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Comparative assessment of pollen micromorphology of *Salvia assurgens* (Lamiaceae), an endemic sage from Mexico

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Abstract

The present study provides novel information about the pollen of *Salvia assurgens*. Pollen grains were collected and described based on their observed characters by light microscope and scanning electron microscopy. The species is distinguished from other Mexican salvias by having small pollen grains (14.2 × 18.2 µm), thin primary muri (0.2 µm thick), elongated primary lumina (1.03 µm long) and secondary lumina with relatively few perforations (9, range 5–14). Pollen characters are similar to those of the majority of American salvias. Regarding Mexican sages, there has been little palynological research, and only 23 species of 32 examined have been quantitatively studied. Standardization is needed in different aspects of palynological studies, especially in relation to measurement protocols and data analysis, as well as the increased use of scanning electron microscopy (SEM), since the majority of differences among species are provided by SEM microscope observation.

Keywords: *Calosphace,* exine ornamentation, Mexican Bajio region, style length, Trans-Mexican Volcanic Belt

Introduction

The genus *Salvia* Linnaeus (1753: 23) is the most diverse within the Lamiaceae, and nearly 1000 species are known. It is distributed worldwide except for Australia (only 1 species) and New Zealand (Drew *et al.* 2017, González-Gallegos *et al.* in press). In the Americas, the native species belong mainly to two subgenera: *Audibertia* Walker, Drew & Sytsma (2015: 837) with 19 species restricted to California (Epling 1940, Walker *et al.* 2015) and *Calosphace* (Bentham 1833: 198) Epling (1939: 3) with 576 species distributed from the southern United States to South America. At the country level, Mexico is the richest, having 294 species of subgenus *Calosphace*, of which 242 are endemic (González-Gallegos *et al.* in press).

There has been little palynological research on *Salvia* (see *e.g.*, Henderson *et al*. 1968, Trudel & Morton 1992). In the Americas, only 43 species of subg. *Calosphace* have been studied, 14 by light microscopy (LM) only (Henderson *et al.* 1968, Saravia & Pinto 2018) and 29 by LM combined with scanning electron microscopy (SEM) (Castro-Morales 2008, Moon *et al.* 2008, Orsini *et al.* 2006, Trudel & Morton 1992). However, it should be noted that 32 of these occur in Mexico, 17 of which are endemic, and they represent 23 of the 104 sections of subg. *Calosphace* (Epling 1939, 1940, 1941, 1944, 1947, 1951, Epling & Mathias 1957, Epling & Játiva 1966). Hence, there is justification to conduct more comparative palynological studies on the Mexican salvias.

Based on LM observations, the pollen grains of *Salvia* are oblate in equatorial view, elliptic in polar view, and hexacolpate (rarely tetra-, penta- or octocolpate). They have either two lateral mesocolpia that are longer and thicker than the four medial ones (Henderson *et al.* 1968, Trudel & Morton 1992) or three wide mesocolpia alternating with three narrow ones (Moon *et al.* 2008). Moon *et al.* (2008) observed by SEM pollen of seven of the eight genera of the subtribe Salviinae (classification according to Harley *et al.* 2004). They found the tribe to be comprised of three clusters of species differentiated by exine ornamentation. These are type I: reticulum with simple perforations

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(*Chaunostoma* Donnell-Smith (1895: 9) and *Lepechinia* Willdenow (1804: sub pl. 21)); type II: bireticulate, muri of primary reticulum thicker than the secondary muri, lumen of primary reticulum contains fewer than five units of secondary lumen (*Perovskia* Karelin (1841: 15)); and type III: bireticulate, muri of the primary reticulum thicker than the secondary reticula or of equal thickness, primary lumen continuous, with shapes that vary from irregular ellipses to polygons, and bearing a network of five or more units of the secondary lumen (*Dorystaechas* Boissier & Heldreich ex Bentham (1848: 261), *Meriandra* Bentham (1829: sub pl. 1282), *Rosmarinus* Linnaeus (1753: 23) *and Salvia*). Taking into account the bireticulate perforations that are characteristic of types II and III, together with additional phylogenetic evidence, Drew *et al.* (2017) recognized all genera of these two clusters as part of *Salvia*, and they are currently treated at the subgeneric level.

Salvia assurgens Kunth in Humboldt *et al.* (1818: 293) is a decumbent perennial herb 20 to 50 cm tall, and the principal diagnostic character is the presence of completely white corollas. However, González-Gallegos *et al.* (2016) included in its synonymy *S. prunifolia* Fernald (1900: 518), a species with flowers that are completely blue or blue with the basal portion of the tube whitish. With this circumscription, *S. assurgens* is endemic to Mexico. The white-flowered populations are restricted to the northwestern portion of the Trans-Mexican Volcanic Belt and occur almost exclusively in the state Michoacán. In comparison, plants with blue flowers are found in the southern portion of the Sierra Madre Occidental in the states of Durango, Jalisco, Nayarit, Sinaloa, and Zacatecas (González-Gallegos *et al.* 2016).

In order to advance our palynological knowledge of the Mexican salvias, the present study describes the micromorphology of the pollen grains of the white form of *S. assurgens.* In addition, we compile information about *Salvia uliginosa* Bentham (1833: 251) a second species of *Salvia* subgenus *Calosphace* section *Uliginosae* (Epling 1935: 54) Epling (1939: 54) observed with LM and SEM (*S. assurgens* also belong to sect. *Uliginosae*). Based on comparisons done with both methods, we discuss the micromorphological variation of 24 Mexican species of *Salvia* subg. *Calosphace*.

