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## Two new hybrid species of *Salvia* (*S.* × *karamanensis* and *S.* × *doganii*) from Turkey: evidence from molecular and morphological studies

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**Abstract:** *Salvia* L. is an ideal exemplar to demonstrate prezygotic isolation mechanisms in sympatric populations due to their well-known staminal lever mechanism. Mechanical, phenological, and ethological isolation mechanisms have been reported among sympatric species of *Salvia*. However, it has been shown that if closely related species are sympatric and flower at the same time, they can potentially hybridize. In this study, we describe two new hybrid species of *Salvia* (*S.* × *karamanensis* Celep & B.T.Drew, and *S.* × *doganii* Celep & B.T.Drew) from Turkey based on morphological and molecular evidence. *Salvia* × *karamanensis* (*S. aucheri* Benth. subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Doğan × *S. heldreichiana* Boiss. ex Benth.) is known from near Karaman city in the central Mediterranean region of Turkey, and *S.* × *doganii* (*S. cyanescens* Boiss. & Bal. × *S. vermifolia* Hedge & Hub.-Mor.) occurs near Sivas in central Anatolia, Turkey. Morphological comparisons between the hybrid species and their putative parents are given with notes on the International Union for Conservation of Nature (IUCN) red list categories, biogeography and ecology of the two hybrid species.

**Key words:** Hybridization, Lamiaceae, *Salvia*, taxonomy

### 1. Introduction

*Salvia* L. is one of the most species-rich genera of flowering plants, with about 1000 species currently accepted (Walker et al., 2007; Will & Claßen-Bockhoff, 2017; Drew et al., 2017; Kriebel et al., 2019, 2020; González-Gallegos et al., 2020). The main regions where the diversity is significant in terms of *Salvia* are the Mediterranean region, Southwest Asia, Mexico and Central/South America, and East Asia (Harley et al., 2004; Drew et al., 2017; Will and Claßen-Bockhoff, 2017; Kriebel et al., 2019). Within the Mediterranean region and Southwest Asia, Turkey has more species of *Salvia* than any other country, and Turkey has the second highest number of *Salvia* species in the world (Mexico has the most) with about 100 species, of which about 53 are endemic (Celep et al., 2015, 2017). It is likely *Salvia* is so diverse in Turkey due to the country's climatic, geologic, topographic, and pollinator diversity (Celep et al., 2020).

Since the publication of the Flora of Turkey and East Aegean Islands vol. 7 (Hedge, 1982a), a number of new taxa, new records, and taxonomic changes have been published involving *Salvia* from Turkey, particularly in the last two decades (Huber-Morath, 1982; Vural and Adigüzel,

1996; Dönmez, 2001; Hamzaoğlu et al., 2005; Behçet and Avlamaz, 2009; Celep et al., 2009a, 2009b, 2011a, 2011b, 2015, 2016; Ilcim et al., 2009; Kahraman et al., 2009, 2010, 2011; Celep and Doğan, 2010; Bagherpour et al., 2011). Based upon molecular phylogenetic results and the classification of Drew et al. (2017) and Kriebel et al. (2019, 2020), Turkish species are represented by three major clades: subg. *Salvia*, subg. *Sclarea*, and the “*S. verticillata* clade”. Although hybridization has not been documented to occur between major clades (i.e. subgenera) of *Salvia*, hybridization has been documented within subgenera (Epling, 1947; Walker et al., 2015; Radosavljević et al., 2019; Nachychko and Sosnovsky, 2020).

Hybridization is an important driver of evolution and can lead to a wide variety of consequences, including sympatric speciation (Rieseberg and Carney, 2005). Celep et al. (2020) reported that mechanical, phenological, and ethological (pollinator behavior) isolation occur among sympatric *Salvia* species in Turkey. While it has been documented that isolation mechanisms (e.g., the staminal lever mechanism in *Salvia*; Claßen-Bockhoff et al., 2003) can limit or prevent hybridization, if closely related *Salvia* species co-occur, flower at the same time and share the

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same chromosome number, they can potentially hybridize (Epling, 1938, 1947; Hedge, 1982a, Wood, 2014; Celep et al., 2020). While species of *Salvia* commonly co-occur, relatively few instances of natural hybridization have been documented. In the New World, hybridization has been reported within subg. *Audibertia* (Epling, 1938, 1947; Emboden, 1971; Walker et al., 2015), subg. *Calosphace* (Epling, 1939; Reisfield, 1987; Wood, 2014), and “subg. *Heterosphace*” (Walker and Elisens, 2001). In the Old World hybridization has been reported within subg. *Sclarea* (Hedge, 1960, 1982a, 1982b; Nachychko and Sosnovsky, 2020) and subg. *Salvia* (Hedge 1982a, 1982b; Radosavljević et al., 2019). The Plant List (TPL: <http://www.theplantlist.org/tpl1.1/search?q=Salvia>; accessed 22 June 2020) listed 17 hybrid species as “accepted” while another 17 hybrid species were listed as “unresolved.” This suggests that hybridization, or at least documented hybridization, is fairly rare within the genus.

Though few hybrids involving *Salvia* have been documented in Turkey, species are often sympatric, and many species within Turkey share identical or similar chromosome numbers, ostensibly allowing for the possibility of hybridization. Hedge (1982a) commented that “Hybrids are not infrequent [in Turkey]” and that “Hybrids are probably more frequent than the few records given might indicate” (Hedge, 1982b). However, there are currently only two formally named hybrids native within Turkey. One documented example of hybridization is *Salvia* × *spiraeifolia* Boiss. & Hohen., with *Salvia bracteata* Poir. and *Salvia suffruticosa* Montbret & Aucher ex Benth. as parental species (Hedge, 1982a, 1982b). These two species have some morphological differences (i.e. flower color and leaf morphology), but their flower morphology and stamen type are the same. *Salvia suffruticosa* has 22–30 mm long yellow flowers and narrow pinnate leaves. *Salvia bracteata* has flowers of 20–30 mm long that are a vibrant shade of pink and have broad pinnate leaves. The resulting hybrids from this cross have a wide range of characteristics. The hybrids display floral colors including shades of yellow, blue, lilac, and pink. It is probable that these various colors are the result of varying degrees of backcrossing between hybrids and the parent species, indicating that the hybrids are fertile (Hedge, 1959). Another example of hybridization in Turkish *Salvia* is *Salvia candidissima* Vahl × *Salvia cyanescens* Boiss. & Bal. This hybrid was first collected near the cities of Kelkit & Köse (Gümüşhane) in northeastern Turkey, on a dry gravel bed (Hedge, 1959, 1982a). The parental species have whitish (*Salvia candidissima*) and blue to purple (*Salvia cyanescens*) flowers; the hybrids varied in color from lilac-blue to white. The wide range of characteristics between the hybrids and the parent species again suggest that backcrossing towards each of the parental species is common (Hedge, 1959). During

