articulation circling the entire filament is found at that same "joint" in sect. *Audibertia* (Fig. 2H). Occasionally, the posterior theca and connective branch is re-expressed from this joint in members of sect. *Audibertia*. Furthermore, *Rosmarinus* typically exhibits arched stamens, while the stamens of sect. *Audibertia* are more or less straight.

***Hybridization in Subgenus Audibertia***—Hybridization has been well documented between species in the California salvias, both in wild collected specimens and through crossing experiments carried out in cultivated individuals (Epling 1938, 1947; Anderson and Anderson 1954; Epling et al. 1962; Grant and Grant 1964; Emboden and Lewis 1967; Emboden 1969, 1971; Grant 1981, 1994; Neisess 1983; Meyn and Emboden 1987; Clebsch 1997). Emboden (1969) suggested that California *Salvia* are relatively young, as shown by the ability of species with different morphologies to hybridize, and probably arose through Pleistocene disruption creating new habitats which could be occupied by hybrid recombinants. However, despite weak barriers to hybridization, members of subg. *Audibertia* maintain their genetic and morphological identity except in disturbed habitats (Emboden 1969). Meyn and Emboden (1987) further argue that the establishment of an introgressed population of any magnitude in *Salvia* is rare as it requires the following conditions: 1) proximity of the parental species, 2) overlap in flowering seasons, 3) effective pollinators, with seasons of activity overlapping the flowering period of the introgressing species and the ability to overcome mechanical and ethological barriers, and 4) disturbance of the habitat to create new "hybrid" or disturbed habitats.

Although our collecting efforts explicitly attempted to avoid sampling individuals of California *Salvia* showing evidence of hybridization, the molecular data document unexpected levels and instances of hybridization and/or chloroplast capture. For example, the placements of *S. munzii* (*JBW 3055*) and *S. mellifera* (*JBW 3145*) within *S. columbariae* (Figs. 4B, S2) in the

cpDNA tree were surprising. Our limited sampling of individuals and genomic regions does not afford us the opportunity to exhaustively discuss all instances of incongruity we have identified in the data. We highlight four examples of hybridization or subsequent chloroplast capture in sect. *Audibertia*.

***Hybridization in Salvia vaseyi***—The evolutionary history of *Salvia vaseyi*, a species that morphologically appears remarkably intermediate between the widespread *S. apiana* and the other, more rugose-leaved members of sect. *Audibertia*, has been a topic of interest for some time, with most hypotheses suggesting past hybridization. Based on morphological and distributional data, Epling (1938) posited that *S. vaseyi* was a product of a *S. apiana* × *S. eremostachya* cross. The reproductive isolation of *S. vaseyi* from *S. apiana* would be facilitated by the fact that *S. vaseyi* generally grows at lower, hotter elevations than *S. apiana*. Additionally, Epling (1938) considered *S. apiana* var. *compacta* a subsequent product of a backcross hybridization event of *S. vaseyi* to parental *S. apiana*. Neisess (1983) later asserted that *S. vaseyi* was derived from a *S. apiana* × *S. mohavensis* cross based on phytochemical, palynological, and trichome evidence. The molecular evidence supports Epling's hypothesis.

