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Potential Hybridization Among Two Species of California Salvia

Ellie Morrison
University of Nebraska at Kearney, elliegmorrison@gmail.com

Bryan Drew
University of Nebraska at Kearney, bdrewfb@yahoo.com

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Ellie Morrison and Bryan Drew

Department of Biology, University of Nebraska-Kearney, NE Kearney, 68849, USA

Correspondence — B. Drew, Department of Biology, University of Nebraska-Kearney, NE Kearney, 68849, USA;
E-mail: bdrewfb@yahoo.com ; Phone: 308-865-8883

Abstract

Hybridization among plants is fairly common, particularly in instances where closely related species are sympatric. One example of a group in which hybridization has been detected is the genus *Salvia* (Lamiaceae; mint family). *Salvia* is a diverse genus consisting of about 1,000 species and is defined primarily by having only two stamens, each with their anther sacs separated by elongated connective tissue. The genus has diversity centers around the world, including Southwest Asia and the Mediterranean region, Mexico/Central America, northern and central South America, and temperate East Asia. The genus also has a smaller species radiation of 19 species in western North America, subgenus Audibertia centered in California, and there has been documented evidence of hybridization within the subgenus. For this study we investigated potential hybridization among *Salvia columbariae* and *Salvia greatae*, two species with a sympatric distribution in the Orocopia Mountains region of Southern California. These species are placed in different sections of subgenus Audibertia, and hybridization has not yet been documented between sections of this subgenus. To examine relationships between the species, we compared molecular phylogenies from nuclear ribosomal DNA and chloroplast DNA. The resulting phylogenies did not show evidence of hybridization between these species. Although no evidence of hybridization was found, it is possible that additional sampling could yield different results.

Introduction

Hybridization was first studied through observations of increasing phenotypic variation in areas of contact between different plant species, predating molecular genomic approaches (Goulet *et al.* 2017). Hybridization can be generally defined as a cross between individuals from separate populations that differ in one or more heritable traits (Harrison 1990). Hybrids can be difficult to detect and describe if the parental taxa are not obvious (e.g., if either parent taxa is extinct or has undergone a range shift away from the hybrid population), and some species are more likely to hybridize than others (Whitney *et al.* 2010). Rates of hybridization in plants vary in different regions and taxonomic groups, though it is more common between species of the same genera than between genera (Whitney *et al.* 2010). Species richness is one factor that can impact the likelihood of hybridization. Areas of high species richness are argued to be more likely to have higher rates of hybridization because there is a larger number of species that are closely enough related to cross with each other (Whitney *et al.* 2010). Whitney *et al.* (2010) also concluded that while external factors such as species richness can have an impact, evidence suggests that the intrinsic properties of a clade are more likely to determine hybridization potential of the group than environmental conditions.

In plants, one way hybridization can be detected is by comparing relationships estimated by nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) phylogenies. These genomic compartments have different inheritance patterns – nrDNA is inherited as a mix from both parents while cpDNA is inherited maternally. Consequently, the chloroplast of one species can persist in populations of closely related species after hybridization events (chloroplast capture), even when there is no morphological evidence of hybridization (Smith and Sytsma 1990, Rieseberg and Soltis 1991, Walker, Drew, and Sytsma 2015). The detection of cpDNA from one species in another species is evidence of hybridization between the two species. A quick and relatively cheap way to produce nr/cpDNA phylogenies is via polymerase chain reactions (PCR). PCRs can be used to amplify target gene regions from DNA extractions. The DNA fragments can be amplified by using primer pairs appropriate for the situation (Shi *et al.* 2012). One genus where hybridization has been detected is the genus *Salvia* (Lamiaceae; mint family) (Fernández-Alonso 2008, Walker, Drew, and Sytsma 2015, Celep, Radars, and Drew 2020).