plant collecting trips in 2012, 2017, and 2018, two putative hybrids were observed. One involving *S. aucheri* Benth. subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Doğan and *S. heldreichiana* Boiss. ex Benth., and the other involving *S. cyanescens* Boiss. & Bal. and *S. vermifolia* Hedge & Hub.-Mor.

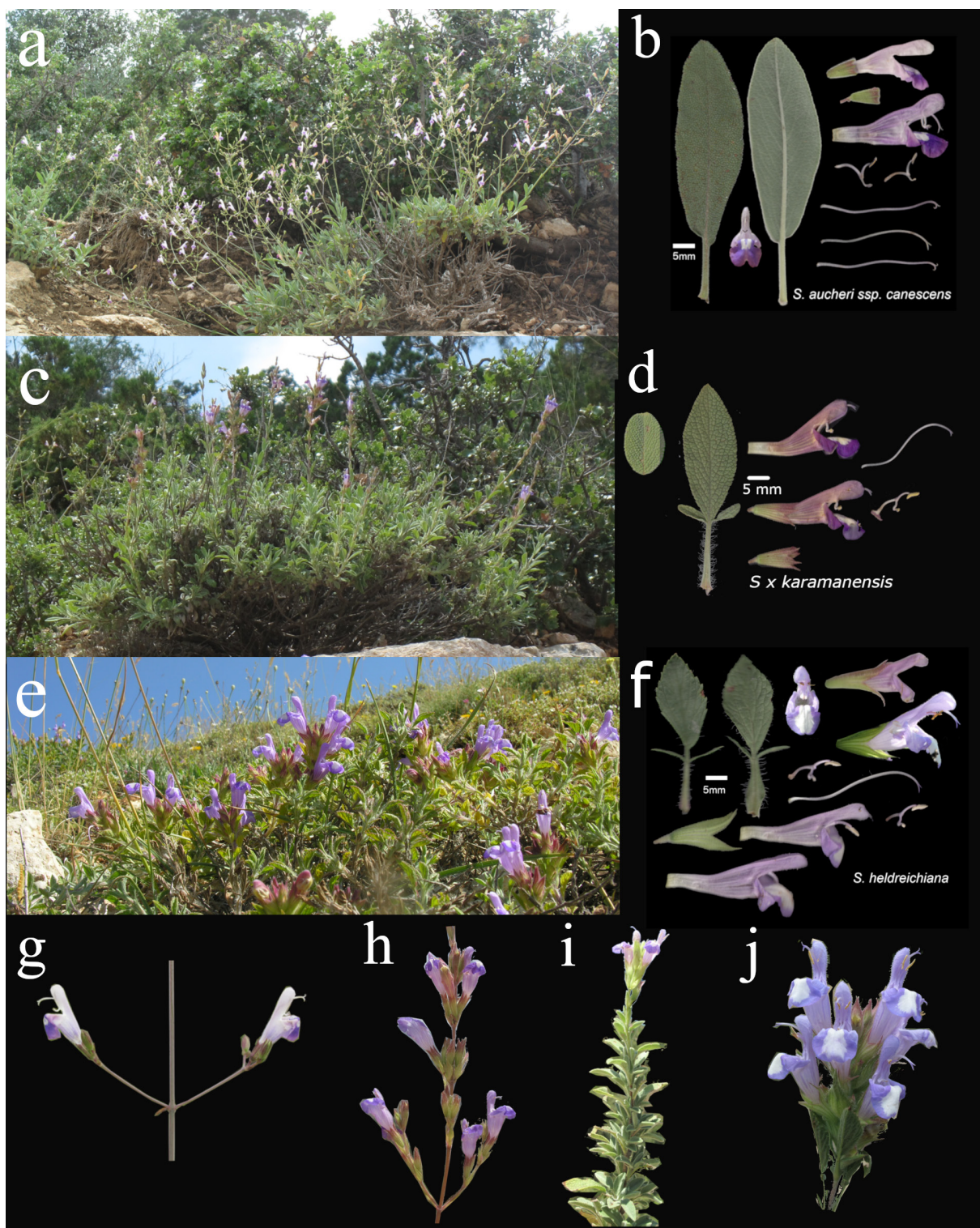
*Salvia aucheri* subsp. *canescens* (Figure 1a, b, g) is endemic to Turkey and naturally occurs only in the Karaman, Ermenek, Mut, and Gülnar regions in the central Mediterranean part of the country at medium to high altitudes (850–1600 m) (Celep et al., 2011). The region is characterized by a Mediterranean climate with hot arid summers and most precipitation occurring during the winter months. *Salvia aucheri* subsp. *canescens* has light lilac or pinkish corollas 15–26 mm in length, and a white-spotted lower corolla lip. The leaves of *Salvia aucheri* subsp. *canescens* are simple and linear-elliptic to elliptic in shape. The stem length is shorter as compared to *Salvia aucheri* subsp. *aucheri* and stem height can range from (20–)30–70 cm (Celep et al., 2011). Inflorescences of *S. aucheri* subsp. *canescens* are widely paniculate and lax. There are usually two flowers (occasionally more) clustered in verticillasters which are clearly distant. *Salvia aucheri* subsp. *canescens* predominately flowers in June.

