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A new species of *Salvia* from the Fulgentes clade (Lamiaceae), from Puebla, Mexico

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Abstract

A new species of *Salvia* from the state of Puebla, Mexico, is described and illustrated: *Salvia gavilanensis* sp. nov., growing in oak forests and oak scrubs, located in the Biosphere Reserve of Tehuacán-Cuicatlán. The new taxon is morphologically similar to *S. microphylla*, but differs by the herbaceous habit (vs. suffrutescent or shrubby), the strigulose pubescence of the leaves (vs. puberulent to tomentulous), bracts with the margin irregularly denticulate and persistent until the beginning of the anthesis (vs. bracts with the margin entire and deciduous before the anthesis), papillae inside the corolla tube with bidentate apex (vs. papillae with trilobate apex), and the lower lip of the corolla shorter in length compared to the upper lip (vs. lower lip of the corolla longer than the upper lip). Additionally, a phylogenetic analysis based on molecular data (nuclear ITS and the plastid regions *trnL-trnF* and *trnH-psbA*) was conducted to explore the relationships of the new species. According to the results, the new species belongs to the Fulgentes clade, which is congruent with the morphology of the species. A key to distinguish *Salvia gavilanensis* from other species of the clade present in the reserve of Tehuacán-Cuicatlán is included.

Keywords: biodiversity, endemic, phylogeny, Tehuacán-Cuicatlán

Introduction

Salvia Linnaeus (1753: 23) is the most diverse genus of the Lamiaceae family, with nearly 1000 species around the world (Harley *et al.* 2004). In Mexico, *Salvia* is the most diverse genus of angiosperms with 306 species and 77% of endemism (Villaseñor 2016; Martínez-Gordillo *et al.* 2017). As a result, the country is considered the main center of diversification for the genus in the New World (Ramamoorthy & Elliot 1998; Jenks *et al.* 2013). In the last five years, at least 16 new species of *Salvia* have been described in different regions of the country (Bedolla-García & Zamudio 2015, 2018; Fragoso-Martínez *et al.* 2015; González-Gallegos 2015; González-Gallegos & Castro-Castro 2016; González-Gallegos & López-Enríquez 2016; González-Gallegos *et al.* 2018; Martínez-Gordillo *et al.* 2016a, 2016b; Zamudio & Bedolla-García 2018), demonstrating that there is still taxonomic work regarding the genus, that needs to be done in Mexico.

During the taxonomic revision of the Lamiaceae for the project Flora del Valle de Tehuacán-Cuicatlán, unusual specimens of *Salvia* were detected. These specimens could not be assigned to any of the described taxa and are here proposed as a new species of *Salvia* from the Fulgentes clade. This clade includes ca. 16 species, distributed from Mexico to Central America, that are divided in the subclades Holwaya and Fulgentes (Fragoso-Martínez *et al.* 2018). Most of its species are characterized by large red flowers with ornithophyllous pollination (Jenks *et al.* 2013).

It is noteworthy that the infrageneric classification of the subgenus *Calosphace* (Benth 1833: 198), the most diverse group of *Salvia* of the New World, has proved to be artificial in comparison with the phylogenies published in recent years (Jenks *et al.* 2013; Fragoso-Martínez *et al.* 2018). Only twelve of the 48 taxonomic sections evaluated in the phylogenies of the subgenus have resulted monophyletic (Fragoso-Martínez *et al.* 2018). As a result, it has become useful to assign new taxa employing a phylogenetic approach and not only by morphological or geographical similarities, which have been used to delimit sections and seem to be prone to homoplasy. Therefore, in addition to

the morphological comparison of the new species with similar taxa, we assessed its phylogenetic position using DNA sequences of three molecular markers (nuclear ITS and two plastid regions: *trnH-psbA* and *trnL-trnF*), analyzed in combination with previous published datasets (Fragoso-Martínez *et al.* 2018; González-Gallegos *et al.* 2018).

Materials and methods

Herbarium and field work:—The new species was identified using the keys of *Salvia* subgen. *Calosphace* by Epling (1939) and contrasted against specimens of similar species from the collections of the National Herbarium of Mexico, Universidad Nacional Autónoma de México (MEXU) and the herbarium of the Facultad de Ciencias, Universidad Nacional Autónoma de México (FCME). The type specimens of the morphologically similar species were reviewed in JSTOR Global Plants (2018).