Salvia contains about 1,000 species with a virtually worldwide distribution, and are within the tribe Mentheae. The genus is differentiated from most other members of the tribe by expressing only two stamens, with

each stamen having the anther sacs (thecae) separated by an elongation of connective tissue which can aid in outcrossing (Claßen-Bockhoff, Wester, and Tweraser 2003). *Salvia* has diversity centers around the world, including Southwest Asia and the Mediterranean region, Mexico/Central America, northern and central South America, and temperate East Asia. The genus also has a smaller radiation of 19 species in western North America, which is centered in the California Floristic Province (CA-FP) of California (Walker, Drew, and Sytsma 2015). The CA-FP sustains hot dry summers and cool wet winters that define the Mediterranean-type climate. *Salvia* are abundant and sometimes dominant within the vegetation of this area. The CA-FP contains native *Salvia* species from two sections within subgenus Audibertia, sections *Audibertia* and *Echinosphace* (Walker, Drew, and Sytsma 2015).

The sections *Audibertia* and *Echinosphace* were originally treated as the genus *Audibertia* (Bentham 1833), but a reorganization resulted in moving the (then) 18 species from both sections into *Salvia* section *Audibertia* (Epling 1938). Later, section *Audibertia* was broken into sections, *Audibertia* and *Echinosphace,* based on morphological and phytochemical data (Neisess 1983). Walker, Drew, and Sytsma (2015) maintained the two sections but combined them into subgenus Audibertia. Section *Audibertia* is separated from other groups of *Salvia* based on chemical compounds, stamen morphology, and molecular phylogenetic evidence (Neisess 1983, Walker, Drew, and Sytsma 2015; Rose et al. 2021). The taxa in this section have the posterior branch of the staminal connective as well as the posterior theca totally aborted (Walker, Drew, and Sytsma 2015). Section *Echinosphace*, while morphologically similar to sect. *Audibertia*, does express the posterior theca, which are generally reduced in size (Neisess 1983, Walker, Drew, and Sytsma 2015). Neither of these sections employ a functional lever mechanism that is commonly seen in the *Salvia*.

Hybridization has previously been observed between species of *Salvia* within California, perhaps most notably between *S. columbariae* and *S. mellifera*, both within section *Audibertia* (Walker, Drew, and Sytsma 2015). The resulting hybrid is common enough that it is referred to as *Salvia × bernardina*. Some specimens of *S. mellifera* were shown to have a "*S. columbariae*-type" chloroplast following molecular phylogenetic analysis (Walker, Drew, and Sytsma 2015). The *Salvia x bernardina* hybrids are viable and often show morphological characteristics intermediate between the parent species, but sometimes there is no morphological evidence of hybridization (Walker, Drew, and Sytsma 2015). Hybridization has been documented between several other species of *Salvia* within section *Audibertia* as well, both in wild and cultivated settings (Epling 1947, Emboden 1971). Hybrid species can arise under certain conditions, though a stable population of hybrids is rare. A stable population of this nature is argued to require effective pollinators, disturbed habitats, the parental species nearby, and an overlap of flowering seasons between parent species (Walker, Drew, and Sytsma 2015). Currently, although hybridization has been well documented among several species within section *Audibertia*, it has not yet been documented among species in section *Echinosphace* or between the two sections of subgenus Audibertia.

For this study we focused specifically on two species from *Salvia* subg. Audibertia, *S. columbariae* (chia sage) from section *Audibertia* and *S. greatae* (Orocopia sage) from section *Echinosphace* (Fig. 1). *Salvia columbariae* is distributed throughout most of California and ranges elsewhere, while *S. greatae* is found only in the Orocopia Mountains region of the Sonoran Desert in Southern California (Fig. 2a). The two species overlap over the entire limited distribution of *S. greatae* (Walker, Drew, and Sytsma 2015). *Salvia columbariae* is an annual plant and has primarily basal leaves and flowers that are pale to deep blue in color with leafy, burgundy-colored bracts subtending the flower heads. Although *S. columbariae* is in section *Audibertia*, it shares several morphological characters with species from section *Echinosphace* such as having a spiny and 5-lobed calyx and two anther thecae per stamen. *Salvia greatae* grows as a rounded shrub and has spiny tomentose leaves and lavender to rose colored flowers. *Salvia columbariae* typically flower from March through June, which overlaps with the flowering season of *S. greatae* from March through April (The Jepson Herbarium 2022). To explore potential hybridization between *Salvia columbariae* and *S. greatae*, we sampled several individuals from both species and analyzed them in the context of a broader *Salvia* phylogeny. We then compared phylogenetic results using cpDNA and the nuclear ribosomal internal and external transcribed spacer regions (ITS and ETS, respectively).