*Salvia heldreichiana* (Figure 1e, f, j) is endemic to Turkey and is primarily distributed in the eastern and central part of the Mediterranean region with a few small populations in central Turkey. This species generally grows on limestone slopes at medium to high altitudes (900–2000 m). *Salvia heldreichiana* also flowers during June and July. The corollas are 20–35 mm and bluish-purple in color with a large white spot on the lower lip. This species has an average stem height of 20–40 (–60) cm. *Salvia heldreichiana* has trisect leaves with larger terminal segments and a spike-like inflorescence (Hedge, 1982a).

*Salvia cyanescens* (Figure 2a, b) is endemic to Turkey and grows at a wide range of altitudes (400–2300 m) on limestone or igneous slopes. *Salvia cyanescens* usually begins flowering in June and continues through September and generally grows in warm and dry areas. The leaves are oblong, broadly ovate to orbicular, and tomentose with a grayish to greenish color. The corolla color of *S. cyanescens* ranges from blue to dark purple (Hedge, 1982a).

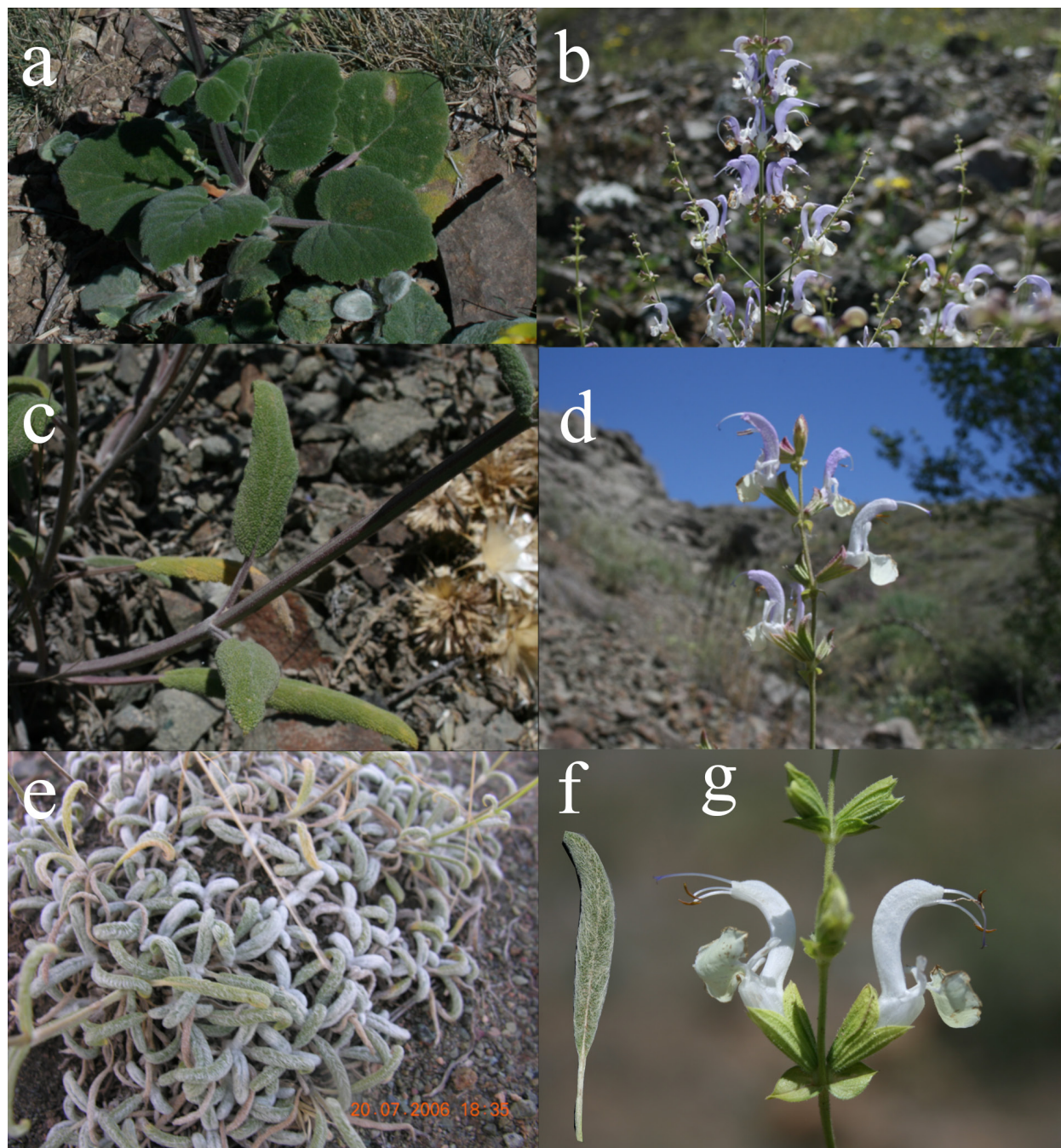
*Salvia vermifolia* (Figure 2e, f, g) is endemic to Turkey and typically occurs at altitudes between 1320 to 1500 m on igneous, gypsum, and serpentine slopes. *Salvia vermifolia* grows up to 60 cm tall and has linear to linear-oblong leaves. The corollas of *S. vermifolia* are mostly pale white with a yellowish lower lip. *Salvia vermifolia* usually begins flowering in June and continues through July (Hedge, 1982a). *Salvia vermifolia* and *S. cyanescens* occur at similar altitudes and are sympatric near Sivas in





**Figure 1.** (a) Habit of *S. aucheri* subsp. *canescens*, (b) basal leaves, corolla, calyx, stamen, and style of *S. aucheri* subsp. *canescens*, (c) Habitus of *S. x karamanensis*, (d) simple and trisect basal leaves, corolla, calyx, stamen, and style of *S. x karamanensis*, (e) Habitus of *S. heldreichiana*, (f) basal leaves, corolla, calyx, stamen, and style of *S. heldreichiana*, (g) verticillaster of *S. aucheri* subsp. *canescens*, (h, i) two different types of inflorescence architecture in *S. x karamanensis*, (j) inflorescence architecture of *S. heldreichiana*.





