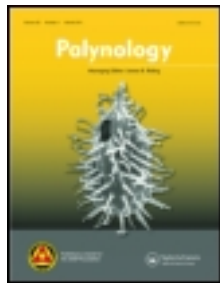


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Pollen morphology of eight species of *Stemodia* (Plantaginaceae) from South America

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The pollen morphology of eight species of *Stemodia* L. (Plantaginaceae) was analysed with the aim of refining the taxonomy of this genus. The pollen grains were examined with both light and scanning electron microscopes. The pollen grains are spheroidal, sometimes prolate-spheroidal, small, with a subcircular or subtriangular amb, 3- or 4-colporate and with a long colpus that reaches the poles, defining a small, circular to slightly elongate apocolpium with irregular margins. The exine is tectate, foveolate or microperforate, 1 μm thick and uniform. The species of *Stemodia* analysed here exhibit morphological homogeneity, which suggests that the genus is stenopalynous.

Keywords: pollen morphology; *Stemodia*; South America

1. Introduction

Stemodia L. (Plantaginaceae) comprises 49 species which live in neotropical areas (Dawson 1968; Turner and Cowan 1993a; Barringer and Burger 2000). A total of 29 species have been recognised for the Americas, 16 of which are exclusive to South America (Turner and Cowan 1993b). There are 9 species in southernmost South America, but only *Stemodia verticillata* (Mill.) Hassl. has a broader distribution. The remaining 8 species are typically endemic to Argentina, Paraguay, Uruguay, southern Bolivia and southern Brazil. The *Stemodia* species are annual or perennial marshy herbs, common in marshlands and lowlands; only *Stemodia verticillata* grows in drier areas, mostly on clay-rich acid soils.

The genus is a natural, well-delimited group, although the identification of some taxa may be complex. For that reason, the genus has been subject to taxonomical, anatomical and cytological studies (Raghavan and Srinivasan 1940; Subramanian and Pondmudi 1987; Turner and Cowan 1993b; Sosa and Seijo 2002; Sosa 2005, 2008; Sosa et al. 2009). However, studies on pollen morphology have been made only on *Stemodia viscosa* Roxb. (Varghese 1968).

The variation of some pollen characters such as shape and size can be related to ploidy level (Stebbins 1971; Soltis and Soltis 1999). In that sense, some authors have observed a positive correlation between the size of pollen grains and ploidy levels (Stebbins 1971; Oliveira et al. 2004; Almada et al. 2006). Polyploidy has been reported in three species: *Stemodia hyptoides* Cham. & Schltdl., which has diploid, tetraploid and hexaploid

cytotypes (Sosa and Seijo 2002; Sosa et al. 2009); *Stemodia lobelioides* Lehm., which has a tetraploid cytotype (Sosa et al. 2009); and *Stemodia viscosa*, which has tetraploid and hexaploid cytotypes (Raghavan and Srinivasan 1940; Subramanian and Pondmudi 1987). However, a correlation between size of pollen grains and ploidy levels has not yet been determined.

Palynological studies are a valuable tool for taxonomic separation. In this sense, studies on pollen morphology have allowed the formation of natural taxonomic groups in the Family Scrophulariaceae *sensu lato* (Hong 1984). Although several palynological studies have been carried out in species from Europe, Asia and Africa (Varghese 1968; Inceoglu 1982; Argue 1984, 1986, 1993; Karim and El-Oqlah 1989; Fernandez et al. 1997), these types of studies in neotropical taxa are relatively scarce (Argue 1980, 1981, 1985; Correa et al. 1995; Santos and Melhem 1999, 2000).

Morphological analysis of South American species of *Stemodia* (Sosa 2009) has shown that different taxa are similar in vegetative characters, which hinders their identification. Additional data on anatomy or palynology may provide further information on taxonomy. In this paper, the pollen morphology of eight species of *Stemodia* from southern South America is described for the first time. The results are discussed in relation to the taxonomy of the genus.