Based on the cpDNA data presented here (Figs. 4B, S2), the only wild collected sample of *S. vaseyi* (*JBW 3101*) included in the study (the cpDNA sequence of the cultivated accession of *S. vaseyi* was not obtained) is most closely related to a collection of *S. apiana* var. *compacta* (*JBW 3100*) made only two kilometers away. These two collections share the exact same cpDNA sequence over the 6,281 nucleotides sampled (*ycf1* was not sequenced for *JBW 3100*). The nrDNA sequence data suggest a different relation of the *S. vaseyi* collection. In the nrDNA analysis, the collection of *S. apiana* var. *compacta* (*JBW 3100*) that matched the cpDNA sequence of *S. vaseyi* (*JBW 3099*) clearly allies with other *S. apiana* collections, and not with *S. vaseyi* (Figs. 3, 4A, S1). This collection of *S. apiana* var. *compacta* (*JBW 3100*) in fact shares the exact nrITS sequence with a collection of *S. apiana* made over 100 km away (*JBW 3192*; nrETS was not sequenced for this accession). In the expanded nrDNA analysis, *S. vaseyi* (*JBW 3101*) is sister to a cultivated *S. vaseyi* collection of unknown origin (*JBW 2530*), and this clade is most closely related to *S. eremostachya* and *S. munzii* (Figs. 3, 4A, S1). The nrDNA sequences of the two *S. vaseyi* accessions differ from that of a collection of *S. eremostachya* (*JBW 3097*) at only six base pairs. The accession of *S. eremostachya* was growing sympatrically with the individual of *S. apiana* var. *compacta* (*JBW 3100*) that shared its chloroplast sequence with *S. vaseyi*.

The molecular evidence clearly indicates that the evolutionary history of at least the one wild collection of *Salvia vaseyi* involves *S. apiana*. These results may indicate a history of *S. vaseyi* that involves a cross between paternal *S. eremostachya* and maternal *S. apiana*, as suggested originally by Epling (1938). However, the molecular evidence also supports an alternative scenario of a close relationship between *S. vaseyi* and *S. eremostachya* with a more recent contact and subsequent chloroplast capture between *S. vaseyi* and *S. apiana*. Disentangling these two (or other possible) evolutionary scenarios for *S. vaseyi* will require increased sampling across the geographical ranges of these three species. The populations of *S. vaseyi* sampled should include areas of contact as well as where neither of the other two species is found. Importantly, populations of *S. vaseyi* have been recently discovered in southwestern Arizona (e.g. Cain et al. 2010), where neither *S. apiana* nor *S. eremostachya* occur.

***Hybridization or Chloroplast Capture in Salvia mohavensis***—*Salvia mohavensis* occurs in the southeastern portions of the Mojave Desert and adjacent Sonoran Desert in California and into Arizona. It has also been found in a disjunct fashion in northwestern Mexico, specifically growing on Cerro del Pinacate and adjacent cinder cones above 1,000 m (Felger 2000). Material of the Mexican Sonoran accession of *Salvia mohavensis* (*PW 504*) included in this study came from garden grown plants from seeds originally collected at Puerto Penasco, Cerro del Pinacate. Floral morphology of this accession is clearly distinctive from western accessions of *S. mohavensis* (personal communication, Petra Wester). The expanded nrDNA analysis places this accession strongly as sister to the other three accessions of *S. mohavensis* (Fig. 3). However, the cpDNA analyses place the Mexican Sonoran accession in a complex of species/accessions unrelated to *S. mohavensis* (Figs. 4B, S2). Its cpDNA is similar to that of some accessions of *S. apiana*, *S. mellifera*, and *S. munzii*, representatives of a subclade within sect. *Audibertia* that shows evidence of active hybridization and subsequent introgression (see below). Of these latter species, only *S. apiana* extends into the Sonoran Desert. Clearly hybridization is in the evolutionary history of the Mexican Sonoran population, but what other species was involved, where the hybridization occurred, and whether subsequent chloroplast capture ensued are not known.