**Figure 2.** (a) Basal leaves of *S. cyanescens*, (b) verticillasters of *S. cyanescens*, (c) basal and stem leaves of *S. × doganii*, (d) flowers of *S. × doganii*, (e, f) basal of *S. vermifolia*, (g) verticillasters of *S. vermifolia*. Except for (e), all photos were taken from the type area of *S. × doganii*.

the central Anatolia region of Turkey. Both species have similar phenological cycles as well as climate preferences.

Here, using phylogenetic and morphological evidence, we document two instances of hybridization between species of *Salvia* in Turkey, neither of which has previously been documented.

## 2. Materials and methods

### 2.1. Plant materials: sampling and outgroups

The phylogenetic analyses contained 30 taxa of *Salvia*. This included 25 wild-collected samples from Turkey (ingroup) and five species to serve as an outgroup. The above sampling included 2 accessions of the putative hybrids *Salvia aucheri*

subsp. *canescens* × *S. heldreichiana* and 3 accessions of *Salvia cyanescens* × *S. vermifolia*. The two accessions each of *S. heldreichiana*, *S. aucheri* subsp. *cyanescens*, *S. aucheri* subsp. *aucheri* as well as three accessions each of *S. vermifolia* and *S. cyanescens* were also included. The two cultivated species, *Salvia rosmarinus* Schleid. and *Salvia yangii* B.T.Drew, served as outgroup taxa to root the tree.

Putative hybrids as well as presumed parental species were identified using keys provided by Hedge (1982a) and specimens are housed in the ADO herbarium (Kırıkkale, Turkey). In addition, the specimens were cross-checked with relevant material housed in the following public herbaria: ADO, ANK, BM, E, G, GAZI, HUB, ISTE, ISTF, K, KNYA and LE.

## 2.2. DNA extraction, amplification, and sequencing

We extracted DNA from silica-dried *Salvia* leaf material using the DNeasy plant mini kit (Qiagen, Hilden, Germany) following Drew and Sytsma (2011). For phylogenetic analyses we employed the chloroplast (cpDNA) markers *rpl32-trnL*, *trnL-trnF*, and *ycf1* and the nuclear ribosomal (nrDNA) internal and external transcribed spacers (ITS and ETS). The cpDNA region *rpl32-trnL* was amplified with the primers given in Shaw et al. (2007) for most taxa. We used the primer pair *rpl32-F* and *rpl32-866r* (Drew and Sytsma, 2012) to amplify some recalcitrant specimens. The *trnL-F* region was amplified with the 'C' and 'F' primers from Taberlet et al. (1991). For *ycf1* we used the primer pairs 4497f-5282r and 4887f-5778r for amplification as described in Drew and Sytsma (2011). Nuclear ribosomal ITS was amplified with the primers Leu1 (Vargas et al., 1998) and ITS4 (White et al. 1990). For external transcribed spacer (ETS) we used the primers 18S-IGS (Baldwin and Markos 1998) and ETS-bdf1 (Drew and Sytsma, 2011) for amplification. For cpDNA and ITS sequencing we used the same primer pairs as in amplification, while for ETS sequencing we used the primers 18S-E (Baldwin and Markos, 1998) and ETS-bdf1. PCR reactions and thermal cycler conditions followed Sytsma et al. (2002). Samples were sequenced via capillary electrophoreses using an Applied Biosystems 3730XL DNA Analyzer (Thermo Fisher Scientific corporation, California, CA, USA).

## 2.3. Sequence and phylogenetic analyses

All sequence contigs were assembled and manually edited using Geneious v 11.1.5 (Kearse et al., 2012) and aligned using Mesquite v. 3.61 (Maddison and Maddison, 2019). Phylogenetic analyses were conducted using MrBayes v. 3.2.7 (Huelsenbeck and Ronquist, 2001) as implemented on CIPRES (Miller et al., 2010). For the MrBayes analyses, we used evolution models as suggested by the Akaike information criterion (AIC) in JModelTest2 (Darriba et al., 2012). For the cpDNA dataset we used the GTR model of evolution, while for the nrDNA dataset we used GTR +  $\Gamma$  + I. We ran both MrBayes analyses for 5 million generations

but otherwise used default parameters. In both instances, adequate mixing is inferred by the standard deviation of split frequencies falling below 0.01, which was achieved prior to 1 million generations.

## 3. Results

### 3.1. Morphological results

*Salvia* × *karamanensis* Celep & B.T.Drew nothosp. nov. (Figure 1c, d, h, i)

*Salvia aucheri* Benth. subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Doğan × *Salvia heldreichiana* Boiss. ex Benth.

Type: TURKEY. Karaman: between Bucakkışla and Ermenek, about 5–7 km from Bucakkışla, *Quercus coccifera* L. forest, growing among calcareous rocks at the edge of the road. 17 June 2017, 862 m, F.Celep 4027 & B.T. Drew (holotype ADO, isotype GAZI).

Diagnosis: *Salvia* × *karamanensis* differs from *Salvia aucheri* subsp. *canescens* by its trisect leaves with larger terminal segments (rarely simple, always simple in *S. aucheri* subsp. *canescens*); longer calyces (9–15 mm, versus 4–7 mm) and corollas (25–35 mm, versus 15–26 mm); and a shorter (5–15 cm, versus 20–50 cm) and little-branched (non-branched) inflorescence. It differs from *S. heldreichiana* by occasionally having simple leaves (but generally with trisect leaves with a larger terminal segment versus always trisect in *S. heldreichiana*), and a longer and little branched inflorescence with well-spaced verticillasters (in *S. heldreichiana* always an elongated ovoid or spike-like inflorescence with very close verticillasters).