2. Materials and methods

Twenty specimens belonging to eight species of *Stemodia* were studied (Plates 1, 2). Pollen samples

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were obtained by removing one or two flowers from specimens of each species deposited in the Herbarium of the Instituto de Botánica del Nordeste (CTES). In some cases, flower buds were obtained from the plants growing in greenhouse conditions; these were previously fixed in formaldehyde-acetic acid-alcohol (FAA) for 24 hours then preserved in 70% alcohol. Pollen grains were acetolysed using the technique of Erdtman (1960). For light microscopy (LM) the pollen samples were mounted in glycerin jelly on glass slides, sealed with paraffin wax and examined with a Wild M 20 microscope. Permanent slides were deposited at the Palynological Laboratory of the Universidad Nacional del Nordeste (PAL-CTES).

For observation with scanning electron microscopy (SEM), acetolysed pollen grains were washed in 96° alcohol which was followed by washing in absolute alcohol. The pollen grains were then air-dried on aluminium plates and sputtered with gold palladium and studied with a JEOL 5800 LV SEM. The description of pollen grains follows the terminology of Punt et al. (1994, 2007). The length of the polar axis (P) and the equatorial diameter axis (E) were measured from 30 pollen grains per specimen using LM. Perforations and thickness of the pollen wall were measured with the SEM.

The Shapiro–Wilk test was used to evaluate the normal distribution of the measurements. Prior to statistical analyses, data with non-normal distribution ($W = 0.891$, $p < 0.0001$) were log-transformed. The measurements of pollen size for diploid species were analysed using a multivariate analysis of variance (MANOVA); when differences were significant, separation of means was performed using the Bonferroni's a posteriori tests ($p < 0.05$). Since we have previously reported three cytotypes in *Stemodia hyptoides* ($2n = 2x = 22$, $2n = 4x = 44$ and $2n = 6x = 66$) (Sosa and Seijo 2002; Sosa et al. 2009), we used MANOVA to analyse the pollen size between these three cytotypes. We also used correspondence analysis to explore the relationship between grain pollen shape (categorical data) and species. All analyses were performed using the Infostat program (version 2010).

2.1. Specimens examined

Stemodia ericifolia (Kuntze) K. Schum.

Argentina. Formosa: Dept. Mataros, Route 39, 6-III-2001, Schinini et al. 35286 (CTES). PAL-CTES 2528. Salta: Dept. Rivadavia, J. Pagé, 13-XII-2005, Sosa et al. 223 (CTES). PAL-CTES 2542.

Stemodia hassleriana Chodat

Paraguay. Dept. Amambay: Bella Vista, 23-XI-2005, Sosa et al. 211 (CTES) PAL-CTES 2529. Bella Vista,

7 km from the city, 23-XI-2005, Sosa et al. 213 (CTES, SP, G). PAL-CTES 2534.

Stemodia hyptoides Cham. & Schltdl.

Argentina. Corrientes: Dept. Mburucuyá, Paso Aguirre, 6-V-2001, Sosa 43 (CTES). PAL-CTES 2544. Dept. Mercedes, El Socorro, 24-II-2006, Sosa et al. 245 (CTES) PAL-CTES 2536. Dept. Saladas, Paso Naranjo, 17-I-2004, Sosa 128 (CTES) PAL-CTES 2545. Misiones: Dept. Capital, Posadas, Zaimán stream, 16-II-2005, Sosa and Rodríguez 138 (CTES) PAL-CTES 2530. Dept. Montecarlo, Puerto Piray, 12-IV-2006, Sosa and Rodríguez 249 (CTES) PAL-CTES 2535. Dept. San Martín, Puerto Rico, 12-IV-2006, Sosa and Rodríguez 251 (CTES) PAL-CTES 2546.

Stemodia lanceolata Benth.

Argentina. Chaco: Dept. 1° de Mayo, Colonia Benítez 19-IV-2001, Sosa and Loizaga de Castro 41 (CTES, SPF) PAL-CTES 2543. Salta: Dept. Orán, Tabacal, 15-XII-2005, Sosa et al. 228 (CTES, ESA) PAL-CTES 2531.

Stemodia lobelioides Lehm.

Argentina. Corrientes: Dept. Monte Caseros, Uruguay river, 6-V-2005, Sosa and Schinini 210 (CTES) PAL-CTES 2540. Uruguay. Dept. Artigas: Bella Unión, 25-XI-2001, Sosa et al. 70 (CTES, HUEFS). PAL-CTES 2532.

Stemodia palustris A. St.-Hil.

Uruguay. Dept. Artigas, near Yacutuyá Mini stream, 25-XI-2001, Sosa et al. 71 (CTES) PAL-CTES 2537; around Guabiyú stream, 5-V-2005, Sosa and Schinini 209 (CTES) PAL-CTES 2541.