The specimen designated as type of *Salvia gavilanensis* *sp. nov.* was collected during a field exploration to the municipality of Caltepec, Puebla, in October 2016. At least four duplicates were obtained from the collection and were distributed to the collections of the FCME and MEXU herbaria. The specimens were prepared according to the methods described in Lot & Chiang (1986). Silica dried foliar tissue and alcohol preserved flowers were collected during field work.

Molecular methods:—DNA extraction from silica gel-dried tissue followed Fragoso-Martínez *et al.* (2018). Genomic DNA from the species in Table 1 was used to amplify by PCR the markers ITS, *trnL-F*, *psbA-trnH*, following the PCR profiles and primers combinations described in Fragoso-Martínez *et al.* (2018). Sanger sequencing was performed in the Laboratorio de Biología Molecular de la Biodiversidad y la Salud, Instituto de Biología, Universidad Nacional Autónoma de México.

TABLE 1. Newly sequenced species of *Salvia* including the recently described species.

Taxon	Voucher information	ITS	<i>trnH-psbA</i>	<i>trnL-trnF</i>
<i>Salvia lineata</i>	P. Tenorio L. 17163 (MEXU)	MK775690	MK790258	MK790260
<i>Salvia gavilanensis</i>	E. Martínez-A. <i>et al.</i> 529 (FCME)	MK775691	MK790259	MK790261

Sequence edition, alignment and analyses:—The sequences were assembled and edited in the software Geneious v.10 (Kearse *et al.* 2012). Subsequently, the alignments were made using the online version of MAFFT v.7 (Katoh & Standley 2013), followed by manual adjustment in PhyDE v.0.9971 (Müller *et al.* 2010). The maximum likelihood analysis was performed using the IQ-TREE algorithm (Nguyen *et al.* 2015) on the IQ-TREE web server (Trifinopoulos *et al.* 2016). Before the phylogenetic analysis, model selection was carried out using the ‘auto’ model selection option, which evaluates 22 substitution models (Chernomor *et al.* 2016). The models selected were GTR+F+I+G4 for the ITS marker, TIM+F+G4 for the *trnL-trnF*, and K3Pu+F+G for the *trnH-psbA* region. After the selection of models, all molecular markers were concatenated in a single matrix and from which the phylogenetic analysis was carried out. The tree resulting from the phylogenetic analysis was drawn using the FigTree v.1.4.2 (Rambaut 2014) software.

Taxonomic description

Salvia gavilanensis Martínez-Ambriz, Fragoso & Mart. Gord., *sp. nov.* (Fig. 1)

Salvia microphyllae affinis, sed habitu herbaceus, foliis supra et subtus strigulosis, bracteis denticulatis margine, persistentibus anthesis initio, tubo corollae ventricosus et invaginatus basis versus, labio infero corollae 6–8.4 mm longo.

Type:—MEXICO. Puebla. Cerro El Gavilán, 18°10'5" N, 97°27'34" W, 2162 m, 14 October 2016, E. Martínez-Ambriz *et al.* 529 (holotype: FCME!, isotype: MEXU!).

Herb perennial, 0.25–0.4 m tall, stems green-purple colored, pubescent with simple trichomes and pedicellate glandular trichomes, yellowish. Leaf blades ovate to ovate-lanceolate, 1.8–3.3 × 0.9–1.7 cm, base shortly cuneate, margin serrate-crenate, slightly revolute, apex acute to short-acuminate, upper surface green, strigulose with simple trichomes and glandular pedicellate trichomes, yellowish, lower surface green, slightly lighter than the upper surface, sparsely



FIGURE 1. Holotype of *Salvia gavilanensis* kept in FCME.