Description: Perennial herb with woody rootstock. Stems 15–50 cm in length, erect, sometimes branched, eglandular pilose below with some glandular villous hairs, densely eglandular pilose above with some glandular hairs having sessile glands. Leaves mostly towards the base of the stem, basal leaves trisect, terminal segment much larger than lateral segments, or occasionally leaves simple but with these leaves distributed along the stem, elliptic to ovate, 7–40 mm in length and 5–20 mm in width. Stem leaves similar shape and size to basal leaves, trisect or rarely simple, rugulose, white pubescent with sessile glands, margin crenulate. Petiole 3–25 mm in length with long ciliate hairs. Inflorescence 5–15 cm long, sometimes very shortly dichotomously branched at the lowest verticillaster (but neither lax nor long as in *S. aucheri* subsp. *canescens*, or densely spike-like as in *S. heldreichiana*), verticillasters 2–8-flowered. Bracts 8–10 mm in length, 3–5 mm in width. Calyx ovate to tubular campanulate, 9–15 mm in length, green to purplish, glandular pilose, some eglandular villous hairs, and sessile glands, ciliate on the nerves. Calyx teeth 3–4 mm in length, bilabiate, upper lip tri-dentate, lower lip bi-dentate, ovate lanceolate, acute to acuminate, margin ciliate (calyx similar to *S. heldreichiana* calyx, in



*S. aucheri* subsp. *canescens* calyx tubular-campanulate, not clearly bilabiate). Corolla bluish-purple with white spot on the lower lip, 25–35 mm in length. Stamen type A (Hedge, 1982a), anthers 3–5 mm in length.

Flowering and fruiting time: *Salvia* × *karamanensis* flowers in June.

Paratypes: Turkey. Karaman: between Bucakkişla and Ermenek, about 5–7 km from Bucakkişla, *Quercus coccifera* L. forest, growing among calcareous rocks at the edge of the road. 862 m. F.Celep & B.T. Drew 837 (ADO), 17 June 2017, ibid F. Celep & B.T. Drew 1102 (ADO), 7 July 2018 (Shrubby, stems branched, leaves simple and dense at the base and along the stem).

Etymology: The specific epithet is derived from name of Karaman city which is near where the type was collected.

Habitat and ecology of *S.* × *karamanensis*: The new hybrid grows on calcareous rocks in open *Quercus coccifera* L. forest.

Distribution and conservation status: *Salvia* × *karamanensis* is known only from the type locality and grows in the central Mediterranean region of Turkey, therefore it is an East Mediterranean floristic region element. The parent species *S. heldreichiana* and *S. aucheri* subsp. *canescens* are sympatric in the Ermenek, Mut, Gülnar, and Karaman regions (where *S. aucheri* subsp. *canescens* is known from), and the life cycles for both species are more or less synchronous. Thus, the hybrid species may be present in other areas where the presumed parental species are sympatric in the central Mediterranean region of Turkey. The current estimated area of occupancy is less than 10 km<sup>2</sup> with the number of known mature individuals being less than 250 (criteria B2 ab (i, iii) of IUCN, 2014). Therefore, it should be classified as CR (critically endangered) according to IUCN (2014) criteria.

*Salvia* × *doganii* Celep & B.T.Drew nothosp. nov. (Figure 2c, d and 3a, b)

*Salvia cyanescens* Boiss. & Bal. × *Salvia vermifolia* Hedge & Hub.-Mor.

Type: Turkey, Sivas, between Altınyayla and Şarkışla, ca. 15 km before Şarkışla, greyish-brownish rock screes, steppe with some shrubs and trees, 14.7.2012, 1323 m, F.Celep 3884 (holotype ADO, isotype GAZI).

Diagnosis: *Salvia* × *doganii* is similar to *S. cyanescens* in floral features but differs from *S. cyanescens* by its narrowly oblong-linear leaves (15–80 mm in length × 5–12 mm in width, versus 30–90 mm in length × 20–75 mm in width in *S. cyanescens*), and differs from *S. vermifolia* by possessing wider narrowly oblong-linear leaves (15–80 mm in length × 5–12 mm in width, versus 20–40 mm in length × 2–4 mm in width); having a white corolla with a lilac upper lip and light yellowish lower lip (in *S. vermifolia* corolla is white with a light yellow lower lip), and generally

having verticillasters with more than two flowers (as in *S. cyanescens*).

Description: Perennial herb, stem erect, 35–70 cm long, branched above, pilose to lanate at the base of the stem, above eglandular papillose with sessile glands. Leaves narrowly oblong-linear, 15–80 mm long × 5–12 mm wide, floccose-tomentose below, margin crenulate-serrate with minute teeth. Petiole up to 45 mm in length on lower leaves, upper stem leaves sessile. Inflorescence paniculate with slender branches, verticillasters 2–6-flowered; bracts broadly ovate-acuminate, 2–7 mm long × 2–5 mm wide. Pedicels 1–3 mm long. Calyx tubular to campanulate, 9–12 mm long, short-pilose (papillose) with sessile glands. Corolla 22–27 mm in length, white with lilac upper lip and light yellow lower lip, ventricose, squamulate. Stamen type B (Hedge, 1982a).

Etymology: The species is named in honor of Turkish botanist, Professor Musa Doğan, who made myriad contributions to *Salvia* taxonomy in Turkey.

Habitat and ecology of *S.* × *doganii*: The newly described hybrid occurs in steppe ecosystems. The plant grows on greyish-brownish volcanic rocky screes.

Distribution and conservation status: *Salvia* × *doganii* is known only from the type locality in the central Anatolia region of Turkey and is a component of the Irano-Turanian floristic region. The known distribution area is less than 1 km<sup>2</sup>, and the total number of known individuals is about 15. In addition, *S.* × *doganii* grows near the recently completed Yapıaltın Dam. Individuals are at an elevated risk of extinction due to potential local climate change caused by the dam and potential future construction activities associated with the dam. *Salvia* × *doganii* should be classified as CR (critically endangered) according to IUCN (2014) criteria.