Stemodia stricta Cham. & Schltdl.

Argentina. Jujuy: Dept. Ledesma, around Zapla river, 16-XII-2005, Sosa et al. 230 (CTES, ESA) PAL-CTES 2539. Salta: Dept. San Martín, near Carapaí river, 14-XII-2005, Sosa et al. 226 (CTES). PAL-CTES 2538.

Stemodia verticillata (Mill.) Hassl.

Argentina. Corrientes: Dept. Capital, Corrientes, 12-IV-2001, Sosa 38 (CTES) PAL-CTES 2547. Jujuy: Dept. Ledesma, around Zapla river, 16-XII-2005, Sosa et al. 231 (CTES) PAL-CTES 2533.

3. Results

Eight *Stemodia* species were analysed: *Stemodia ericifolia*, *Stemodia hassleriana*, *Stemodia hypoides*, *Stemodia lanceolata*, *Stemodia lobelioides*, *Stemodia palustris*, *Stemodia stricta* and *Stemodia verticillata*. The shape of the pollen grains, type of aperture and sculpture of the exine exhibit remarkable uniformity. The parameters determined are summarised in Table 1.

3.1. General pollen description of the species of *Stemodia* studied

Pollen grains are oblate-spheroidal, prolate-spheroidal or rarely prolate; 3-colporate or sometimes 4-colporate. Colpi are long, reaching the poles to form an apocolpium of around 7 μm long; ora are circular or slightly lalongate with irregular margins. Polar diameter ranges from 12.6 to 23.2 μm and the equatorial axis from 9.5 to 20.4 μm . Amb (in polar view), the pollen outline is subcircular or subtriangular. Exine is 1 μm thick, uniform across the surface of the grain, sexine and nexine of equal thickness. Tectate, foveolate or microperforate.

The species studied are described as a pollen type; nevertheless, some differences can be observed.

Shape: pollen grains of *Stemodia* species are predominantly spheroidal (Plate 1, figures 5, 7, 12; Plate 2, figures 6, 12), although prolate grains were observed in *Stemodia hassleriana* (Plate 1, figure 10) and occasionally in *Stemodia lobelioides* (Plate 2, figures 4, 5).

Size: pollen grains of the described species are small, from 9 to 23 μm (Table 1).

Aperture: in all cases the pollen grains are predominantly 3-colporate (Plate 1, figures 1–3, 8, 9, 11; Plate 2, figures 10, 11), but sometimes 4-colporate pollen grains may also occur such as in *Stemodia lobelioides* and *Stemodia palustris* (Plate 2, figures 1, 2, 7).

Exine: the exine is tectate-perforate as in *Stemodia hypoides*, *Stemodia hassleriana* (Plate 1, figures 6, 10) and *Stemodia palustris* (Plate 2, figures 7, 8), or tectate-foveolate as in *Stemodia lanceolata* (Plate 1, figure 12) and *Stemodia lobelioides* (Plate 2, figure 3).

Observations from light microscopy were confirmed by those of pollen grains during scanning electron microscopy.

Results from MANOVA showed significant differences in the size of pollen grains (Wilks's $\lambda = 0.38$,

Table 1. Measurements of *Stemodia* pollen. Schi.: Schimini A, minimum, average and maximum value obtained from measurements of the P = polar axis and E = equatorial diameter; P/E = polar/equatorial diameter and shape. The chromosome numbers are from Sosa and Seijo (2002) and Sosa et al. (2009).

