



## *Salvia fimbriatocalyx*, a new species of *Salvia* (Lamiaceae) from Oaxaca, Mexico

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### Abstract

In this study, we describe and illustrate *Salvia fimbriatocalyx*, a new species from Oaxaca, Mexico, which is morphologically similar to *Salvia leptostachys*, a species from section *Angulatae*. The new species differs from the latter by having persistent, lanceolate bracts with cuneate base; calyx lobes with a densely fimbriate margin; corolla tube longer (3–3.5 mm long), and the lower lip of the corolla shorter (3–3.5 mm long). Three molecular markers (ITS, *trnL-trnF* and *trnH-psbA*) were sequenced for the new species, and its phylogenetic position is discussed.

### Resumen

En este trabajo se describe e ilustra a *Salvia fimbriatocalyx*, una especie nueva de Oaxaca, México, que es morfológicamente similar a *Salvia leptostachys*, una especie de la sección *Angulatae*. La nueva especie difiere de la anterior por tener brácteas persistentes, lanceoladas, con base cuneada; lóbulos del cáliz con el margen densamente fimbriado, tubo de la corola más largo (3–3.5 mm de largo), y el labio inferior de la corola más corto (3–3.5 mm de largo). Tres marcadores moleculares (ITS, *trnL-trnF* and *trnH-psbA*) fueron secuenciados para la nueva especie y su posición filogenética se discute.

**Keywords:** *Angulatae*, endemism, phylogeny, sages

### Introduction

The genus *Salvia* Linnaeus (1753: 23) is the most diverse genus in the Lamiaceae family. Most of the New World species of *Salvia* belong to subgenus *Calosphace* (Bentham 1833: 198) Epling (1939: 4), which is the most species-rich clade within the genus, comprising ca. 580 taxa (González-Gallegos *et al.* 2020). Mexico has been considered the main center of diversification of the subgenus *Calosphace* (Jenks *et al.* 2013), due to the great diversity of species that harbors (306 spp.), and its high level of endemism (77%; Martínez-Gordillo *et al.* 2013, 2017).

Oaxaca is the most biologically diverse state in Mexico, and ca. 40% of the flowering plants that are distributed there are endemic (Villaseñor & Ortiz 2014). Regarding sage diversity, Oaxaca is where most of the species are distributed (93 spp.), followed by the states of Jalisco (91 spp.) and Guerrero (82 spp.; Martínez-Gordillo *et al.* 2017). However, this diversity is continuously increasing as more expeditions are being made and more taxonomists collaborate to describe it. In the last five years six new species of *Salvia* subgenus *Calosphace* from Oaxaca have been described (Martínez-Gordillo *et al.* 2016a, 2016b, 2017, González-Gallegos *et al.* 2018), which suggests that the basic taxonomic work in Mexican *Calosphace* is far from over.

During the revision of the Lamiaceae of Oaxaca some specimens that resemble the vegetative body of *Salvia leptostachys* Bentham (1833: 258) were found. Despite the morphological affinities of the new taxon with species from

section *Angulatae* (Epling 1935: 67) Epling (1939: 234), its phylogenetic position, although inconclusive, suggests a possible relationship with species from sections *Farinaceae* (Epling 1935: 87) Epling (1939: 186) and *Pennellia* Epling (1939: 211). These results support the need for a reevaluation of the current infrageneric classification of *Calosphace*, based on less labile character combinations, as well as more variable molecular markers to resolve relationships in recently derived lineages of the subgenus.

## Materials and methods

**Laboratory work:**—DNA was isolated from leaf tissue of an herbarium specimen of the new species (*S. Molina 651*, FCME), employing a modification of the 2 × CTAB method (Doyle & Doyle 1987) described in Fragoso-Martínez *et al.* (2017). Genomic DNA was used to amplify the nuclear ribosomal ITS marker, and the plastid *trnL-trnF* and *trnH-psbA* regions. The PCR profiles and primers used followed Fragoso-Martínez *et al.* (2018) and Sanger sequencing was performed at the Laboratorio de Biología Molecular de la Biodiversidad y la Salud, Instituto de Biología, Universidad Nacional Autónoma de México.

**Sequence edition, alignment and analysis:**—The sequences were assembled and edited using Sequencher 4.10 (Gene Codes Corp., Ann Arbor, MI). This data was uploaded to GenBank, with the following accession codes: ITS (MW561597), *trnH-psbA* (MW560897) and *trnL-trnF* (MW560896).