Figure 1 (*opposite*) Photographs of Species. *Salvia greatae* and *S. columbariae*. **A)** *S. greatae* flower with short corolla tube; **B)** *S. greatae* flower with short corolla tube; **C)** *S. greatae* plant in native habitat; **D-E)** *S. columbariae* inflorescences; **F)** *S. columbariae* plant in native habitat. — Images A–C by B. Drew; images D–F from Calflora.

Figure 2 (*preceding page*) Distribution and Collection Map. **A)** Known distribution of *Salvia greatae*. **B)** Map of new sample collection sites. The Salton Sea is in the lower left corner, with the Orocopia Mts. to the North and the Chocolate Mts. to the South.

Methods and Materials

In total we sampled 121 accessions. The nrDNA dataset contained 121 accessions, while the cpDNA dataset contained 74 accessions. Both datasets included new sequences for 10 accessions of *S. columbariae*. We also newly sequenced 11 accessions of *S. greatae* for the nrDNA dataset and 10 accessions for the cpDNA dataset. The remaining samples were downloaded from GenBank and corresponded to the data matrices from Walker, Drew, and Sytsma (2015). Accessions of the newly sequenced samples were collected on April 5th, 2020 in the Orocopia Mountains region of Southern California (Fig. 2b). All of the *S. columbariae* samples were collected within 3 meters of *S. greatae* plants, and the paired collections were at least 100 meters from one another. In total, the sampling represented most of the limited geographic range of *S. greatae*.

We extracted DNA from dried leaf material following the procedure of Drew and Sytsma (2011). The *ycf1-rps15* and *psbA-trnH* cpDNA regions as well as the ITS and ETS (nrDNA) nuclear ribosomal regions were chosen as molecular markers based on Walker, Drew, and Sytsma (2015). The *psbA-trnH* region was amplified and sequenced according to Walker and Sytsma (2007), while the other three markers were amplified and sequenced with primers as described by Drew and Sytsma (2011). PCR reactions and thermal cycler conditions were used following Sytsma *et al.* (2002), and PCR amplification efficacy was determined by gel electrophoresis. Sequencing was performed using an Applied Biosystems 3730xl automated DNA sequencer at the University of Arizona Genetics Core. Phylogenetic analyses were conducted using MrBayes v 3.2.7 as described by Ronquist *et al.* (2012). We conducted 10 million generations using default parameters. The resulting trees were visualized using Figtree v1.4.4 (Rambaut 2018).

Results

In the nrDNA dataset, both *Salvia columbariae* and S. *greatae* were monophyletic with high support (Posterior Probability [PP] =1.00; Fig 3). There was generally poor support for relationships within each of these clades. *Salvia columbariae* was sister to the remaining taxa of section *Audibertia*, and other relationships were identical to those obtained by Walker, Drew, and Sytsma (2015). In the cpDNA tree, the 10 accessions of *S. greatae* newly sequenced for this study formed a well-supported (PP =1.00) group, but this group did not include the accession used in Walker, Drew, and Sytsma (2015), *S. greatae* jbw2511 (Fig. 4). The 10 newly sequenced accessions of *S. columbariae* formed a clade with accessions of *S. columbariae* downloaded from GenBank, but this clade also included one accession each of *S. munzii* and *S. mellifera*, echoing the results from Walker, Drew, and Sytsma (2015) (Fig. 4). Again, *Salvia columbariae* was recovered as sister to the remaining members of section *Audibertia*. No evidence of hybridization between *S. columbariae* and *S. greatae* was found.

Discussion

This study was the first to look explicitly for hybridization between sections of subg. Audibertia. Although no evidence of hybridization between *S. columbariae* and *S. greatae* was found, this is perhaps not surprising as the species are in different sections within *Salvia* subg. Audibertia. Hybridization is somewhat common within section *Audibertia*, but has not been documented within section *Echinosphace* or between the two sections. Intrasectional hybridization has been documented elsewhere in *Salvia*, including but not limited to *S. officinalis* and *S. fruticosa* (both within section *Salvia*), and *S. columbariae* and *S. mellifera* (both within section *Audibertia*) (Radosavljević *et al.* 2019, Walker, Drew, and Sytsma 2015). Hybridization among sections has been documented in *Salvia*, but in nature this has only been found within subg. Calosphace (Drew, unpublished data).