***Two Examples of Chloroplast Capture with Salvia columbariae***—In the expanded nrDNA sampling, thirteen collections of *Salvia columbariae* were included, all of which form a well-supported monophyletic lineage sister to "core *Audibertia*" (Fig. 3). *Salvia columbariae* is a morphologically distinct member of sect. *Audibertia*. As described earlier, it is the only species in the section that is an annual (all others are woody shrubs or subshrubs), the only species with lobed to pinnatifid leaves (all others have simple, unlobed (rarely hastate) leaves), and the only member to consistently express the posterior theca of its stamen (all others show complete abortion of the posterior theca and posterior connective branch). Three accessions of *S. columbariae* were included in the cpDNA analysis, all of which share the same relationship as is suggested by the nrDNA data; *S. columbariae* is sister to "core *Audibertia*". In the cpDNA analysis, however, two accessions of additional taxa are included within the *S. columbariae* lineage. One collection of *S. munzii* (*JBW 3055* - southern San Diego County) and one collection of *S. mellifera* (*JBW 3145* - Monterey County) contain a "S. *columbariae*-type" chloroplast (Fig. 4B). *Salvia columbariae* was not observed in close proximity with either of these collections, although based on habitat, association, and locality it would not be surprising to find *S. columbariae* in either of these locales. In the nrDNA analysis the *S. mellifera* collection (*JBW 3145*) shares an identical ITS sequence with eight other collections of *S. mellifera* (Figs. 3, 4A, S1). Likewise, the collection of *S. munzii* (*JBW 3055*) shares an identical ITS sequence with another collection of *S. munzii* (*JBW 3209*) made over 200 km to the south (Figs. 3, 4A, S1).

These collections of *Salvia mellifera* and *S. munzii* appear to represent a well-supported case of chloroplast capture (Fig. 4). *Salvia mellifera* has been documented on numerous occasions to hybridize with *S. columbariae*, and viable hybrids have been observed both in the wild and in garden experiments (Munz 1927; Epling 1938; Emboden 1971; Neisess 1983). This hybrid is common enough in the wild to have earned the formal name *Salvia* × *bernardina* Parish ex Greene,

and typically exhibits morphological characters intermediate between the two parents (Neisess 1983; Epling 1938). The collection of *S. mellifera* (*JBW 3145*) from Monterey County in this study, however, shows no morphological characters suggesting intermediacy with *S. columbariae*. No examples of hybridization between *S. munzii* and *S. columbariae* have been documented in the literature. However, Epling (1938) indicated that *S. munzii* hybridizes with *S. apiana*, a species that has been suggested to hybridize with as many as ten other species in sect. *Audibertia*. This might suggest that *S. munzii* crossing with *S. columbariae* is not unreasonable. Another possibility is that *S. munzii* obtained its *S. columbariae*-type cpDNA via hybridization with an individual of *S. mellifera* that possessed the *S. columbariae*-type cpDNA. Though we are not aware of documented hybrids between these two species, *S. mellifera* and *S. munzii* are morphologically similar. As with the *S. mellifera* collection, the collection of *S. munzii* (*JBW 3055*) from southern San Diego County also shows no morphological intermediacy with *S. columbariae*.

Future expanded sampling of nuclear loci, along with cpDNA sequences, may be necessary to provide phylogenetic resolution within the backbone of the "core *Audibertia*." However, the many examples of discordance between cpDNA and nrDNA imply that a more complete evolutionary history of subg. *Audibertia* may only be possible by sampling many unlinked nuclear loci and a larger set of accessions across the geographical range of each species.