Materials and methods

Study area and pollen sampling:—Pollen grains of *Salvia assurgens* were obtained from a wild population in a periurban area located north of Pátzcuaro, Michoacán, (Cerro Blanco: 19°31'57.8"N, 101°36'2.65"W; 2180 m). Samples were extracted from mature flower buds randomly selected among 10 individuals and taken to the laboratory to be processed following Erdtman´s (1986) acetolysis protocol. The specimen (*B. Bedolla & D. Madrigal 487*, IEB63000) and pollen vouchers were deposited in the IEB Herbarium, Instituto de Ecología A. C., Centro Regional del Bajío (Pátzcuaro, Michoacán), Mexico.

Measurement of pollinic traits:—In order to describe the quantitative and qualitative properties of pollen grains, measurements were made using a light microscope (LM: Microscopio optical Zeiss Primo Star) and a scanning electron microscope (SEM: Field Emission Scanning Electron Microscope FEI Quanta 250 FEG). For LM measurements, pollen grains were hydrated in water and mounted on slides with glycerin to be photographed at ×100 magnification. From a sample of 40 pollen grains, five polar view measurements were obtained: i) equatorial diameter (E), ii) length of one of the longest colpi (LC), iii) width of one of the biggest mesocolpi (WM) and iv) exine thickness (ET). Twenty-eight pollen grains were measured in equatorial view for the following: i) polar axis length (P) and ii) exine thickness (ET). Ornamentation was registered in both, polar and equatorial views. The shape quotient was calculated for each grain from the polar and equatorial diameter (P/E, see data analyses). The exine ornamentation description was prepared using SEM. From a high pollen concentration suspension in alcohol (70%), a pollen drop was let to evaporate on an aluminium slide, and once the sample was dehydrated, a gold coat was applied using a metal ionizer Quorum Q150RS. After this, 10 pollen grains in equatorial view were photographed. Measurements were made within three rectangular areas, each 5 μm^2 (~ 2.23 × 2.23 µm) were placed on each image. In each case, care was taken to make sure that in each square portions of the exine were perpendicular to the observer. Six traits were registered: i) thickness of the muri on the primary reticulum (TMPR), ii) thickness of the muri on the secondary reticulum (TMSR), iii) diameter of the biggest primary lumina (DPL), iv) diameter of the biggest secondary lumina (DSL), v) number of primary lumina (NPL) and vi) number of secondary lumina (NSL). Three measurements were taken for the first four traits, and the other two were counted per square. The measurements were taken on the LM and the SEM photographs using ImageJ 1-52a (Rasband 2020). Additionally, the measures in LM, taken directly too with an eyepiece micrometer.

Data analyses:—The descriptions of pollen micro structure were based on quantitative measurements, including the minimums, maximums, and standard deviations of each palynological feature, by the method of observation and measurement: LM, on photos and with an eyepiece micrometer, and SEM. Additionally, eight palynological traits were also compiled for *Salvia uliginosa*, a South American species also belonging to Sect. *Uliginosae*, and for 23 of 32 Mexican *Salvia* subg. *Calosphace* species previously studied. The species studied by Henderson *et al.* (1968) were not included because they lack quantitative information about the palynological traits. The traits here evaluated for each species were: i) length of the polar axis (P), ii) equatorial diameter (E), iii) number of colpi (NC), iv) length of one of the longest colpi (LC), v) exine thickness (ET), vi) thickness of the muri on the primary reticulum (TMPR), vii) type and diameter of primary lumina (T-DPL), viii) number and type of secondary lumina, and ix) shape ratio, calculated using the values P and E. In order to classify the species using the shape ratio, we followed Erdtman's proposal (1986): peroblate <0.5, oblate 0.5−0.75, suboblate 0.75−0.88, oblate-spheroidal 0.88−1, prolate-spheroidal 1−1.14, subprolate 1.14−1.33, prolate 1.33−2, and perprolate >2.0.

At the same time, we reported minimum and maximum style length for each *Salvia* (including *S. assurgens*), in addition to indicating the section to which it belongs in accordance with Epling's classification (1939). Terminology used for the palynological traits follows Punt *et al.* (1994) and Moon *et al.* (2008). In this manner, the analyses focused on the comparison of i) the consistency of measurements of palynological traits according to the measurement method with LM, and ii) palynological traits between *S. assurgens* and previously studied Mexican salvias.

Results

Based on the measurements made from the LM and SEM photographs, the pollen of *Salvia assurgens* is characterized as follows (Table 1): suboblate (14.2 × 18.2 µm, P/E= 0.78) (Fig. 1 B, E), hexacolpate with no equidistant colpi, 6.1(±0.9) µm long, two terminal mesocolpia with length of 7.8(±0.8) µm, operculum absent (Fig. 1 C, D), exine ornamentation semitectate, bireticulate, 0.8(±0.4)−1(±0.3) µm thick. Based on SEM observations, pollen grains have a primary reticulum with the muri being smooth, elongate, well defined, 0.2(±0.03) µm wide, supratectal. The primary lumina are irregularly elongated with the greatest diameter being 1.03(±0.03) µm, and muri of the secondary reticulum thinner (0.09 ±0.02 µm wide) and lower height than the primary reticulum, forming a net below the tectum, bearing 5−14 secondary lumina expanded per primary lumina (Fig. 1 F, Table 2). Upon comparing the LM measurements made by the photographs and those made by eyepiece micrometer, a substantial difference in the precision of the measurements was detected. In particular, the measurements made directly with the eyepiece micrometer tend to overestimate the measurements of each palynological trait, resulting in a different shape ratio (Table 1).