To evaluate the phylogenetic position of the new species we combined the sequences generated in this study with the most comprehensive dataset of *Salvia* subgenus *Calosphace* from Fragoso-Martínez *et al.* (2018). To expand the taxon sampling of the latter dataset we included sequences from other species of subgenus *Calosphace* from recent studies (González-Gallegos *et al.* 2019, Martínez-Ambríz *et al.* 2019). The alignments were made using the online version of MAFFT v.7 (Katoh & Standley 2013), followed by visual inspection and manual adjustment in PhyDE v.0.9971 (Müller *et al.* 2010). The matrix was concatenated with Geneious version 10 (<http://www.geneious.com>, Kearse *et al.* 2012); its total length was 2,193 bp, from which 718 bp corresponded to the ITS region, 505 bp to the *trnH-psbA* intergenic spacer (IGS) and 970 bp to the *trnL-trnF* region. Molecular substitution models were selected previous to phylogenetic inference, using the “auto” option which evaluates 22 different models (Chernomor *et al.* 2016) on the W-IQ-TREE web server (Trifinopoulos *et al.* 2016). The models selected were GTR+F+I+G4 for the nuclear dataset, the K3Pu+F+G4 model for the *trnH-psbA* IGS and the TIM+F+G4 for the *trnL-trnF* region. Maximum likelihood analyses were performed on three different matrices: plastid, nrITS and concatenated, using the IQ-TREE algorithm (Nguyen *et al.* 2015) on the W-IQ-TREE web server. The resulting maximum likelihood trees are included as Supplementary Materials 1–3. Due to space restriction, a resumed version of the phylogenetic tree from the concatenated matrix (Supplementary material 1), displaying the core *Calosphace* clade, is shown in Fig. 3. For this figure, clade extraction and tree edition were achieved using the phytools package (Revell 2012) in R (R Core Team 2014) and FigTree version 1.4.2 (Rambaut 2014), respectively.

## Taxonomy

*Salvia fimbriatocalyx* Mart.Gord. & Fragoso, *sp. nov.* (Figs. 1, 2)

*Salviae leptostachydi affinis, sed bracteis lanceolatis, base cuneatis, calycis margine dense fimbriato, corollae tubo 3–3.5 mm longo, incluso in calyce; labio infero 3–3.5 mm longo.*

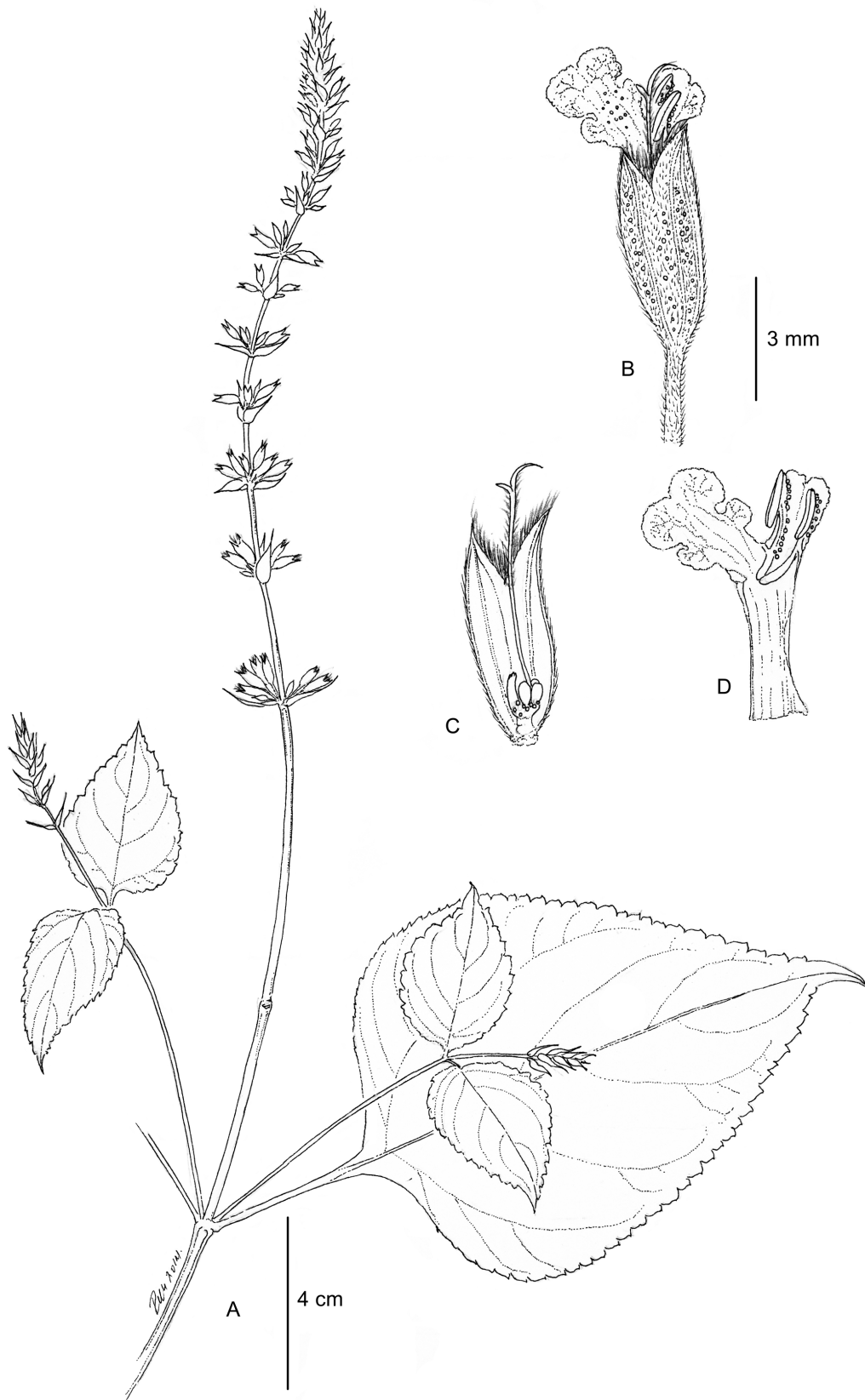
Type:—MEXICO. Oaxaca, San Pedro Huamelula, Piedra Sola, 16°0'16.9"N, 95°44'1.6"W, 272 m, 29 October 2010, *S. Molina 651* (holotype MEXU!; isotype SERBO!).