### 3.2. Molecular results

The combined nrDNA alignment was 1062 characters in length, with ITS accounting for 664 base pairs and ETS contributing the remaining 398 nucleotides. Two main clades were recovered within the ingroup (Figure 4). The first clade consisted of *S.* × *doganii* and putative parent species *S. cyanescens* and *S. vermifolia*, while the second clade consisted of *S.* × *karamanensis*, presumed parent species *S. aucheri* ssp. *canescens* and *S. heldreichiana*, and several related taxa. Both major clades were recovered with posterior probability [PP] values of 1.00. Within the *S.* × *doganii* clade there were no well-supported (all PP values < 0.70) relationships. The three accessions of *S.* × *doganii* were interspersed with the parental species. Within the *S.* × *karamanensis* clade, support for relationships was also low; however, the two accessions of *S.* × *karamanensis* did not cluster together. *Salvia* × *karamanensis* btd 1102 was recovered in a clade (0.91 PP) with the two accessions of *S. heldreichiana*, while *Salvia* × *karamanensis* Celep



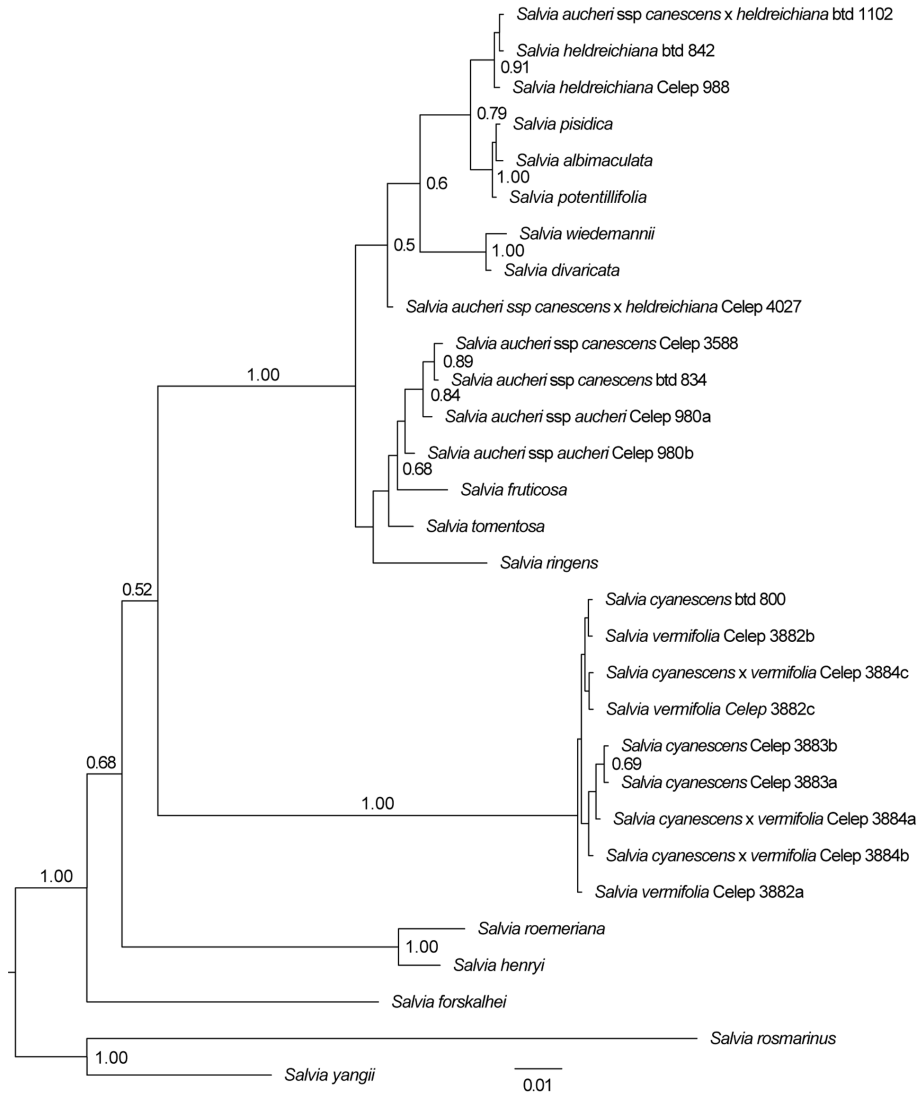
**Figure 3.** A. Habit of *S* × *doganii*. B. Flowers and verticillaster of *S* × *doganii*.

4027 was sister to a clade consisting of related species (*S. pisidica*, *S. albimaculata*, *S. potentillifolia*, *S. divaricata*, and *S. wiedemannii*) and the aforementioned *S. heldreichiana*/ *Salvia* × *karamanensis* btd 1102 clade. The accessions of *S. aucheri* ssp. *canescens*, *S. aucheri* ssp. *aucheri*, and three related species made up a poorly supported second clade (PP = 0.53). After a close inspection of the alignment data, it was discovered that the intermediate topological position (intermediate between putative parents) of *Salvia* × *karamanensis* Celep 4027 was the result of 16 polymorphic characters (Figure 5). These 16 polymorphic characters were intermediate between *S. aucheri* ssp. *canescens* and *S. heldreichiana*. Additionally, one character

(134 from the ITS dataset) of *S. aucheri* ssp. *aucheri* Celep 980b was polymorphic; intermediate between *S. aucheri* ssp. *canescens* and *S. heldreichiana*.

The combined cpDNA data matrix contained 2686 characters, with *ycf1* containing 1047 characters, the *rpl32-trnL* region containing 857 characters, and the *trnL-trnF* region contributing 782 nucleotides. The cpDNA analysis recovered the same two major ingroup clades as found in the nrDNA tree, with the first clade containing *S.* × *doganii*, *S. cyanescens* and *S. vermifolia*, and the second containing *S.* × *karamanensis*, *S. aucheri* ssp. *canescens* and *S. heldreichiana*, and related taxa (Figure 6). As in the nrDNA phylogeny, both major clades were recovered





**Figure 4.** Bayesian inference “all compatible groups” consensus tree based on nrDNA ITS and ETS data (ITS+ETS). Only posterior probability values above 0.65 are shown except for nodes of interest.

with PP = 1.00. Within the *S.* × *doganii* clade, two major subclades were recovered: one containing two accessions of *S. cyanescens* and two accessions of *S.* × *doganii* (PP = 0.92), and the second containing (PP = 0.86) the three accessions of *S. vermifolia* (which together formed a clade, PP = 1.00), one accession of *S. cyanescens*, and one accession of *S.* × *doganii*. In the *S.* × *karamanensis* clade there was little well-supported resolution. However, a subclade was recovered (PP = 0.99) consisting of one accession of *S. heldreichiana*, both accessions of *S. aucheri* ssp. *aucheri*, and three related species (*S. divaricata*, *albimaculata*, and *S. wiedemannii*), as well as another subclade containing the other accession of *S. heldreichiana* and one accession of *S. aucheri* ssp. *canescens* (PP = 1.00). The two accessions

of *S.* × *karamanensis* formed a clade, but this clade had scant support (PP = 0.1).