TABLE 1. Palynological characters of *Salvia assurgens* from LM. The measurements were taken on photographs and directly with an eyepiece micrometer.

Pollen		Photographs measured with digital program (ImageJ)		Direct measured with eyepiece micrometer	
View	Character	Mean	Range (min–max)	Mean	Range (min–max)
Equatorial	polar axis (P) (µm)	14.2 ±1.2	12.2–16.8	21.55	20–24
Equatorial	exine thickness (ET) (µm)	0.8 ±0.4	0.3–1.6	2	0
Polar	equatorial diameter (E) (µm)	18.2 ±1.5	15.3–21.1	22.95	19–25
Polar	largest colpus length (LC) (µm)	6.1 ±0.9	4.4–8.1	8.3	6–10
Polar	largest mesocolpi width (LT) (µm)	7.8 ±0.8	6.3–9.8	10.9	10–12
Polar	exine thickness (ET) (µm)	1 ±0.3	0.4–1.6	2	0
Equatorial and polar	ornamentation	bireticulate		bireticulate	
	P/E*	0.78 suboblate		0.93 oblate	

*Ratio obtained from the average values of P and E

TABLE 2. Pollen traits of *Salvia assurgens* obtained by using SEM.

Feature	Mean	Range (min–max)
Average thickness of primary muri (TMRP) (µm)	0.2 ±0.03	0.13–0.26
Average thickness of secondary muri (TMSR) (µm)	0.09 ±0.02	0.06–0.15
Largest diameter of the primary lumina (DPL) (µm)	1.03 ±0.3	0.5–2.34
Largest diameter of the secondary lumina (DSL) (µm)	0.27 ±0.05	0.18–0.42
Average number of lumina in the primary reticule per 5 $µm^2$ (NPL)	5.47 ±1.2	3–8
Average number of secondary lumina per 5 $µm^2$ (NSL)	9.0 ±2.2	5–14