**Perennial herb**, 0.4–0.7 m tall. **Stems** pubescent, trichomes short, multicellular. **Leaves** with petioles 1.4–3.8 cm long, blade broadly rhomboid-ovate, base cuneate, decurrent, apex acuminate, margin crenate-serrate, ciliate, trichomes multicellular, upper leaf surface pubescent on veins, trichomes minute, lower leaf surface pubescent. **Inflorescence** in racemes, 14.5–17 cm long, verticillasters 0.6–2.3(–3.1) cm apart, 9–12 flowers per verticillaster, peduncles 0.6–1.2 cm long. **Bracts** persistent, lanceolate, 0.6–1.2 cm long, apex caudate. Pedicel 2.5–4(–7) mm long, with simple trichomes. **Calyx** 5.5–7.5 mm long, almost the same length as the corolla tube, outer surface pubescent with yellow spherical glands, inner surface glabrous; upper lip 3-veined, margin densely fimbriate. **Corolla** blue, 6–7 mm long, tube 3.5–5

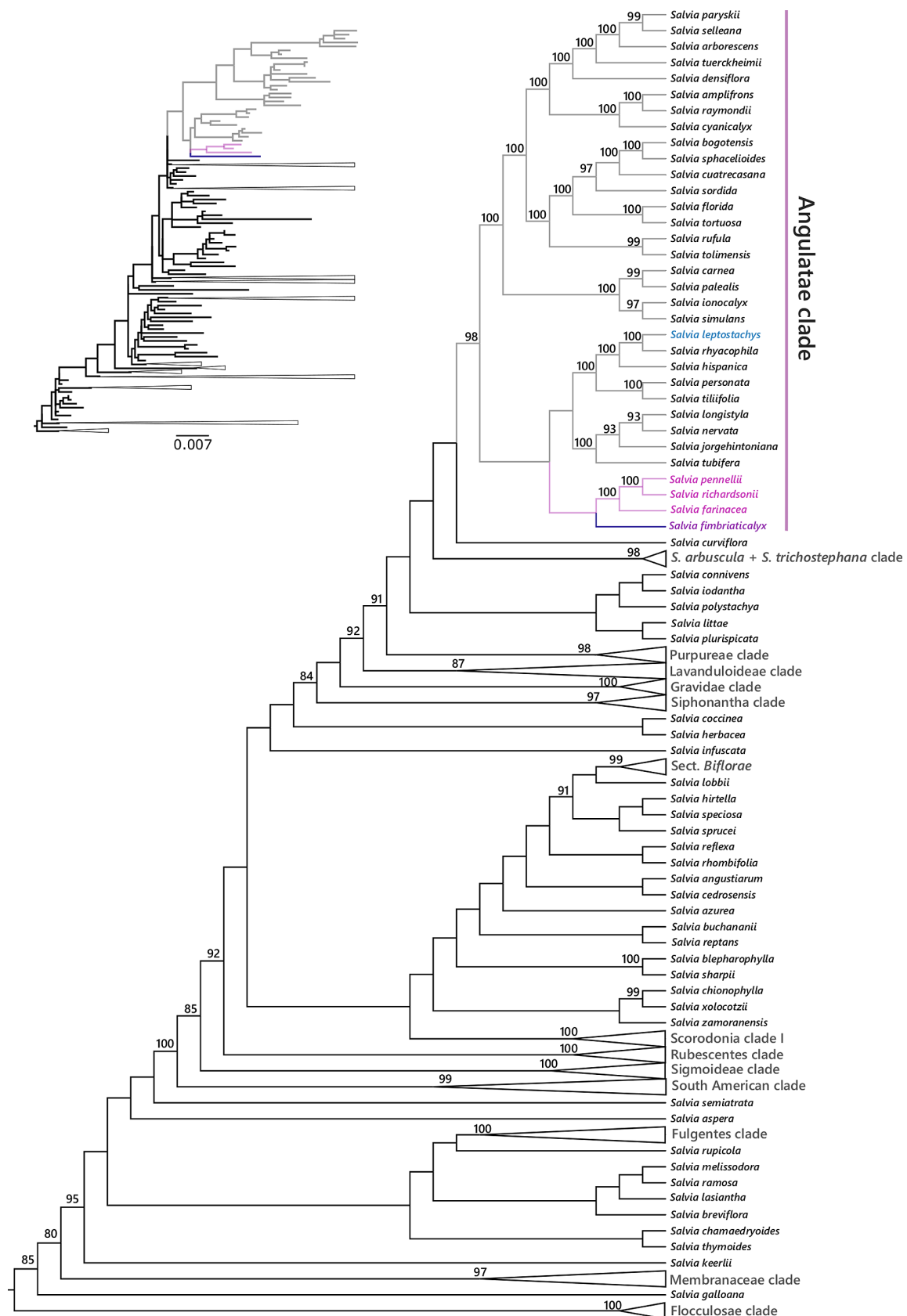
mm long, completely included in the calyx, slightly ventricose, internally epillate, lobes subequal, upper lobe 2–3 mm long, margin ciliate, lower lobe 3–3.5 mm long, tetralobate, deflexed, with glands on the apex. **Stamens** included in the upper corolla lobe, fused close to the corolla opening, filaments ending with a glabrous tooth, gubernaculum entire with a retrorse tooth, glabrous, junction between the filament and