Species	Voucher	N	Chromosome number	P (μm)	E (μm)	P/E	Shape
<i>S. ericifolia</i> ^(d)	Schi. et al. 35286 (CTES)	30	2n = 22	16.3 (17.7) 19.04	15 (17.34) 19	0.96 (1) 1.27	Spheroidal
	Sosa et al. 223 (CTES)	30	2n = 22	13.6 (15) 16.32	13.6 (14.7) 16.3	0.91 (1) 1.2	Spheroidal
	Sosa et al. 211 (CTES)	30	2n = 22	13.6 (15.8) 19.04	9.5 (12.4) 13.6	1 (1.3) 1.78	Prolate-spheroidal
<i>S. hassleriana</i> ^(b)	Sosa et al. 213 (CTES)	30	2n = 22	13.6 (16) 17.68	9.5 (13.6) 17.7	1 (1.2) 1.5	Prolate-spheroidal
	Sosa et al. 249 (CTES)	30	2n = 22	12.6 (14.3) 14.9	12.9 (14.1) 16.3	1 (1.02) 1.1	Spheroidal
<i>S. hypoides</i> ^(a)	Sosa 251 (CTES)	30	2n = 22	13.6 (14.6) 15	13.6 (14.7) 16.3	0.9 (1) 1.1	Spheroidal
	Sosa 128 (CTES)	30	2n = 44	15 (16.4) 19.04	14.9 (16.4) 17.7	0.84 (1) 1.18	Spheroidal
	Sosa 245 (CTES)	30	2n = 44	16.3 (17.3) 19.04	16.3 (17.3) 19.04	0.92 (1) 1.08	Spheroidal
<i>S. lanceolata</i> ^(c)	Sosa 43 (CTES)	30	2n = 66	16.3 (18.2) 20.4	16.3 (17.9) 19	0.92 (1) 1.08	Spheroidal
	Sosa 138 (CTES)	30	2n = 66	16.3 (17.5) 19.04	16.32 (17.8) 19	0.9 (1) 1.2	Spheroidal
	Sosa 41 (CTES)	30	2n = 22	13.6 (15) 16.3	13.6 (15.2) 16.3	0.8 (1) 1.1	Spheroidal
<i>S. lobelioides</i>	Sosa et al. 228 (CTES)	30	2n = 22	13.6 (15.3) 17.68	13.6 (15.6) 16.3	0.9 (1) 1.1	Spheroidal
	Sosa et al. 70 (CTES)	30	2n = 44	16.3 (18.7) 23.12	13.6 (17.3) 20.4	0.86 (1) 1.4	Spheroidal
<i>S. palustris</i> ^(d)	Sosa 210 (CTES)	30	2n = 44	17.68 (20) 23.12	17.7 (19.8) 20.4	0.86 (1) 1.3	Spheroidal
	Sosa et al. 71 (CTES)	30	2n = 22	14.9 (16.5) 17.7	14.9 (15.6) 17.7	0.84 (1) 1.2	Spheroidal
<i>S. stricta</i> ^(d)	Sosa 209 (CTES)	30	2n = 22	14.9 (16.5) 17.7	15 (16) 17.7	0.91 (1) 1.2	Spheroidal
	Sosa et al. 226 (CTES)	30	2n = 22	13.6 (16.2) 17.7	14.9 (16.1) 17.7	0.8 (1) 1.2	Spheroidal
<i>S. verticillata</i> ^(c)	Sosa et al. 230 (CTES)	30	2n = 22	14.96 (16.6) 17.7	14.9 (16.4) 17.7	0.9 (1) 1.2	Spheroidal
	Sosa 38 (CTES)	30	2n = 22	13.6 (14.5) 16.3	13.6 (14.5) 15	0.9 (1) 1.1	Spheroidal
	Sosa et al. 231 (CTES)	30	2n = 22	13.6 (15.8) 17.7	13.6 (15.7) 17.7	0.8 (1) 1.2	Spheroidal

Note: Different letters between diploid species indicate significant differences ($p < 0.05$).