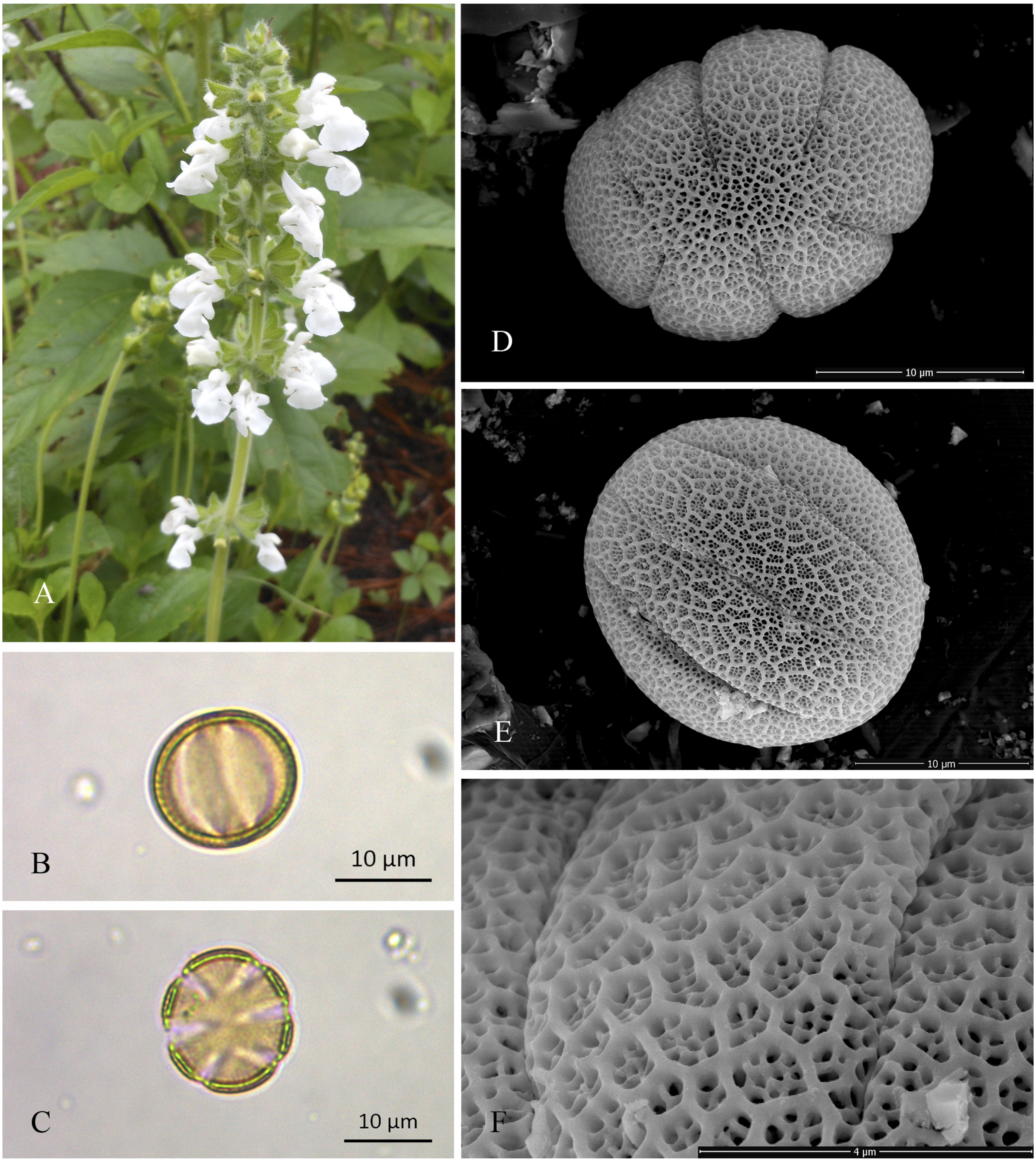

FIGURE 1. Flowers and pollen grains of *Salvia assurgens*. A. Inflorescence, B & C, Pollen grains viewed with a light microscope, B. Equatorial view, C. Polar view. D–F, SEM micrographs. D. Polar view of the pollen grain. E. Equatorial view of the pollen grain. F. magnified view of the bireticulate exine showing the coarse secondary reticulum.

Heterogeneity in quantity and quality of the available information was detected among the 24 American species of *Salvia* previously studied (see supplementary material, Table S1). Nevertheless, in all cases median values of size traits were available (*i.e.* P and E), and as a result it was possible to obtain a shape ratio even when the original source did not provide one (Fig. 2 A–B, Table S1). However, the range (min–max) or any other dispersion measurements were not always available (Fig. 2, Table S1). The next characters in terms of quantity of information are the length of the longest colpi and exine thickness (available in 23 of 24 species). However, fewer species had the range information (Fig. 3, Table S1). For other characters, such as muri thickness of primary reticulum and secondary lumina number, fewer species had median values, and in some cases the measurements were reported categorically (*e.g.*, thick *vs.* thin) (Table S1). In this manner, quantitative inspection of palynological traits among *S. assurgens* and the rest of species was focused on size (P and E), shape ratio, biggest colpi length, and exine thickness. It has to be emphasized that for four of the species that were evaluated in more than one study (*i.e. S. ballotiflora* Bentham (1833: 270), *S. coccinea* Buc'hoz ex Etlingler (1777: 23), *S. elegans* Vahl (1804: 238), and *S. farinacea* Bentham (1833: 274), notable intraspecific variation in the size and shape ratio was detected (Fig. 2 A, B).

In comparison with other species of *Salvia* subg. *Calosphace, S. assurgens* posesses the smallest pollen grains (*i.e.* 14.2 × 18.2 μm) reported to date (Fig. 2 A), and its shape ratio places it near the extreme of grains that are very elongate in the equatorial axis (Fig. 2 B: suboblate). Only *S. occidentalis* Swartz (1788: 14) and *S. coccinea* have pollen grains that are more elongate than *S. assurgens* (Fig. 2 B). On the other hand, exine thickness is similar to that of species with twice or three times larger pollen grains (Fig. 2 A, 3 A): *S. ballotiflora*, *S. farinacea*, *S. hispanica* Linnaeus (1753: 25), *S. microphylla* Kunth in Humboldt *et al.* (1818: 295), *S. reflexa* Hornemann (1807: 34) and *S. uliginosa*. When the relationship between style length and pollen size is examined (P and E), a positive correlation was observed, with bigger pollen size associated with longer styles (Fig. 4 A–B).

Discussion

Palynological findings of this study allow us to postulate that *S. assurgens* is distinguishable from the 23 evaluated species by: i) pollen grains that are the smallest reported until now for subgenus (14.2 × 18.2 μm), ii) exine with a thin primary muri (0.2 μm), an elongate primary lumina (length: 1.03 μm) and secondary lumina with relatively few perforations (9, range 5–14). This study complements the palynological understanding of pollen morphology in *Uliginosae* through the comparison of three of its species: the Mexican *S. tricuspidata* Martens & Galeotti (1844: 78), the South American *S. uliginosa*, and *S. assurgens,* the focus of the present study. Due to the lack of quantitative palynological data of *S. tricuspidata* (see Henderson *et al.* 1968), it cannot be compared in detail with the other two; however, with the information available it is possible to infer that *S. uliginosa* can be distinguished from *S. assurgens* by possessing larger pollen grains: 25 × 28.5 μm (*vs.* 14.2 × 18.2 μm), being octocolpate (*vs.* hexacolpate) and rounded primary lumina (*vs.* elongate). Therefore, proposing one or more palynological features that unite the species of sect. *Uliginosae* is not possible at this point; nevertheless, neither it is suggested that they should be split. It is necessary to include more species of sect. *Uliginosae* in future works.