strigulose, with simple trichomes, more densely distributed along the veins and glandular pedicellate trichomes, yellowish, in addition to peltate translucent trichomes; petioles 0.5–1.8 cm long, strigose. Inflorescences terminal, racemiform, (2.1–)3.5–9.6(–11.1) cm long; peduncle (0.5–)0.9–2.8(–3.2) cm long, internodes 0.9–2.3 cm long, 2–6 flowers per node; bracts persistent until the beginning of the anthesis, 3–8 mm long, ovate to lanceolate, margin irregularly denticulate, apex acuminate to caudate, upper surface pilose, with simple trichomes and glandular pedicellate trichomes, lower surface glabrescent, with parallel venation. Flower with pedicel 1–3 mm long, hirtellous. Calyx 0.8–1.1 cm long, slightly infundibuliform, upper lip 7-veined, pilose, with simple trichomes, glandular pedicellate trichomes, yellowish, and peltate translucent trichomes. Corolla red, 1.85–2.8 cm long, tube 1.2–1.6 cm long, distally slightly ventricose and invaginated near the base, with a pair of linear papillae inside the tube, erect, with bidentate apex, upper lip 6.7–11.3 mm long, lower lip 6–8.4 × 5–6 mm, without maculae. Stamens inserted, filaments 6–6.5 mm long, connective apex slightly oblique, connective 8.4–9.6 mm, puberulent, abaxially with a distal abaxial tooth, filiform staminodes. Style 2.6–3.5 cm long, exerted, pilose; gland of the gynobase larger than the ovary. Mericarps not observed.

Etymology:—The name of the species refers to the only site from where it is known and has been collected: Cerro El Gavilán in Puebla, Mexico.

Distribution, habitat and phenology:—*Salvia gavilanensis* is endemic to Puebla, Mexico (Fig. 2). It grows in oak forests and oak scrubs, with calcareous soils, at an elevation of 2160 m. Flowering occurs in October.