the thecae with glands. **Style** densely pubescent, dorsal and ventrally, 6–7 mm long. **Nectary** disc surface with yellow glands, nectary horn long and terete, twice the length of the immature mericarps. **Mericarps** ovoid, 2 mm long, brown and smooth.



**FIGURE 1.** Scanned image from the holotype of *Salvia fimbriaticalyx*.



**FIGURE 2.** *Salvia fimbriatocalyx*. A) Branch with leaves and inflorescence. B) Lateral view of the flower. C) Dissected calyx with style, ovary, and nectary horn. D) Dissected corolla with androecium. Illustration drawn from *S. Molina 382* (FCME) by Ramiro Cruz Durán.



**FIGURE 3.** Phylogenetic relationships of the core *Calosphace* clade based on three molecular markers (nrITS, *trnH-psbA* and *trnL-trnF*) and an increased taxon sampling including the new species. Bootstrap values  $\geq 80\%$  are shown above the branches. The names in pink correspond to the taxa related to the new species; however, the relationship lacks support (BS $<80\%$ ). The name in blue corresponds to species that resembles *Salvia fimbriaticalyx* the most. In the phylogram on the left branch lengths are displayed. Some previously reported clades have been collapsed to ease visualization. An extended version of the phylogenetic tree is provided in the supplementary material 1.