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Appendix 1.Voucher information and GenBank accessions numbers for sequenced taxa. Taxon name and authority is followed by provenance, collector and voucher no.; GenBank accession numbers of *atpB-rbcL*, nrETS, nrITS, *matK-trnK*, *psbA-trnH*, *rps16*, *trnK*, *trnL-F*, *trnG-trnS*, *ycf1*. RSA = Rancho Santa Ana Botanical Garden, RBGE = Royal Botanic Garden Edinburgh. The TreeBASE submission number is S16712.***Ingroup:*** ***Salvia apiana*** Jepson. U. S. A. California: Los Angeles Co., *Jay Walker 2509* (WIS); KP852933, DQ667214. U. S. A. California: San Diego Co., *Jay Walker 3058* (SD); KP853017, KP852934, KP852765, KP852715, KP852612, KP852562, KP852663, KP852890, KP852845. U. S. A. California: San Diego Co., *Jay Walker 3065* (WIS); KP852935, KP852766. U. S. A. California: San Diego Co., *Jay Walker 3072* (WIS); KP853018, KP852716, KP852613, KP852563, KP852664, KP852891, KP852846. U. S. A. California: San Diego Co., *Jay Walker 3080* (WIS); KP852767. U. S. A. California: San Diego Co., *Jay Walker 3081* (WIS); KP852936, KP852768. U. S. A. California: Riverside Co., *Jay Walker 3088* (WIS); KP852937, KP852769. U. S. A. California: Ventura Co., *Jay Walker 3179* (WIS); KP852938, KP852770. U. S. A. California: Ventura Co., *Jay Walker 3180* (WIS); KP853019, KP852939, KP852771, KP852717, KP852614, KP852564, KP852665, KP852892, KP852847. U. S. A. California: Santa Barbara Co., *Jay Walker 3185* (WIS); KP852940, KP852772. U. S. A. California: Los Angeles Co., *Jay Walker 3192* (WIS); KP852773. U. S. A. California: Los Angeles Co., *Jay Walker 3196* (WIS); KP852941, KP852774. U. S. A. California: Los Angeles Co., *Jay Walker 3198* (WIS); KP852942, KP852775. U. S. A. California: Riverside Co., *Jay Walker 3201* (WIS); KP852943, KP852776. U. S. A. California: San Bernardino Co., *Jay Walker 3202* (WIS); KP853020, KP852944, KP852777, KP852718, KP852615, KP852565, KP852666, KP852893, KP852848, KP853066. MEXICO. Baja California Norte, *Jay Walker 3208* (WIS); KP853021, KP852778, KP852719, KP852616, KP852566, KP852667, KP852894, KP852849. MEXICO. Baja California Norte, *Jay Walker 3211* (WIS); KP852945, KP852779; ***Salvia apiana*** **var.** ***compacta*** Jepson. U. S. A. California: San Diego Co., *Jay Walker 3082* (WIS, SD); KP852946, KP852780. U. S. A. California: San Diego Co., *Jay Walker 3084* (WIS); KP852947, KP852781. U. S. A. California: Riverside Co., *Jay Walker 3100* (WIS); KP853022, KP852948, KP852782, KP852720, KP852617, KP852567, KP852668, KP852895, KP852850. ***Salvia brandegeei*** Munz. Cultivated RSA, *Jay Walker 2551* (WIS); KP853022, KP852948, KP852782, KP852720, KP852617, KP852567, KP852668, KP852895, KP852850. MEXICO. Baja California