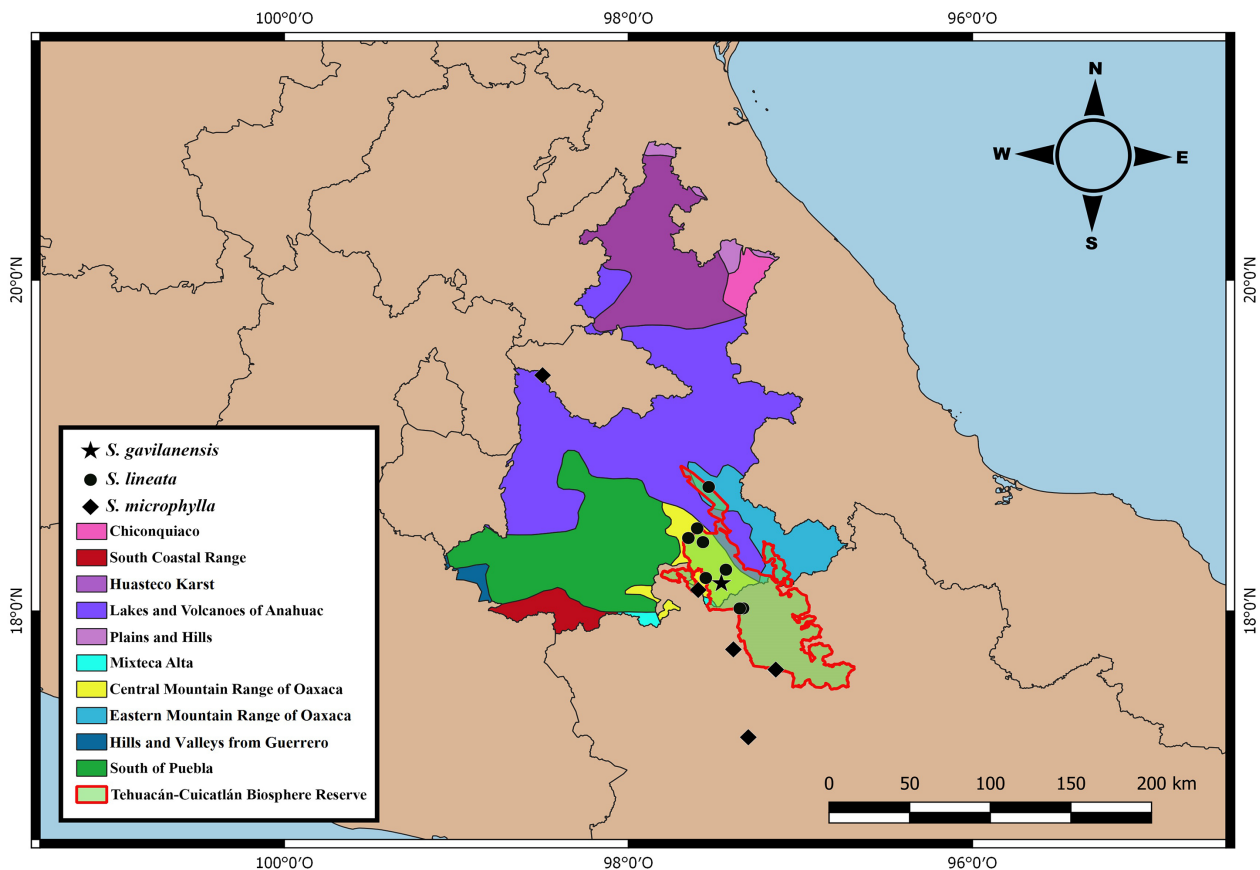


FIGURE 2. Distribution map of *S. gavilanensis*, *S. lineata* and *S. microphylla*, considering physiographic regions. According to Cervantes-Zamora *et al.* (1990).

Discussion:—*Salvia gavilanensis* is compared with *S. microphylla* Kunth (1818: 295) and *S. lineata* Benth. (1835: 724), which are the species that share distribution and morphological similarities with the former (Table 2). *Salvia microphylla* was considered the most closely related species to *S. gavilanensis*, due to its morphological similarity, since they share leaves ovate, similar in size, racemiform inflorescences and flowers similar in color and length. However, *S. gavilanensis* can be distinguished by the herbaceous habit, strigulose pubescence of the leaves, bracts with the margin irregularly denticulate and persistent until the beginning of the anthesis, calyx slightly infundibuliform, the slightly ventricose corolla tube, papillae inside the corolla tube with bidentate apex (Fig. 3K) and the lower lip of the corolla with smaller size (6–8.4 × 5–6 mm).

As for the characters that help to differentiate between *S. lineata* and *S. gavilanensis*, the former species lacks pubescence in the leaves, the inflorescence is longer (11–27.5 cm long), calyx tubular, corolla tube conspicuously ventricose, the papillae inside the corolla tube are curved with a bilobate apex (Fig. 3L), the lower lip of the corolla is longer (8–11 mm long), and the staminal lever glabrous and straight in comparison with the staminal lever puberulent and curved in *S. gavilanensis* (Fig. 3H).

According to the phylogenetic analysis conducted, *Salvia gavilanensis* belongs to the Fulgentes clade (Fig. 4), that also comprises *Salvia microphylla* and *S. lineata*. This result is congruent with the morphology, since the three species share with the remaining taxa of the clade the following characters: pubescence composed of simple and glandular trichomes, an upper lip of the calyx 5–7 veined, a ventricose corolla (Fig. 3 D–F) in red to pinkish colors, inserted stamens and pubescent style. Here, we provide an identification key for the species comprised in the clade, that are distributed in the area of the Tehuacán-Cuicatlán reserve.

TABLE 2. Morphological comparison among *S. gavilanensis*, *S. lineata* and *S. microphylla*.

Characters	<i>S. gavilanensis</i>	<i>S. lineata</i>	<i>S. microphylla</i>
Habit	Herbaceous	Herbaceous	Suffrutescent or shrubby
Leaf shape	Ovate to ovate-lanceolate	Ovate-deltoid	Ovate, deltate or elliptic
Leaf size (cm)	1.8–3.3 × 0.9–1.7	1.8–4.7 × 1.3–3.4	1–2.5 × 0.7–1.6
Base	Shortly cuneate	Rounded or truncate	Truncate or occasionally cordate
Inflorescence length (cm)	3.5–9.6	11–27.5	4–7.3
Peduncle length (cm)	0.9–2.8	4–12.8	1–2.3
Internodes length (cm)	0.9–2.3	1.2–6.3	0.4–1.5
Flowers per node	2–6	2–6	2–4
Bracts length (mm)	3–8	4–7	4–6
Calyx length (cm)	0.8–1.1	0.9–1	1.1–1.3
Corolla length (cm)	1.85–2.8	2.5–3.6	2.2–2.6
Corolla tube length (cm)	1.2–1.6	1.8–2.8	1.5–1.7
Length of the upper lip (mm)	6.7–11.3	6–8	6–10
Staminal lever	Curved	Curved	Straight
Connective pubescence	Puberulent	Glabrous	Puberulent
Style length (cm)	2.6–3.5	2.4–3.9	2.1–2.7

Key to the species of *Salvia* Fulgentes clade from the Tehuacán-Cuicatlán reserve.