Although we did not find evidence of hybridization between the two species, the two species share some common morphological characters that suggest natural hybridization may be possible. For example, similar morphological characteristics between *S. columbariae* and *S. greatae* are a potential phenotypic indicator of shared genetic material. *Salvia columbariae* shares a 5-lobed calyx with the members of sect. *Echinosphace* (including *S. greatae*), a feature that is not found in other species within section *Audibertia*. Also, both *S. columbariae* and *S. greatae* have two anther thecae per stamen. *Salvia columbariae* is the only species within section *Audibertia* to possess two anther thecae per stamen (all 4 species of section *Echinosphace* also express this trait). In addition, both species have relatively small, similarly colored flowers, and overlapping flowering times, suggesting at least the possibility of cross pollination.

Figure 3 Tree with cpDNA Results. The resulting tree of cpDNA sequencing (*Salvia columbariae and Salvia greatae*) combined with previously sequenced related taxa. Taxa colored in blue were identified as hybrids in Walker, Drew, and Sytsma (2015).
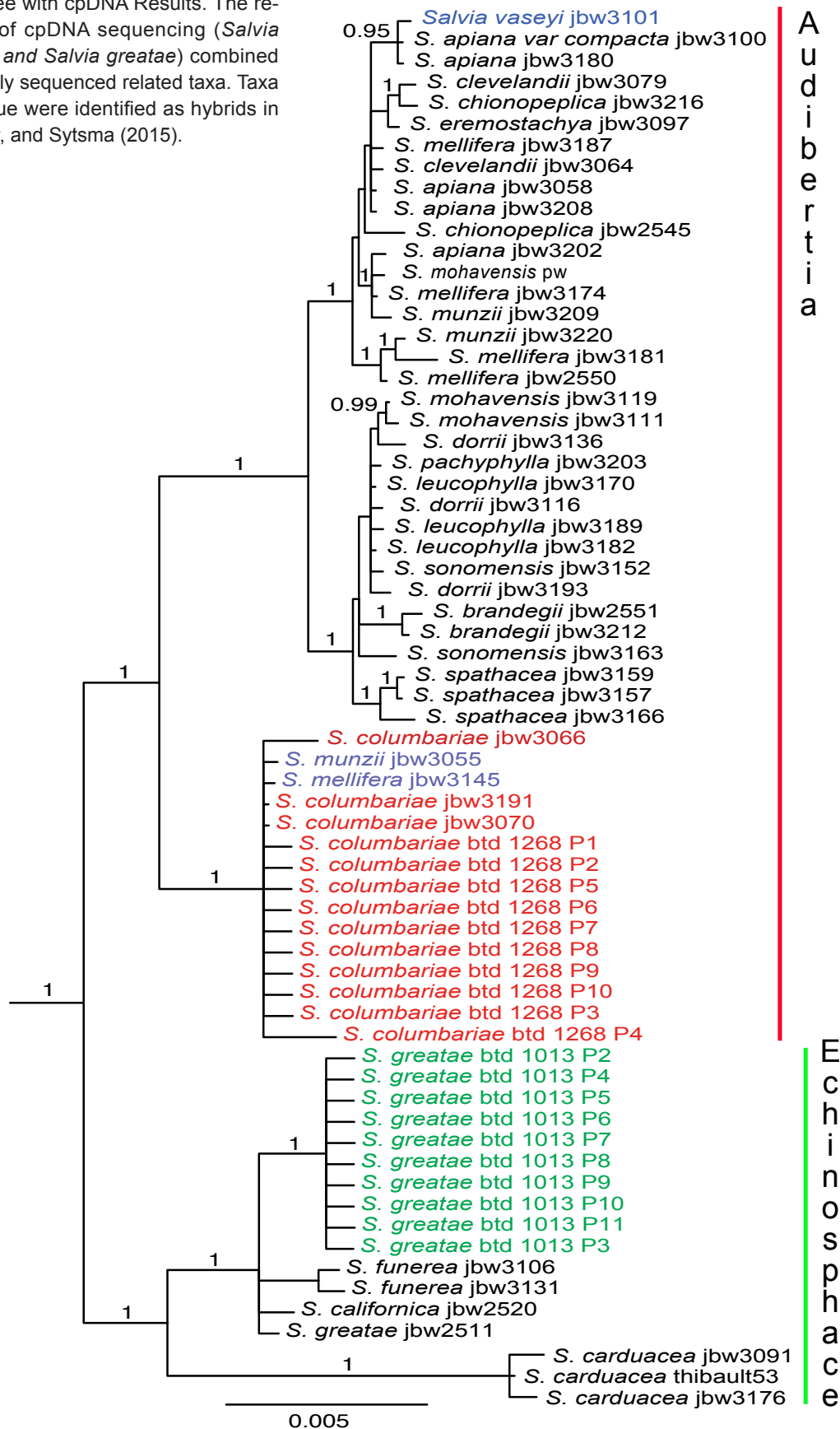