In accordance with Moon *et al.* (2008), exine ornamentation found in *Salvia assurgens* agrees with that reported for all of *Salvia* (group III), in having bireticulate exine, primary muri that are thicker than or of similar thickness to the secondaries, the primary muri are continuous and vary in shape from irregular ellipses to polygons, and each primary lumina has more than 5 perforations. However, it has to be pointed out that *S. assurgens* can be placed on group IIIb where the majority of species belong to subgenera *Calosphace* and others are of the Old World, which are characterized by having a primary muri thicker than the secondary ones, the shape of the primary muri is rounded (in *S. assurgens* and other species is elongate) and continuous, and the secondary lumina are regular and with more than 10 perforations (in *S. assurgens*: mean 9, range 5–14). It has to be mentioned that 22 of the 23 Mexican species reviewed (except for *S. hispanica*), as well as *S. uliginosa* (a South American taxon), coincide with the characteristics of subgroup IIIb. *Salvia hispanica* belong to group IIIa where there are species of the genus *Dorystaechas*, *Meriandra*, *Rosmarinus* and sages of the subgenera *Audibertia* that are characterized by presenting a primary muri twice as thick as the secondary muri, with rounded and irregular or sometimes discontinuous primary lumen shape; however, the macromorphological and molecular characters locate it in *Calosphace* (Fragoso *et al.* 2018, González-Gallegos *et al.* 2016, González-Gallegos *et al.* in press). Among the group IIIb, *S. axillaris* Mociño & Sessé ex Bentham (1833: 270) (Mexican species) is the most different species due to the few perforations in the secondary lumina (4–5), large, and by having a triangular shape (Castro-Morales 2008) (Table S1); however, the rest of the characters coincide with the characteristics of *Calosphace*.

Table S1 shows two relevant aspects: 1) interspecific variation and 2) the absence of robust features that enable us to recognize taxonomic groups (*i.e.* sections). With respect to variation among species, *S. assurgens* and the rest of the 24 species included could be distinguished by using the evaluated traits (see Table 1 and 2), which coincides with previous findings (Orsini *et al.* 2006, Kahraman *et al.* 2009, Saravia & Pinto 2018). In particular, colpi length can be a very useful taxonomic feature to differentiate species, as reported by Orsini *et al.* (2006) (Table S1, Fig. 3 A). Regarding the recognition of sections or clades (Fragoso *et al.* 2018), there is not sufficient evidence of a unique palynological feature or a combination of features that consistently allows identifying or grouping the American *Salvia* species. For example, polygonal primary lumina are present in species of sect. *Angulatae* (Epling 1935: 67) Epling (1939: 234) and *Polystachyae* Epling (1939: 213), marginal arrangement of secondary lumina has been observed in some species of sects. *Polystachyae* and *Lavanduloideae* Epling (1939: 34), and rounded primary lumina have been described in most species of sect. *Uliginosae* (except *S. assurgens*) and *Tomentellae* (Epling 1935: 55) Epling (1939: 18) (paraphyletic sections), and from two unrelated species of sect. *Fulgentes* Epling (1939: 273) (*S. microphylla)* and sect. *Polystachyae* (*S. polystachya* Cavanilles (1791: 17)). In other words, the above mentioned palynological features are sparsely distributed on the *Salvia* phylogeny. Even so, this topic requires an ample and detailed study, including a uniform and inclusive species sample from throughout the sections and clades of *S.* subg. *Calosphace* phylogeny.

Although infraspecific variation naturally exists in palynological traits such as size, variation is also attributable to the methods used to gather the data. In the first place, variation may be due to different measurement techniques. Palynological measurements in Lamiaceae are often taken directly by using an eyepiece micrometer in LM (Saravia & Pinto 2018, Trudel & Morton 1992). In contrast, in other studies the measurements were obtained from photographs using the Carnoy 2.0 software (Moon *et al.* 2008, Schols *et al.* 2002) or with a camera lucida (Orsini & Casale 1996, Orsini *et al.* 2006). Comparing both methods (from photographs *vs.* direct measurements) allowed us to detect an over estimation when using the eyepiece micrometer. Nevertheless, for *S. ballotiflora, S. coccinea*, *S. farinacea*, and *S. reflexa,* palynological values were underestimated when measured directly with LM (Moon *et al.* 2008, Trudel & Morton 1992). In both cases, direct observation with LM may increase error due to lack of resolution, effects of light (*e.g.*, halos), and observer expertise. In the second place, variation in pollen grain size may be affected by the chemical treatment procedures used to prepare samples for observation. Thus, acetolysis and the glycerin set up can cause swelling of the pollen grains (Kapp *et al.* 2000), and the level of distortion may be very variable among samples. In third place, interspecific data variation can be also attributed to the lack of standardization when measuring the pollen grains. For example, some studies (Castro-Morales 2008, Moon *et al.* 2008, Saravia & Pinto 2018) do not indicate if polar and equatorial measurements were made on the same pollen grain; only Orsini *et al.* (2006) pointed this out. This may explain some of the observed variation in traits that are not directly measured, such as the shape ratio.