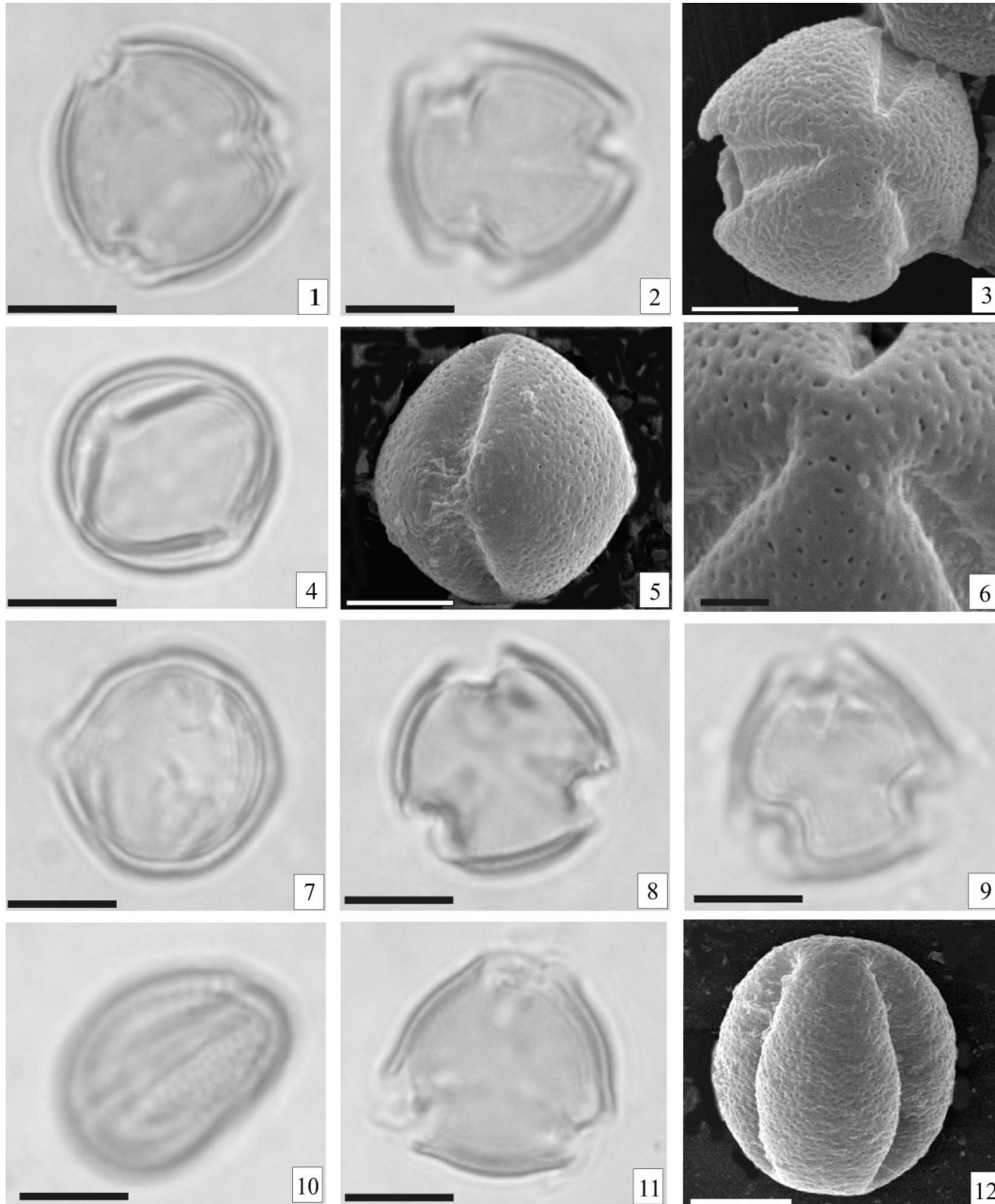


Plate 1. Light and scanning electron microscopy micrographs of *Stemodia* pollen grains. *S. hyptoides* (Sosa 138): Figure 1, polar view, optical cross-section; Figure 2, optical surface; Figure 3, polar view; Figure 4, equatorial view, optical cross-section; Figure 5, equatorial view; Figure 6, polar view showing tectum in apocolpium. *S. ericifolia* (Schinini et al. 35286): Figure 7, equatorial view, surface; Figure 8, polar view, optical cross-section; Figure 9, optical surface. *S. hassleriana* (Sosa 211): Figure 10, equatorial view, optical surface. *S. lanceolata* (Sosa et al. 228): Figure 11, polar view, optical cross-section; Figure 12, equatorial view. Scale bar: Figure 6 = 1 μm , figures 1–5 and 7–12 = 10 μm .

F=40.07, $P < 0.0001$) among the diploid species of *Stemodia* analysed. The largest pollen grains were observed in *Stemodia palustris*, *Stemodia ericifolia* and *Stemodia stricta* ($P = 16.47 \mu\text{m}$, $E = 16.23 \mu\text{m}$), while the smallest ($P = 14.27 \mu\text{m}$, $E = 14.13 \mu\text{m}$) were observed in *Stemodia hyptoides* 2x. *Stemodia hassleriana* had a $P = 15.98 \mu\text{m}$ and a $E = 12.93 \mu\text{m}$, also

indicating a smaller size. *Stemodia lanceolata* and *Stemodia verticillata* produced intermediate-sized pollen grains. *Stemodia hyptoides* showed significant differences in pollen size between ploidy levels (Wilks's $\lambda = 0.27$, $F = 66.76$, $P < 0.0001$). The pollen grains were smaller in the diploid cytotype ($P = 14.27 \mu\text{m}$, $E = 14.13 \mu\text{m}$) than in the tetraploid