#### 4. Discussion

The parent species of *S.* × *karamanensis* (*S. aucheri* subsp. *canescens* and *S. heldreichiana*) are within *Salvia* subg. *Salvia* (Drew et al., 2017; Hu et al., 2018; Kriebel et al., 2019, 2020). In this clade, corollas have a ± straight upper lip and a three-lobed lower lip with one larger middle lobe flanked by two smaller revolute lateral lobes. The corolla tube is gradually wider towards the throat, straight, and annulate (hairy ring at the lower part of the corolla tube). The length of the upper lever arm is slightly longer than the lower one or subequal. The anther connective tissue

Taxon/Character #	23	41	42	48	64	131	134	189	597	608	775	915	953	1005	1020	1024
<i>Salvia x karamanensis</i> Celep 4027	R	S	W	R	Y	K	Y	Y	Y	S	R	R	S	S	W	S
<i>Salvia x karamanensis</i> btd 1102	G	G	T	A	C	G	C	C	C	G	G	G	C	G	A	G
<i>S. aucheri</i> ssp <i>canescens</i> Celep 3588	A	C	A	G	T	T	T	T	?	?	A	A	G	C	T	C
<i>S. aucheri</i> ssp <i>canescens</i> btd 834	A	C	A	R	T	T	T	T	T	C	A	A	G	C	T	C
<i>S. heldreichiana</i> Celep 988	G	G	T	A	C	G	C	C	?	?	G	G	C	G	A	G
<i>S. heldreichiana</i> btd 842	G	G	T	A	C	G	C	C	C	G	G	G	C	G	A	G
<i>S. aucheri</i> ssp <i>aucheri</i> Celep 980a	A	C	A	A	T	T	C	T	T	C	G	A	G	C	T	C
<i>S. aucheri</i> ssp <i>aucheri</i> 980b	A	C	A	A	T	T	Y	T	T	C	G	A	G	Y	T	C

**Figure 5.** Selected characters from the nrDNA alignment highlighting polymorphic characters present in *S. x karamanensis* 4027. Characters 23-608 are nrITS characters, while characters 775-1024 are from the ETS alignment.

is more or less the same length as the filament, and the lower lever arm bears some fertile pollen grains which are positioned close to the corolla tube entrance (Figure 1d, Stamen Type A, according to Hedge, 1982a). The style is exerted from the upper corolla lip and slightly curved due to the straight upper lip of the corolla.

*Salvia aucheri* subsp. *canescens* has  $2n = 16-18$ , and *S. heldreichiana* has  $2n = 14-16$  (Hedge, 1982a; Martin et al., 2011). Though their corolla and stamen morphology are very similar, *S. aucheri* subsp. *canescens* has corollas 15–26 mm long, while *S. heldreichiana* has 20–35 mm long corollas. These results suggest that while differences in chromosome number and corolla length may impede hybridization in many or most instances where the species occur sympatrically, hybridization may be common between the species when both have chromosome numbers of  $2n = 16$  and corollas between 20–26 mm long with the similar staminal lever mechanism.

*Salvia x karamanensis* was growing near the parental species and we observed many intermediate forms, which suggests backcrossing with the putative parents. For example, we observed both trisect (from *S. heldreichiana*) and simple leaved (from *S. aucheri* subsp. *canescens*) specimens of *S. x karamanensis*. The corolla and calyx morphology of *S. x karamanensis* is more similar to *S. heldreichiana* than *S. aucheri* subsp. *canescens*. However, the inflorescence architecture is intermediate between its parents; neither ovoid or elongated and spike-like as in *S. heldreichiana* (Figure 1j) nor widely elongated and paniculate like *S. aucheri* subsp. *canescens* (Figure 1g, Table).

*Salvia x doganii* (as well as *S. cyanescens* and *S. vermifolia*) is part of the *Salvia* subg. *Sclarea* clade (Drew et al., 2017; Kriebel et al., 2019, 2020). Flowers in this clade have falcate upper lips and three-lobed lower lips with one large middle lobe. The corolla tube is gradually wider towards the throat and squamulate. The corolla tube entrance is very narrow, and the lower staminal lever arm is sterile, spoon-shaped, and completely blocks the corolla tube entrance. The length of the upper lever arm is considerably longer than the lower one, and the anther connective tissue is also considerably longer than the filament (Stamen Type B according to Hedge,

1982a). The style is exerted from the upper corolla lip and is highly curved due to the falcate shape of the corolla upper lip. *Salvia cyanescens* (Figure 2b) and *S. vermifolia* (Figure 2g) are similar in corolla size and stamen morphology, and they have chromosome numbers of  $2n = 22$  (Hedge, 1982a; Martin et al., 2011, pers. comm. with Prof. E. Martin for the chromosome number of *S. vermifolia*).

*Salvia x doganii* was collected between Altınyayla and Şarkışla (Sivas) in the central part of Turkey. The hybrid was growing sympatrically with the putative parental species and we observed many intermediate morphological forms, suggesting backcrossing between the hybrid species and the parents. The hybrid is morphologically more similar to *S. cyanescens* in terms of corolla color (corolla white with lilac upper lip and yellow lower lip in both) relative to *S. vermifolia*. In the area, *S. cyanescens* was represented only by individuals with broadly ovate to orbicular leaves (Figure 2a) while *S. vermifolia* had oblong-linear leaved individuals (Figure 2e, f). However, *S. x doganii* had leaves intermediate between these two species (Figure 2c).