Norte, *Jay Walker 3212* (WIS); KP852950, KP852784, KP852722, KP852620, KP852569, KP852670, KP852897, KP852852. ***Salvia californica*** Brandegee. Cultivated RSA, *Jay Walker 2520* (WIS); KP853025, DQ667213, KP852951, KP852723, KP852621, KP852570, KP852671, DQ667424, KP852853, KP853068. Salvia carduacea Benth. U. S. A. California: Riverside Co., *Jay Walker 3091* (WIS); KP853026, KP852785, KP852724, KP852622, KP852571, KP852672, KP852898, KP852854, KP853069. U. S. A. California: Ventura Co., *Jay Walker 3176* (WIS); KP853027, KP852786, KP852725, KP852623, KP852572, KP852673, KP852899, KP852855. U. S. A. California: Riverside Co., *Thibault 53* (WIS); KP853028, KP852952, KP852787, KP852726, KP852624, KP852573, KP852674, KP852900, KP852856; ***Salvia chionopeplica*** Epling. Cultivated RSA, *Jay Walker 2545* (WIS); KP853029, DQ667227, KP852954, KP852728, KP852626, KP852575, KP852676, AY570472, KP852858, KP853071. ***Salvia clevelandii*** (A. Gray) Greene. Cultivated RSA, *Jay Walker 2508* (WIS); KP852955, DQ667219. U. S. A. California: San Diego Co., *Jay Walker 3064* (SD); KP853031, KP852956, KP852788, KP852730, KP852627, KP852576, KP852677, KP852901, KP852859. U. S. A. California: San Diego Co., *Jay Walker 3079* (WIS); KP853032, KP852957, KP852789, KP852731, KP852628, KP852577, KP852678, KP852902, KP853072. MEXICO. Baja California Norte, *Jay Walker 3216* (WIS); KP853030, KP852790, KP852729, KP852629, KP852578, KP852679, KP852903. MEXICO. Baja California Norte, *Jay Walker 3217* (WIS); KP852979, KP852810. ***Salvia columbariae*** Benth. U. S. A. California: San Diego Co., *Jay Walker 3061* (WIS); KP852958, KP852791. U. S. A. California: San Diego Co., *Jay Walker 3066* (WIS); KP853033, KP852959, KP852792, KP852732, KP852630, KP852579, KP852680, KP852904, KP853073. U. S. A. California: San Diego Co., *Jay Walker 3070* (WIS); KP853034, KP852960, KP852793, KP852733, KP852631, KP852580, KP852681, KP852905, KP852860. U. S. A. California: San Diego Co., *Jay Walker 3083* (WIS); KP852961, KP852794. U. S. A. California: Riverside Co., *Jay Walker 3089* (WIS); KP852962, KP852795. U. S. A. California: Riverside Co., *Jay Walker 3099* (WIS); KP852963, KP852796. U. S. A. California: San Bernardino Co., *Jay Walker 3109* (WIS); KP852964, KP852797. U. S. A. California: Inyo Co., *Jay Walker 3134* (WIS); KP852965, KP852798. U. S. A. California: Monterey Co., *Jay Walker 3149* (WIS); KP852966, KP852799. U. S. A. California: Monterey Co., *Jay Walker 3153* (WIS); KP852967, KP852800. U. S. A. California: Ventura Co., *Jay Walker 3178* (WIS); KP852968, KP852801. U. S. A. California: Los Angeles Co., *Jay Walker 3191* (WIS); KP853035, KP852969, KP852802, KP852734, KP852632, KP852581, KP852682, KP852906, KP852861. MEXICO. Baja