1. Stems and leaves with dendritic trichomes *S. karwinskii*
- Stems and leaves with simple trichomes or glabrous 2
2. Leaves glabrous on both sides *S. lineata*
- Leaves pilose, pubescent, puberulent, strigulose, tomentulose or villous at least on one of the two sides 3
3. Corolla longer than 3 cm 4
- Corolla shorter than 3 cm 5
4. Stems and leaves villous; inflorescences 16–32.4 cm long, with 1–3 flowers per node *S. stolonifera*
- Stems and leaves pilose to pubescent, sometimes tomentulose; inflorescences 7.9–18.5 cm long, with 4–8 flowers per node *S. fulgens*
5. Leaves pilose, puberulent or tomentulose on both sides; inflorescences with 2–4 flowers per node, bracts with entire margin; lower lip of the corolla 8–12 mm long *S. microphylla*
- Leaves strigulose on both sides; inflorescences with 2–6 flowers per node, bracts with irregularly denticulate margin; lower lip of the corolla 6–8.4 mm long *S. gavilanensis*

Regarding the phylogenetic position of *S. gavilanensis* within the Fulgentes clade, it is the sister species of the complex formed by *S. microphylla* and the species belonging to section *Flocculosae* (Fig. 4). Morphologically, all the species included in this complex resemble *S. microphylla*, and they are characterized by a corolla size shorter than 3 cm long, an upper lip of the calyx apiculate (Fig. 3 A–C) and a reduction or absence of the papillae inside the corolla tube, compared to the rest of the species belonging to the Fulgentes clade (Fig. 5). These characters have been related to a possible reversion from the ornithophily to melittophily (Fragoso-Martínez *et al.* 2018; Martínez-Ambriz, 2019), showing that the pollination syndromes in *Calosphace* are labile.