Figure 4 Tree with nrDNA Results. The resulting tree of nrDNA sequencing (*Salvia columbariae and Salvia greatae*) combined with previously sequenced related taxa. Taxa colored in blue were identified as hybrids in Walker, Drew, and Sytsma (2015).
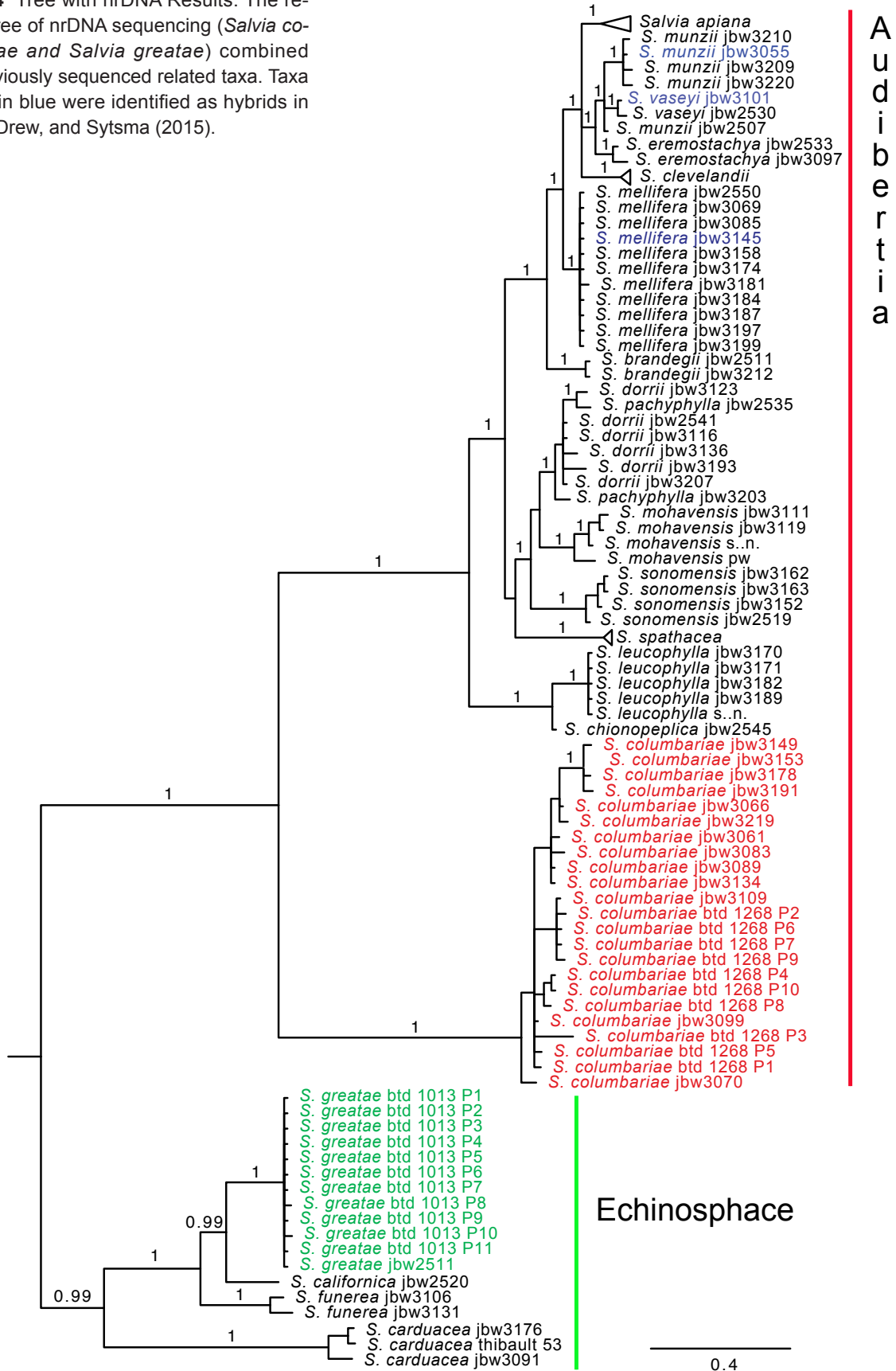
Though we were unable to demonstrate hybridization between *S. columbariae* and *S. greatae* here, it is certainly possible that hybridization has occurred between the two species and thus far eluded our detection. We only sampled 11-12 individuals of species within the range of *S. greatae*, so hybrids could exist that were not sampled. In addition, the current range of *S. greatae* (and *S. columbariae*) is likely a relatively recent occurrence, and the species was most likely located southward during recent glaciations (Van Devender 2002, Butterfield *et al.* 2019). Given that plant species migrate at different rates, if hybridization between *S. columbariae* and *S. greatae* occurred during glacial cycles, evidence of that hybridization, at least within *S. columbariae*, might be best sought elsewhere. It is also more likely to detect ongoing hybridization as opposed to if it has occurred in the recent or distant past, as signals of hybridization may be muted over time by mutation, subsequent backcrossing between the hybrid and one of the parent taxa, natural selection against the introgressed alleles, or genetic drift (Whitney *et al.* 2010).