California Norte, *Jay Walker 3219* (WIS); KP852970, KP852803. ***Salvia dorrii*** (Kellogg) Abrams. Cultivated RSA, *Jay Walker 2541* (WIS); KP852971. U. S. A. California: San Bernardino Co., *Jay Walker 3116* (WIS); KP853036, KP852972, KP852804, KP852735, KP852633, KP852582, KP852683, KP852907, KP852862, KP853074. U. S. A. California: San Bernardino Co., *Jay Walker 3123* (WIS); KP852973, KP852805. U. S. A. California: Inyo Co., *Jay Walker 3136* (WIS); KP853037, KP852974, KP852806, KP852736, KP852634, KP852583, KP852684, KP852908, KP852863. U. S. A. California: Los Angeles Co., *Jay Walker 3193* (RSA); KP853038, KP852975, KP852807, KP852737, KP852635, KP852584, KP852685, KP852909, KP852864. U. S. A. California: San Bernardino Co., *Jay Walker 3207* (SD); KP852976, KP852808. ***Salvia eremostachya*** Jepson. Cultivated RSA, *Jay Walker 2533* (WIS): KP852977. U. S. A. California: Riverside Co., *Jay Walker 3097* (WIS); KP853039, KP852978, KP852809, KP852738, KP852636, KP852585, KP852686, KP852910, KP852865, KP853075. ***Salvia funerea*** M. E. Jones. U. S. A. California: San Bernardino Co., *Jay Walker 3106* (WIS); KP853040, KP852980, KP852811, KP852739, KP852637, KP852586, KP852687, KP852911, KP852866, KP853076. U. S. A. California: Inyo Co., *Jay Walker 3131* (RSA); KP853041, KP852981, KP852812, KP852740, KP852638, KP852587, KP852688, KP852912. ***Salvia greatae*** Brandegee. U. S. A. California: Riverside Co., *Jay Walker 2511* (WIS); JF301331, DQ667215, KP853043, KP852742, KP852589, KP852690, AY570481, KP852868, JF289062. ***Salvia leucophylla*** E. Greene. U. S. A. California: Santa Barbara Co., *Jay Walker 3170* (SD); KP853044, KP852982, KP852813, KP852743, KP852639, KP852590, KP852691, KP852913, KP852869, KP853077. U. S. A. California: Santa Barbara Co., *Jay Walker 3171* (RSA); KP852983, KP852814. U. S. A. California: Ventura Co., *Jay Walker 3182* (WIS); KP853045, KP852984, KP852815, KP852744, KP852640, KP852591, KP852692, KP852914, KP852870. U. S. A. California: Los Angeles Co., *Jay Walker 3189* (SD); KP853046, KP852985, KP852816, KP852745, KP852641, KP852592, KP852693, KP852915, KP85287. Cultivated, *Jay Walker s.n.* (WIS); KP852986, DQ667210. ***Salvia mellifera*** E. Greene. U. S. A. California: San Diego Co., *Jay Walker 2550* (WIS); JF301338, DQ667220, KP853047, KP852746, KP852642, KP852593, KP852694, DQ667427, KP852872, JF289064. U. S. A. California: San Diego Co., *Jay Walker 3069* (SD); KP852987, KP852817. U. S. A. California: Riverside Co., *Jay Walker 3085* (WIS); KP852988, KP852818. U. S. A. California: Monterey Co., *Jay Walker 3145* (SD); KP853048, KP852989, KP852819, KP852747, KP852643, KP852594, KP852695, KP852916, KP852873. U. S. A. California: San Luis