Despite that several studies measured 10 to 30 pollen grains, nevertheless the majority reported only a single shape ratio value; in these situations, it is unclear if the ratio was calculated on the basis of measurements of the polar length axis and equatorial diameter, or if the ratio is a measurement from pollen grains measured simultaneously by the polar axis and equatorial diameter. The ideal scenario is that the shape (P/E) is obtained for each pollen grain. However, this has logistical complications when measurements are made with conventional methods (*e.g.* LM and SEM). It is possible that other methods, such as X-ray tomography for high-resolution images (*e.g.* Li *et al.* 2016), allow measurements to be made on the same pollen grain. However, these methods are still expensive, of limited access, and therefore not feasible for multi-taxonomic comparative studies. Another alternative may be a "statistical approach", where the measurement of pollen grains is performed based on a random selection of both the observation fields (in LM or SEM) and the pollen grains per sample per species *(e.g.* Orsini *et al.* 2006, Gonzaga *et al.* 2019). In this way, the pollen shape would be calculated as the ratio between the means of the length of the polar axis (P) and the equatorial diameter (E). Thus, the P/E will be a value without variation, but it will be a proxy measure of the shape of an average pollen grain per species.

A positive correlation in angiosperms (including Lamiaceae) has been proposed between pollen grain size and style length (Plitmann & Levin 1983, Moon *et al.* 2008). This idea is partially supported for 23 of the 25 species included in the present study (Fig. 4 A–B). For instance, *Salvia gesneriiflora* Lindley & Paxton (1851: 49) and *S. coccinea* possess the longest styles, having lengths of 47−66 and 25−32 mm, respectively (Zamudio *et al.* in press), and they also have the largest pollen grains (48.9 × 48.5 and 26.9 × 58 µm, respectively). However, these results have to be taken cautiously, since the tendency of this correlation is due to all the high values being provided by ornithophillous species. When analyzing the rest of species (which are melittophilous), the correlation between pollen grain size and style length is not positive. For example, *S. assurgens* has a style length of 10−12 mm, but shorter styles exist in other *Salvia* species (*S. hispanica*: 6.5−8 mm, and *S. lavanduloides* Kunth in Humboldt *et al.* (1818: 287): 5−6.3 mm), and these have pollen grains with a longer polar axis. This subject requires further study in the context of a broad taxonomic sample that takes into account pollination syndromes as a covariate.