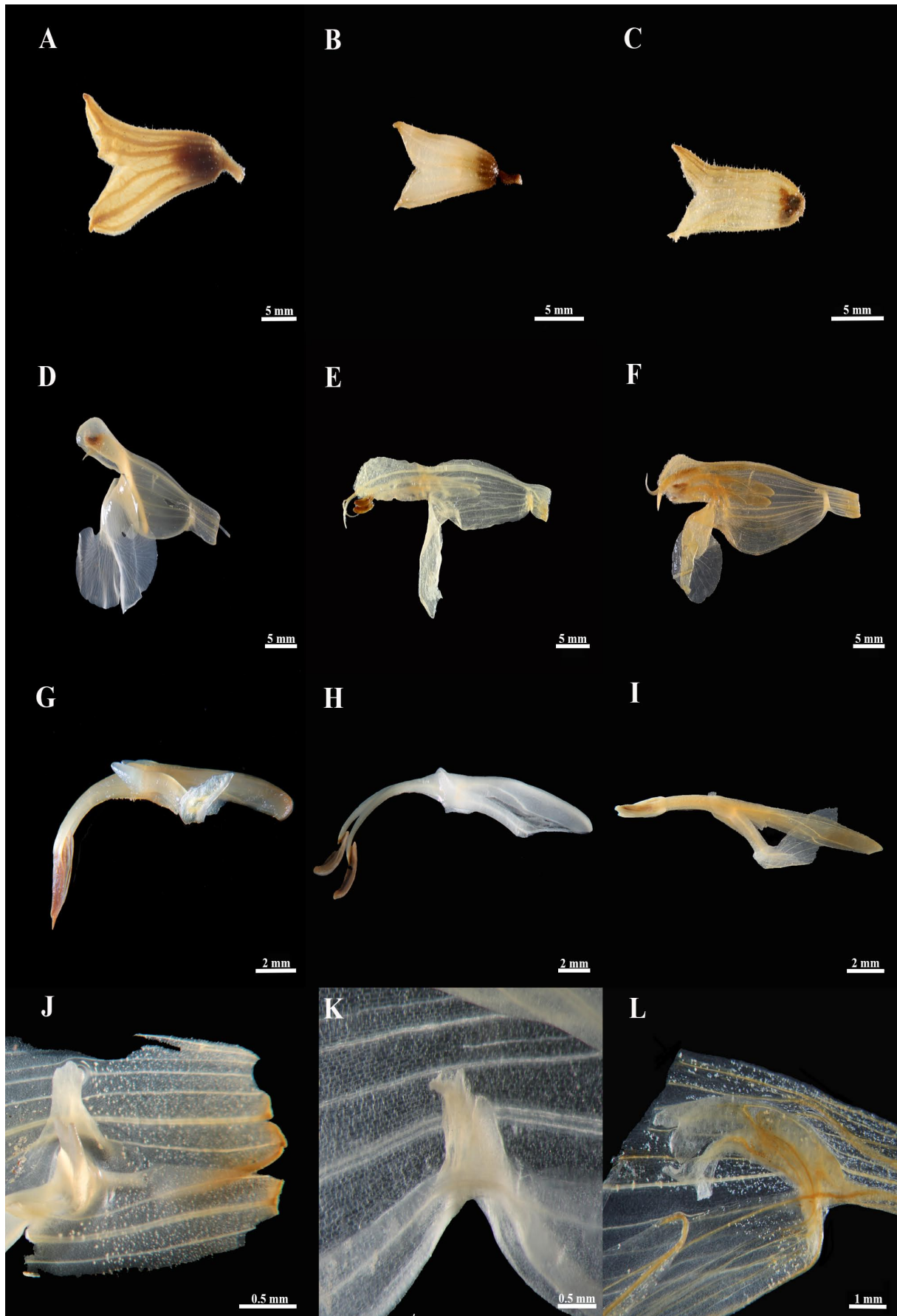


FIGURE 3. Distinctive floral morphological characters among *S. microphylla* (A. Calyx, D. Corolla, G. Stamens, J. Papillae inside of the corolla tube); *S. gavilanensis* (B. Calyx, E. Corolla, H. Stamens, K. Papillae inside of the corolla tube) and *S. lineata* (C. Calyx, F. Corolla, I. Stamens, L. Papillae inside of the corolla tube).

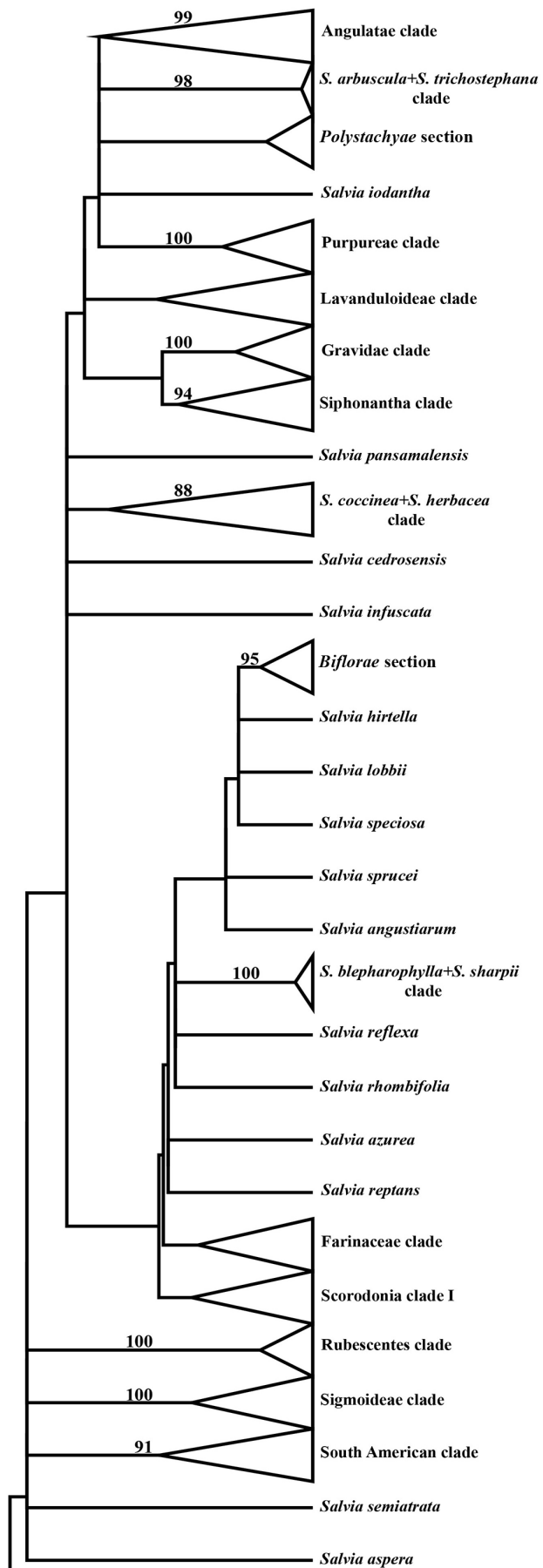


FIGURE 4. Continuation of the tree in Fig. 5.