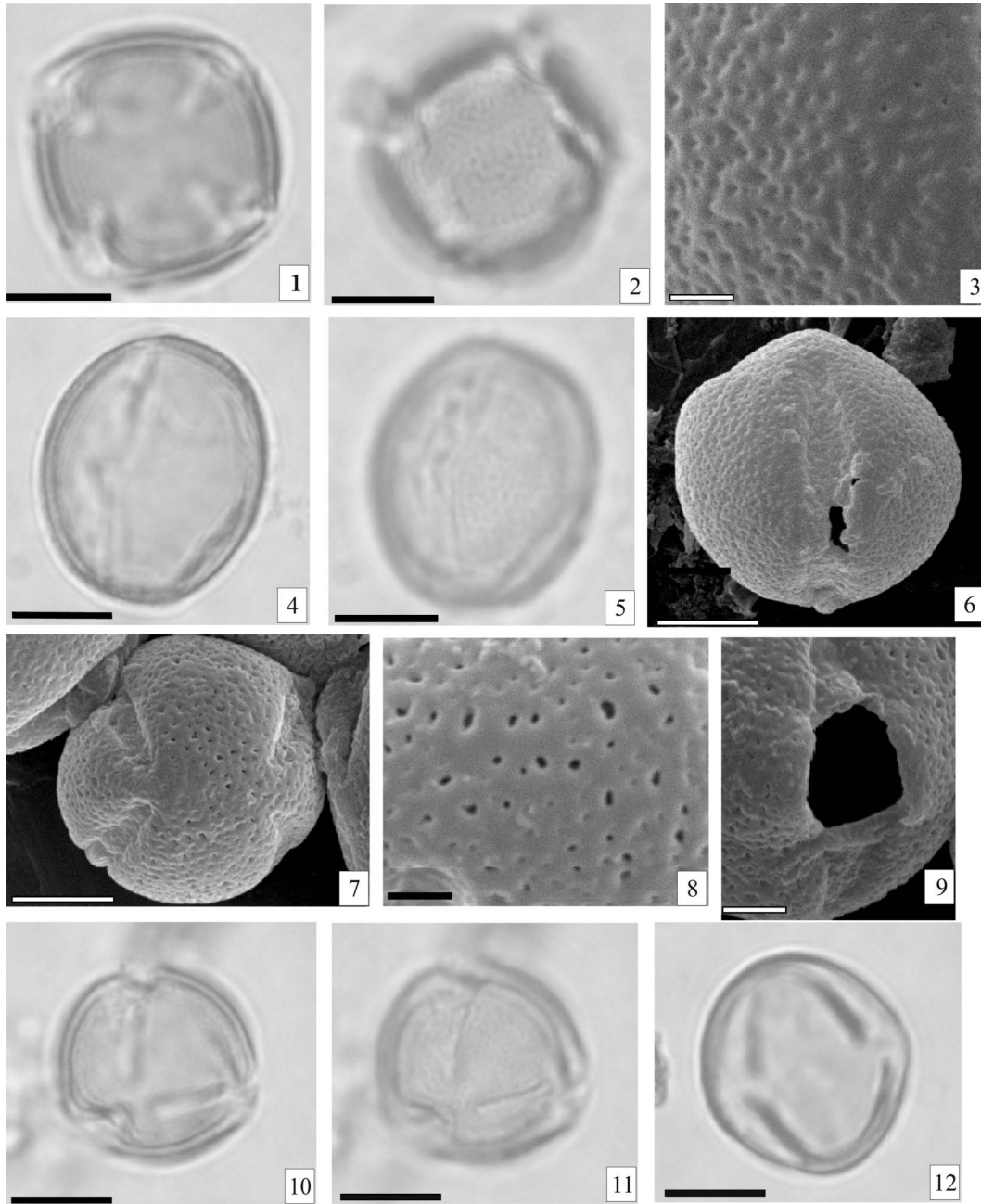


Plate 2. Light and scanning electron microscopy micrographs of *Stemodia* pollen grains. *S. lobelioides* (Sosa 210): Figure 1, polar view, optical cross-section; Figure 2, optical surface; Figure 3, vista equatorial view; Figure 4, equatorial view, optical cross-section; Figure 5, equatorial view, optical surface; Figure 6, equatorial view. *S. palustris* (Sosa 209): Figure 7, polar view; Figure 8, equatorial view showing aperture; Figure 9, equatorial view showing tectum in mesocolpium. *S. verticillata* (Sosa 23): Figure 10, polar view, optical cross-section; Figure 11, optical surface; Figure 12, equatorial view, optical surface. Scale bar: Figure 3, 8 = 1 μm , 9 = 2 μm ; figures 1, 2, 4–7, 10–12 = 10 μm .

($P = 15.57 \mu\text{m}$, $E = 15.57 \mu\text{m}$) and the hexaploid ($P = 17.85 \mu\text{m}$, $E = 17.85 \mu\text{m}$), which had the largest pollen grains (Figure 1).

The correspondence analysis between species and pollen grain shape is shown in Figure 2. The first two dimensions expressed 98.14% of total inertia of the

crossing contingency. In its first axis (inertia of 82.32%) the graph suggests that most species had spheroidal grains except for *Stemodia hassleriana*, which showed prolate or subprolate pollen grains. Accordingly, all species show great uniformity in their pollen features.

4. Discussion