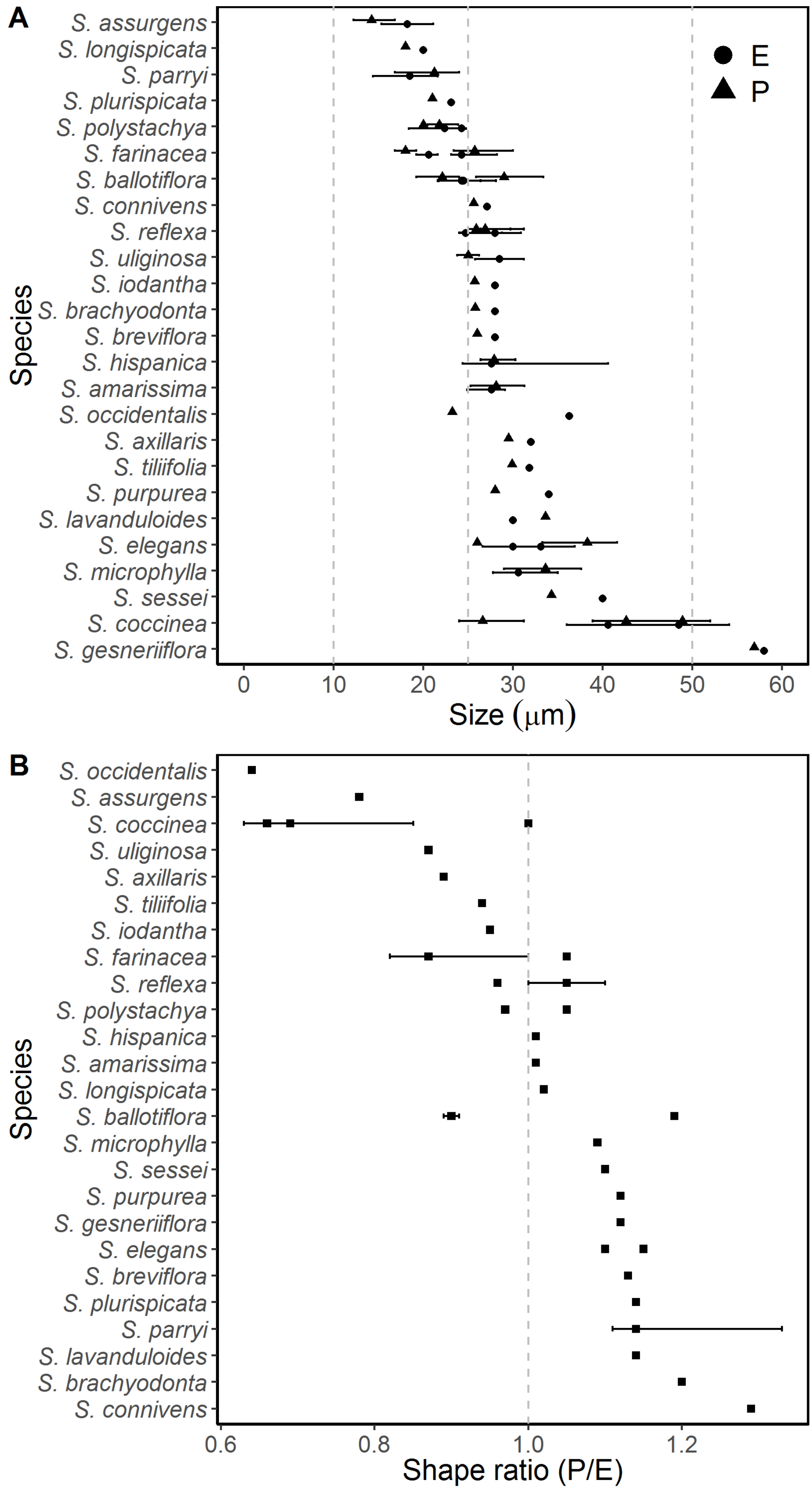

FIGURE 2. A. Equatorial diameter length (E) and polar axis (P) B. Shape ratio of pollen grains of *Salvia assurgens*, *S. uliginosa* (South American) and 23 Mexican *Salvia* species. Average obtained from the consulted sources is shown, notice that some species have more than one published data. Range is indicated by using horizontal bars (min–max). In A. Vertical lines indicate threshold for size of the pollen grain according to Erdtman (1986) (very small: <10, small: 10–25, medium: 26–50, large: 51–100, very large: >100 micrometers). In B, the vertical discontinued line indicates a ratio equal to 1, for a spherical pollen grain.

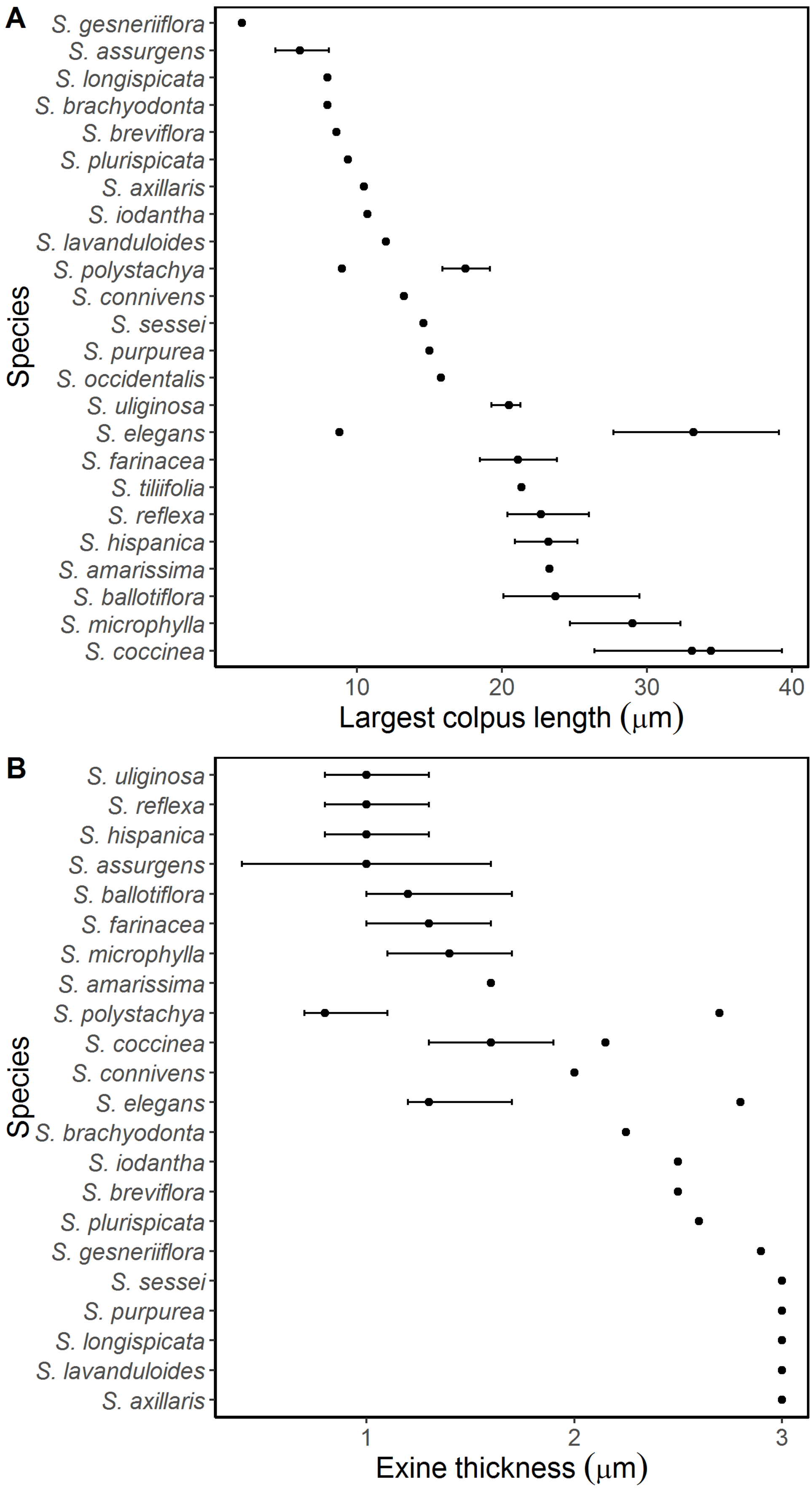

FIGURE 3. A. Biggest colpi length, B. Exine width at the equatorial zone; *Salvia assurgens*, *S. uliginosa* (South American) and other Mexican *Salvia* species (A: 24 spp; B: 22 spp). Average values are shown for each. Range is indicated by horizontal bars (min–max). The two values in S. coccinea are from different studies.