Obispo Co., *Jay Walker 3158* (WIS); KP852990, KP852820. U. S. A. California: Santa Barbara Co., *Jay Walker 3174* (WIS); KP853049, KP852991, KP852821, KP852748, KP852644, KP852595, KP852696, KP852917, KP852874. U. S. A. California: Ventura Co., *Jay Walker 3181* (WIS); KP853050, KP852992, KP852822, KP852749, KP852645, KP852596, KP852697, KP852918, KP852875. U. S. A. California:: Santa Barbara Co., *Jay Walker 3184* (WIS); KP852993, KP852823. U. S. A. California: Los Angeles Co., *Jay Walker 3187* (WIS); KP853051, KP852994, KP852824, KP852750, KP852646, KP852597, KP852698, KP852919, KP852876. U. S. A. California: Los Angeles Co., *Jay Walker 3197* (WIS); KP852995, KP852825. U. S. A. California: Los Angeles Co., *Jay Walker 3199* (WIS); KP852996, KP852826 ***Salvia mohavensis*** E. Greene. U. S. A. California: San Bernardino Co., *Jay Walker 3111* (WIS); KP853052, KP852997, KP852827, KP852751, KP852647, KP852598, KP852699, KP852920, KP852877. U. S. A. California: San Bernardino Co., *Jay Walker 3119* (WIS); KP853053, KP852998, KP852828, KP852752, KP852648, KP852599, KP852700, KP852921, KP852878, KP853078. Cultivated, *Jay Walker s.n.* (WIS); KP852999, DQ667212. MEXICO: Sonora, *Petra Wester 504* (WIS); KP853054, KP852829, KP852757, KP852649, KP852600, KP852701, KP852922, KP852879. ***Salvia munzii*** Epling. U. S. A. California: San Diego Co., *Jay Walker 3055* (WIS); KP853055, KP853000, KP852830, KP852753, KP852650, KP852601, KP852702, KP852923, KP852880. MEXICO. Baja California Norte, *Jay Walker 3209* (WIS); KP853056, KP853001, KP852831, KP852754, KP852651, KP852602, KP852703, KP852924, KP852881. MEXICO. Baja California Norte, *Jay Walker 3210* (WIS); P852832. MEXICO. Baja California Norte, *Jay Walker 3220* (WIS); KP853057, KP853002, KP852833, KP852755, KP852652, KP852603, KP852704, KP852925, KP852882, KP853079. ***Salvia pachyphylla*** Munz. U. S. A. Cultivated RSA, *Jay Walker 2535* (WIS); KP853003, DQ667230. U. S. A. California: San Bernardino Co., *Jay Walker 3203* (WIS); KP853058, KP853004, KP852834, KP852756, KP852653, KP852604, KP852705, KP852926, KP852883, KP853080. ***Salvia sonomensis*** E. Greene. Cultivated RSA, *Jay Walker 2519* (WIS); KP853005. DQ667218. U. S. A. California: Monterey Co., *Jay Walker 3152* (WIS); KP853060, KP853006, KP852835, KP852759, KP852655, KP852606, KP852707, KP852927, KP852885. U. S. A. California: San Luis Obispo Co., *Jay Walker 3162* (WIS); KP853007, KP852836. U. S. A. California: San Luis Obispo Co., *Jay Walker 3163* (WIS); KP853061, KP853008, KP852837, KP852760, KP852656, KP852607, KP852708, KP852928, KP853081. ***Salvia spathacea*** E. Greene. U. S. A. California: Monterey Co., *Jay Walker 3154* (RSA); KP853009, KP852838. U. S. A. California: San Luis Obispo Co., *Jay Walker 3157* (WIS); KP853062, KP853010, KP852839, KP852761, KP852657, KP852608, KP852709, KP852929, KP852886. U. S. A. California: San Luis Obispo Co., *Jay Walker 3159* (WIS); KP853063, KP853011, KP852840, KP852762, KP852658, KP852609, KP852710, KP852930, KP852887, KP853082. U. S. A. California: San Luis Obispo Co., *Jay Walker 3166* (SD); KP853064, KP853012, KP852841, KP852763, KP852659, KP852610, KP852711, KP852931, KP852888. ***Salvia vaseyi*** (Porter) Parish. Cultivated RSA, *Jay Walker 2530* (WIS); KP853013, DQ667226. U. S. A. California: Riverside Co., *Jay Walker 3101* (RSA); KP853065, KP853014, KP852842, KP852764, KP852660, KP852611, KP852712, KP852932, KP852889, KP853083.***Outgroup*: *Dorystaechas hastata*** Boiss. & Heldr. Ex Benth. Cultivated RBGL, *RBG-L-1972-0177D* (RBGL); JF301312, DQ667252, KP853015, KP852714, DQ667360, KP852560, KP852661, AY570454, KP852843, JF289014. ***Lepechinia chamaedryoides*** Epling. Cultivated RSA, *Jay Walker 2537* (WIS); JF301317, DQ667231, KP853016, KP852713, DQ667343, KP852561, KP852662, AY570459, KP852844, JF289031. ***Meriandra benaglensis*** (Roxb.) Benth. YEMEN. *Lavranus & Newton 15796* (MO); JF301326, DQ667329, DQ667414, DQ667518, JF289044. ***Salvia axillaris*** Moc. et Sees ex Benth. MEXICO. Oaxaca, *Jay Walker 3038* (WIS); JF301330, DQ667294, KP852618, DQ667480, JF289060. ***Salvia cedrocensis*** Greene. Cultivated RSA, *Jay Walker 2539* (WIS); KP852953, DQ667228, KP852727, KP852625, KP852574, KP852675, AY570470, KP852857, KP853070. ***Salvia glutinosa*** L. Cultivated, *Jay Walker 2568* (WIS); KF307496, DQ667250, KP853042, KP852741, DQ667359, KP852588, KP852689, AY570480, KP852867, JF289061. ***Salvia patens*** Cav. Cultivated RBGE, *RBG-E-1973-91972535* (RBGE); JF301333, DQ667253, DQ667361, DQ667442, JF289066. ***Salvia roemeriana*** Scheele. Cultivated, *Jay Walker 2525* (WIS); JF301340, DQ667211, KP853059, KP852758, KP852654, KP852605, KP852706, AY570491, KP852884, JF289069.