**Distribution, habitat and phenology:**—*Salvia fimbriaticalyx* is endemic to Mexico and is known from the District of Tehuantepec in the state of Oaxaca. This species is found in tropical deciduous forests, growing on rocky substrates; at 90 to 272 m. The new species has been collected with flowers and fruits during the rainy season in the months of September and October.

**Etymology:**—The epithet “*fimbriaticalyx*” refers to the margin of the calyx lobes, which is densely fimbriate (Fig. 1, 2).

**Discussion:**—Due to its morphological characteristics *Salvia fimbriaticalyx* should be placed in section *Angulatae*, one of the most diverse of subgenus *Calosphace* (52 spp.; González-Gallegos *et al.* 2020). However, molecular evidence suggests that this section is not monophyletic, with most of its species found in two different clades and being related to species from sections: *Briquetia* Epling (1939: 267), *Carneae* (Epling 1935: 89) Epling (1939: 228), *Curtiflorae* Epling (1939: 337), *Farinaceae*, *Maxonia* Epling (1939: 263), *Pennellia*, *Potiles* Epling (1939: 105), *Scorodonia* Epling (1939: 166), among others (Fragoso-Martínez *et al.* 2018).

In the phylogenetic analysis we conducted (Fig. 3, Supplementary material 1), *Salvia fimbriaticalyx* is included in the *Angulatae* clade (98% Bootstrap Support; BS), possibly related to a clade formed by species of sections *Farinaceae* and *Pennellia* (BS 100%). Nevertheless, this relationship lacks support (BS <80%) and is inconclusive. The species that resembles *Salvia fimbriaticalyx* the most in morphological terms, *Salvia leptostachys*, is included in the sister subclade to that where the new species is, along with other species of sections *Angulatae* and *Potiles* (Fig. 3; BS 100%). Although the relationships within this subclade are resolved and supported, the relationships among the three subclades (i.e., the one that includes *S. leptostachys*, the one with the new species and a third one including species from section *Curtiflorae*) remain unsupported (BS <80%).

The fact that the new species is not closely related to morphologically similar taxa suggests that some of the characters employed to define sections are labile and/or homoplastic (Fragoso-Martínez *et al.* 2018) or need to be re-analyzed considering the phylogeny. Additionally, the lack of resolution and support exhibited in the relationships of the new taxon show the need to explore new molecular markers or high-throughput sequencing strategies (e.g., Anchored Hybrid Enrichment; Fragoso-Martínez *et al.* 2017), to tackle recalcitrant relationships in this subgenus.

Epling’s (1939) sectional classification of *Calosphace* remains the greatest effort made to categorize the diversity of the Neotropical sages into smaller units that can be more manageable. These sections constitute practical units that are defined by combinations of characters that can be evaluated during specimen identification. The new species could potentially be related to taxa from sections *Farinaceae* and *Pennellia*, but resembles morphologically species from sect. *Angulatae*.

Upon analyzing the morphology of these three sections, the number of shared characteristics is overwhelming, while the differences among them seem to be minimal (Table 1). Some of the characters shared by the species of these sections are: interrupted spikes as inflorescences, mostly 6–12 flowers per node; calyces with upper lips 3-veined (also 5- and 7-veined in *Angulatae* and *Farinaceae*, respectively); corolla tubes epapillate; stamens inserted in the galea, stamen connective with a retrorse tooth and style pubescent with the posterior branch longer than the anterior (Epling 1939). On the other hand, sections *Angulatae* and *Farinaceae* differ mainly in the density and quality of the indumentum found on the surface of the calyx, which varies from glabrous to hirtellous in the former and is farinaceous or lanose in the latter. The species from these two sections differ from *Pennellia* in the size of the flowers, being larger in this section (corolla tube 12–14 mm long vs. 3.5–11 mm in *Angulatae* and *Farinaceae*; Epling 1939).

As seen above, there are many similarities among the three sections, that could potentially be used as synapomorphies in future studies in the light of the phylogenetic relationships. The fact that *Angulatae* and *Farinaceae* are non-monophyletic sections and/or have disjunct distributions could have obscured these patterns in previous studies. Broadening taxon sampling in these sections will allow uncovering groups of species that are closely related and could aid the search of reliable morphological characters to re-circumscribe more natural infrageneric groups. Clades such as *Angulatae*, that encompass great morphological and taxic diversity, lacking apparent morphological synapomorphies are a reminder that our knowledge of the subgenus is still under construction, and that there are a number of potential research lines that can be followed.