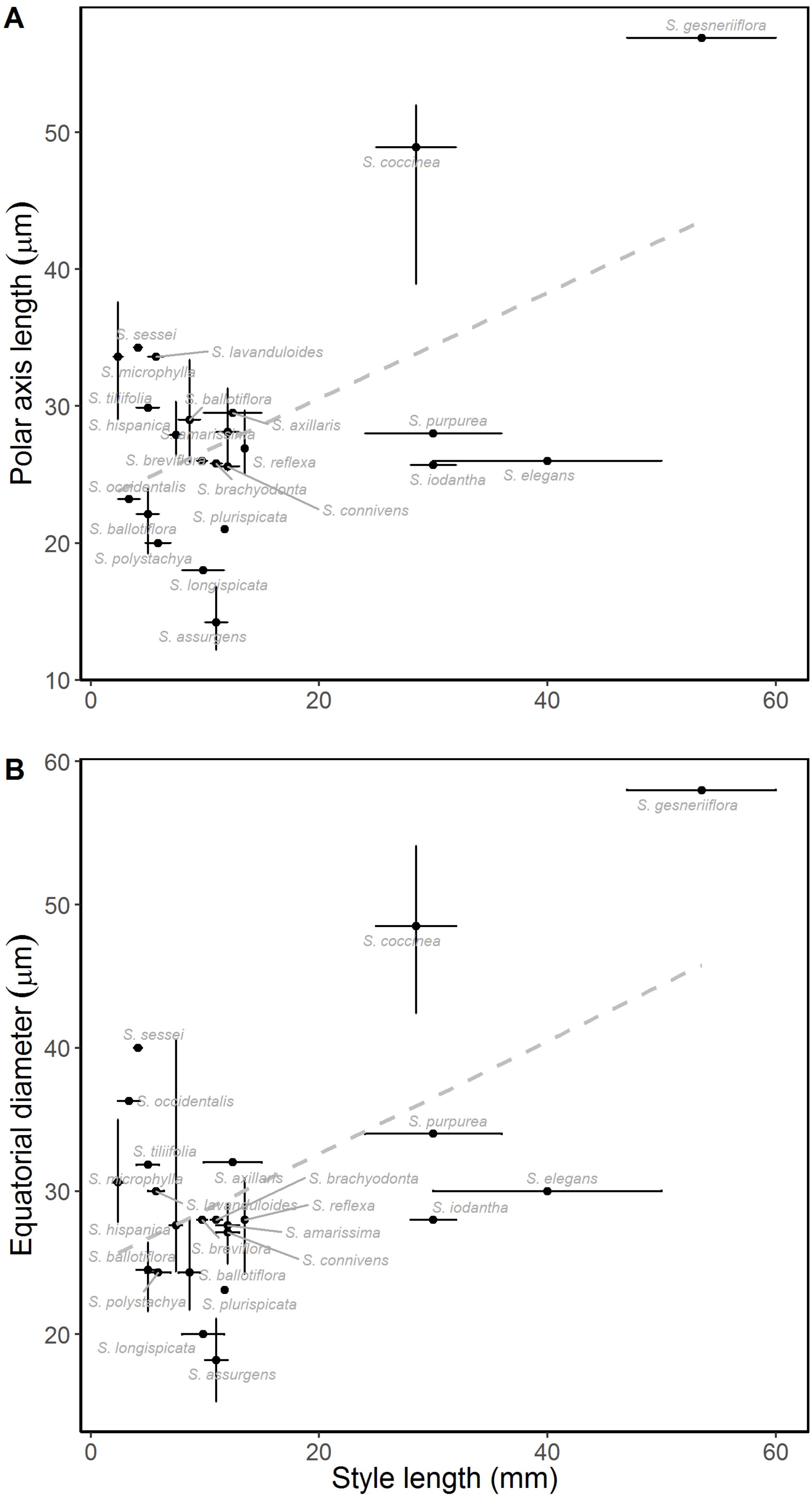

FIGURE 4. Exploration of the relationship between the length of the polar axis (A) and the equatorial diameter (B) with respect to style length of 23 Mexican *Salvia* species (including *S. assurgens*). Range is indicated by horizontal and vertical bars (min–max) for the style length and both pollen attributes. The dashed gray line is a trend line, it does not represent a fitted linear model.

Conclusions and perspectives

The comparative description of pollen microstructure in *S. assurgens* corroborates the potential of palynological research to complement our taxonomic knowledge in *Salvia* subg. *Calosphace*. However, in this study we have detected the necessity of increasing the species sample and to standardize the measuring and data analyses protocols. In particular, we recommend: 1) standardizing measuring procedures and the reporting of values of palynological characters, 2) taking the measurements from photographs, and 3) if possible, obtaining the information from both, LM and SEM, since they are complementary. In fact, beyond colpi size and number, the main differences among species are obtained from traits measured through SEM. Standing out among these traits are the size of perforations (*i.e.* primary lumina), ornamentation, primary and secondary muri thickness, and number of perforations inside each primary lumen (*i.e.* secondary lumina) (Orsini *et al.* 2006, Trudel & Morton 1992). Future projects need to include the blue-flowered populations of *Salvia assurgens* in order to compare analytically the two floral morphs.

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