Recently, several molecular phylogenetic studies based on nuclear and chloroplast markers have been used to suggest or demonstrate putative hybridization events within Lamiaceae and the other families (e.g., Bendiksby et al., 2011; Drew et al., 2014; González-Gallegos et al., 2015; Walker et al., 2015; Dirmenci et al., 2018, 2019; Xiang et al., 2018). Although in general phylogenetic relationships between the hybrid and putative parent species were poorly resolved here, there is evidence in both trees that support our hybridization hypotheses. In the nrDNA tree the two accessions of *S. x karamanensis* did not cluster together. *Salvia x karamanensis* btd 1102 was in a moderately supported (PP = 0.91) clade with two accessions of *S. heldreichiana*, while *S. x karamanensis* Celep 4027 was in a position intermediate relative to the placement of *S. heldreichiana* and *S. aucheri* subsp. *canescens*. The fact that *S. x karamanensis* Celep 4027 had 16 polymorphic characters that were intermediate between *S. heldreichiana* and *S. aucheri* subsp. *canescens* is strong evidence that *S. x karamanensis* is indeed a hybrid species and that *S. heldreichiana* and *S. aucheri* subsp. *canescens* are indeed the parents (Figure 4).





**Table.** Comparison of morphological characteristics of the new hybrids and their parent species. Blue highlighted species belong to the subg. *Salvia* clade with *S. × karamanensis* and its parents, yellow highlighted species belong to the subg. *Sclarea* clade with *S. × doganii* and its parents.

	<i>S. aucheri</i> subsp. <i>canescens</i>	<i>S. × karamanensis</i>	<i>S. heldreichiana</i>	<i>S. cyanescens</i>	<i>S. × doganii</i>	<i>S. vermifolia</i>
Leaf morphology	Simple and linear-elliptic to elliptic	Mainly trisect with larger terminal segment, occasionally simple	Trisect with larger terminal segments	Simple, oblong to broadly ovate to suborbicular	Narrowly oblong	Linear to linear-oblong
Inflorescence architecture	Widely paniculate and lax	Shortly dichotomic at the lower verticillasters, or unbranched	Ovoid or elongated spike	Paniculate with slender branches	Paniculate with slender branches	Paniculate with slender branches
Inflorescence height	20–50 cm	5–15 cm	5–15 cm	(10–) 25–50 cm	20–40 cm	10–30 (–40) cm
Verticillasters and flower number	Verticillasters very distant, usually 2 flowers per verticillaster	Verticillasters distant, 2–6 flowered	Verticillasters extremely close, over and over, 2–6 flowered	Verticillasters distant, 2–6 flowered	Verticillasters distant, 2–6 flowered	Verticillasters distant, 2-flowered
Bract size	1.5–4 mm length, 0.5–1.5 mm width	8–10 mm length, 3–5 mm width	20–35 mm length, 7–12 mm width	2.5–6 mm length, 2–4.5 mm width	2–7 mm length, 2–8 mm width	4–7 mm length, 4–5 mm width
Corolla color	Light lilac or pinkish with a white spot on the lower lip	Blueish-purple with a white spot on the lower lip	Blueish-purple with a white spot on the lower lip	Lilac to violet	White with lilac upper lip and light yellow lower lip	White with yellow lower lip
Corolla length (mm)	15–26 mm	25–35 mm	20–35 mm	16–32 mm	22–27 mm	15–25 mm
Calyx morphology	Ovate to tubular-campanulate	Tubular infundibular	Tubular infundibular	Tubular-campanulate, usually purple-tinted	Tubular-campanulate, yellowish green with or without purple tinted	Tubular-campanulate, yellowish green
Calyx size (mm)	4–7 mm	9–15 mm	14–17 mm	7–12 mm	9–12 mm	10–12 mm
Calyx teeth shape	Triangular, acute to acuminate	Ovate-lanceolate to triangular	Ovate-lanceolate, acute to acuminate	Triangular, acute to acuminate, upper lip tridentate	Triangular, acute to acuminate, upper lip tridentate	Triangular, acute to acuminate, upper lip tridentate
Calyx teeth length	1–2 mm	ca. 4 mm lower teeth, upper ones smaller	8–9 mm	4–6 mm	5–6 mm	5–6 mm



Celep 988 (and three related species). The pattern of relationship between *S. heldreichiana* and taxa of *S. aucheri* suggests possible past hybridization between the species and subsequent chloroplast capture. The cpDNA dataset also supports a hybridization hypothesis for *S. × doganii*. One accession of *S. × doganii* (Celep 3884b) was part of a clade (PP = 0.86) consisting of the three *S. vermifolia* accessions and one accession of *S. cyanescens* (btd 800), while the other two accessions of *S. × doganii* formed a clade (PP = 1.00) with a second accession of *S. cyanescens* (Celep 3883b). This latter clade was sister (PP = 0.92) to the third accession of *S. cyanescens* (Celep 3883a). The fact that one accession of *S. × doganii* was recovered with accessions of *S. vermifolia* while the other two were recovered with accessions of *S. cyanescens* is the type of pattern expected if *S. × doganii* is actually a hybrid with *S. vermifolia* and *S. cyanescens* as parental species. Admittedly, the support values in both the cpDNA and nrDNA trees were suboptimal. However, the signal that was recovered reinforces our hybridization hypotheses.

Morphological and phylogenetic evidence both indicate that *S. × karamanensis* and *S. × doganii* are of hybrid origin.

The morphological and genetic evidence also support that *S. aucheri* subsp. *aucheri* and *S. heldreichiana* are the parent species of *S. × karamanensis* and that *S. cyanescens* and *S. vermifolia* are the parent species of *S. × doganii*. With the addition of these two newly documented and formally named hybrids, there are about 100 accepted native species and 4 named native hybrids of *Salvia* known to occur in Turkey.

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