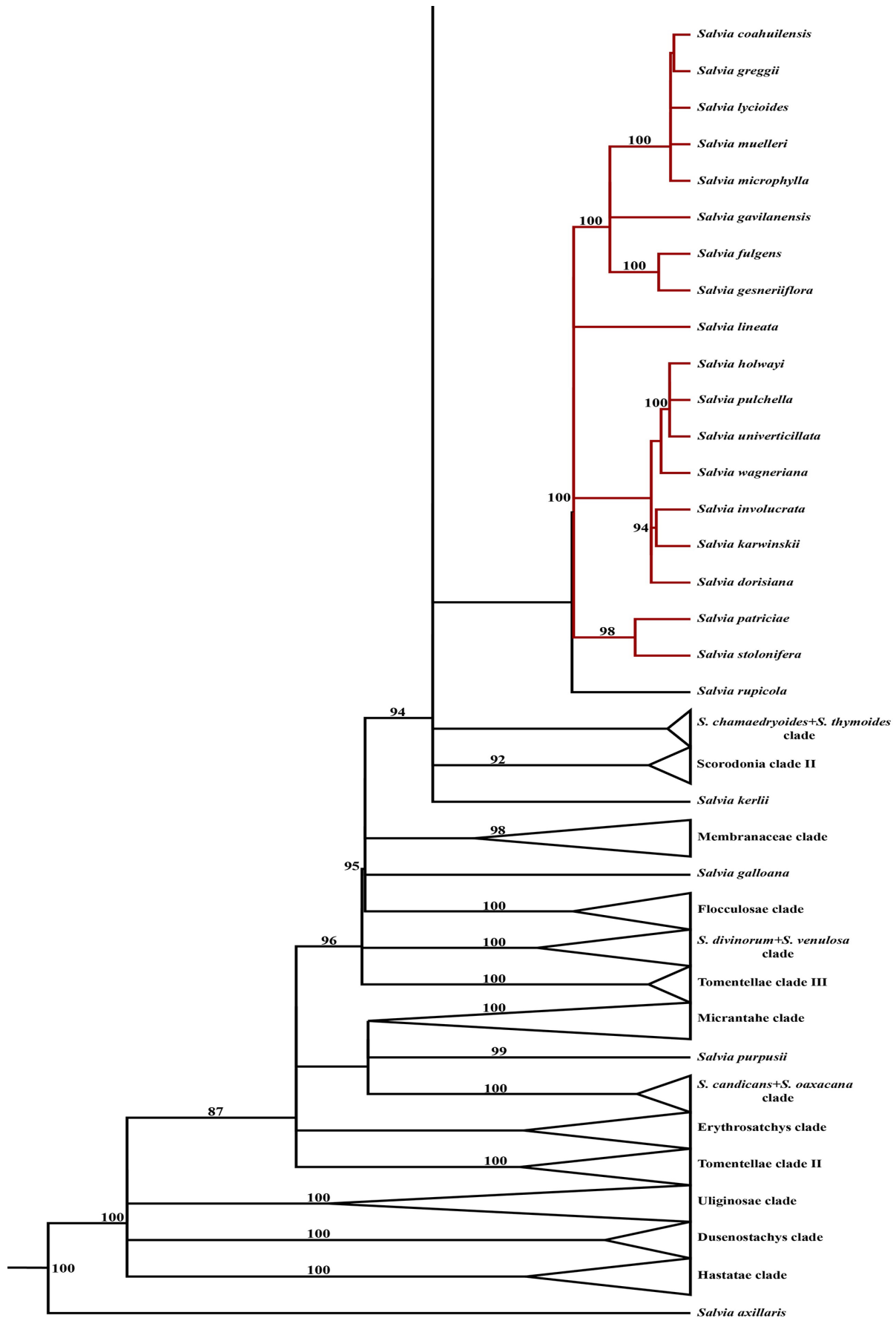


FIGURE 5. Phylogenetic relationships of *Salvia* subgenus *Calosphace*, including the new species, based on the analysis of three molecular markers (ITS, *trnL-trnF*, *trnH-psbA*) under the Maximum likelihood criterion. Bootstrap values ≥ 80 are show above the branches.

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