The focal species of this study have different numbers of chromosomes. *Salvia columbariae* has a diploid number of 2n = 26, while *S. greatae* has a diploid number of 2n = 30 (Jepson Herbarium eflora project 2022). However, it has been shown that members of *Salvia* with different chromosome numbers can produce viable hybrid offspring, such as *Salvia transsylvanica* x *Salvia involucrata* (Tychonievich & Warner 2011). In this previous study, the resulting hybrid offspring failed to backcross with either parent species, but were highly fertile when self-pollinated. The *S. transsylvanica* plants had a diploid chromosome count of 16, while *S involucrata* were shown to have a diploid chromosome count of 2n = 22 (Tychonievich & Warner 2011). The resulting hybrids were allopolyploids and had a diploid chromosome count of 38. The authors concluded that the fertility of the *S. transsylvanica* x *S. involucrata* plants may be due to the allopolyploid character of the hybrid (Tychonievich & Warner 2011). Moreover, Walker, Drew, and Sytsma (2015) demonstrated that *Salvia columbariae* (2n = 26) has interbred with *Salvia mellifera* (2n = 30) and *Salvia munzii* (2n = 30) in the past. These results show that it is possible to produce a viable hybrid between *Salvia* species with different numbers of chromosomes and serves as evidence of the possibility of hybridization of our target species, *S. columbariae* and *S. greatae*.

One other factor is that may impede or prevent hybridization between the two species is mechanical isolation. *Salvia greatae* has pleurotribic flowers, in which the two anthers are facing each other and positioned on the either side of the corolla when fertile. This morphology tends to place pollen on the sides of pollinators. *Salvia columbariae*, by contrast, has nototribic flowers, in which the two fertile anthers are positioned in the middle of the upper corolla lobe and the fertile pollen is facing downwards. This strategy tends to place pollen on the back of pollinators. Although this mechanical isolation does not necessarily preclude hybridization (Emboden 1971), it is likely to at least inhibit it.

The outcome may have been different if we had used more or different samples. The number of samples used was chosen to fit the time and budget constraints of the project. The range of *S. greatae* is also quite small (Fig. 2a), and our sampling covered virtually the entire geographic range of the species. According to our nrDNA results, there is considerable sequence variation in *S. columbariae*. This is not surprising, as *S. columbariae* has a much broader distribution than *S. greatae* and therefore a larger gene pool. To investigate different outcomes, future research should continue to examine the potential for hybridization between these species by sampling additional accessions of *S. columbariae* across a broader geographic range.

Acknowledgements

Web Resources

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