The new species lacks the farinaceous indumentum characteristic of the species from section *Farinaceae* and has flowers with corolla tubes shorter than those of *Pennellia* (3.5–5 mm long vs. 12–14 mm long). Thus, we decided to place *Salvia fimbriaticalyx* provisionally in section *Angulatae*, until a more comprehensive taxonomic work in *Calosphace* is achieved. The new species fits the circumscription of *Angulatae*, having characteristics such as: the herbaceous habit, the leaf shape ovate with a cuneate base and an acuminate apex, the presence of persistent bracts, and the calyx surface pubescent, but not farinaceous. Moreover, *Salvia fimbriaticalyx* is included in the *Angulatae* clade and morphologically resembles species from section *Angulatae*.

**TABLE 1.** Morphological comparison among sections *Angulatae*, *Farinaceae* and *Pennellia*, based on Epling (1939).

Character	<i>Angulatae</i>	<i>Farinaceae</i>	<i>Pennellia</i>
Habit	annual and perennial herbs	perennial herbs	perennial herbs
<b>LEAF</b>			
Shape	ovate, deltoid-ovate, elliptic	various shapes including ovate, deltoid-ovate, elliptic	oblong-ovate
<b>INFLORESCENCE</b>			
Type	interrupted spike	interrupted spike	interrupted spike
Number of flowers per node	6–12	(2–)6–12	6–12
<b>BRACT</b>			
Persistence	persistent and deciduous	deciduous, rarely persistent	late deciduous
<b>CALYX</b>			
Upper lobe venation	3(5)-veined	3(7)-veined	3-veined
Pubescence	glabrous to hirtellous	farinaceous to tomentose	hirtellous
<b>COROLLA</b>			
Color	blue, white, pink	blue, purple	blue, purple
Tube size (mm)	3.5–9(–13)	5–9(–11)	(10–)12–14
Inner surface	epapillate	epapillate	epapillate
<b>STAMENS</b>			
Insertion	inserted in the galea	inserted in the galea	inserted in the galea
Connective	with retrorse tooth	with retrorse tooth	with retrorse tooth
<b>STYLE</b>			
Indumentum	present	present	present
Posterior stigmatic branch	longer than anterior	longer than anterior	longer than anterior

For practicality, we contrast the morphology of the new species with its most similar species: *Salvia leptostachys* (Table 2). The vegetative body of *S. fimbriaticalyx* resembles that of *S. leptostachys*, but the new species is a perennial herb, while the latter is annual. *Salvia fimbriaticalyx* has persistent, lanceolate bracts (*vs.* deciduous, ovate-lanceolate bracts); the margins of the calyx lobes are densely fimbriate (*vs.* pubescent); the corolla tube is longer (3–3.5 mm long *vs.* 2–3.1 mm long) and the lower corolla lobe is shorter (3–3.5 mm long *vs.* 3.5–5.8 mm long).

Finally, *Salvia fimbriaticalyx* has been collected at elevations of 90–272 m, while the morphologically similar species can be found at higher elevations, of 1000 m or more.

**Additional specimens examined:**—MEXICO. Oaxaca: San Pedro Huamelula, La Lomita, 90 m, 15°59'24"N, 95°42'51.6"W, 29 September 2010, *S. Molina* 382 (SERBO!, FCME!).

**TABLE 2.** Morphological comparison between *S. fimbriatocalyx* and *S. leptostachys*.

Character	<i>S. fimbriatocalyx</i>	<i>S. leptostachys</i>
Habit	perennial herb	annual herb
Plant size (m)	0.4–0.7	0.5–1
LEAF		
Petiole length (cm)	1.4–3.8	1.4–5
Shape	rhomboid-ovate	ovate, deltoid-ovate
Base	cuneate, decurrent	cuneate
Margin	crenate-serrate	serrate
INFLORESCENCE		
Length (cm)	14.5–17	12.5–45
Number of flowers per node	9–12	2–10
BRACT		
Shape	lanceolate	ovate-lanceolate
Length (mm)	6–12	2–6.2
Base	cuneate	truncate
Persistence	persistent	deciduous
CALYX		
Pedicel length (mm)	2.5–4(–7)	1.5–2.1
Length (mm)	5.5–7.5	5.4–8.7
Lobe margin	fimbriate	hirtellous
COROLLA		
Tube length (mm)	3.5–5	2–3.1
Size of lips	subequal lips	lower lip longer than upper lip
Upper lip length (mm)	2–3	1.6–3
Lower lip length (mm)	3–3.5	5.3–8

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