Palynological studies in some species of the tribes Veroniceae (Hong 1984; Karim and El-Oqlah 1989; Fernández et al. 1997; Martínez-Ortega et al. 2000; Saeidi-Mehrvarz and Zarrei 2006), Buchnereae (Santos and Melhem 2000), Caprariae (Santos and Melhem 1999), Gratiolae (Varghese 1968; Argue 1985, 1986; Correa et al. 1995), Mimuleae (Argue 1980, 1981, 1984), Manuleae (Argue 1993), Verbasceae (Karim and El-Oqlah 1989; Kheiri and Khayami 2006), Anthirrineae (Karim and Al-Oqlah 1989; Bigazzi and Tardelli 1990) and Rhinanthaeae (Inceoglu 1982; Wang et al. 2003) have revealed that the Family Scrophulariaceae *sensu lato* is eurypalynous.

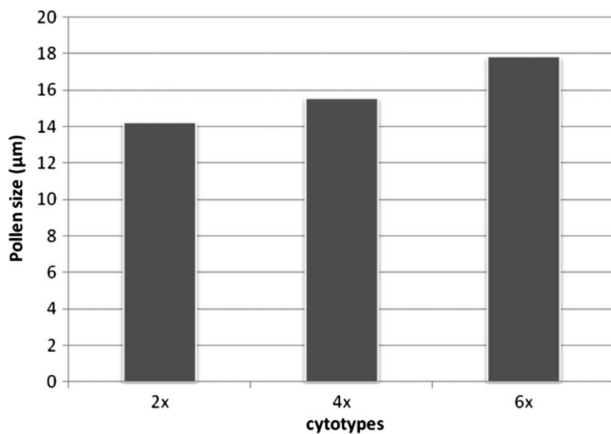


Figure 1. Average size of spheroidal pollen grains (μm) of *Stemodia hypnoides* with diploid ($2n = 2x = 22$), tetraploid ($2n = 4x = 44$) and hexaploid ($2n = 6x = 66$) cytotype.

The Family Scrophulariaceae *sensu lato* has been considered a heterogeneous group based on morphological characters of the flowers (Wettstein 1895). Phylogenetic analysis based on molecular data (Olmstead and Reeves 1995; Olmstead et al. 2001; Albach et al. 2005; Oxelman et al. 2005; Rahmanzadeh et al. 2005) has shown that the family is unequivocally polyphyletic. According to these studies, the Tribe Gratiolae would have been included in the Family Veroniceae. This tribe was later referred to Plantaginaceae according to APG II (2003). The great variability observed in palynological characters may be related to the polyphyly of the family. Rahmanzadeh et al. (2005) have identified the monophyly of the tribes Gratiolae and Linderniae and intended to raise them to the rank of family.

According to Varghese (1968), Argue (1985, 1986) and Correa et al. (1995), the Tribe Gratiolae that contains *Stemodia* exhibits considerable diversity in pollen morphology. In general, the pollen grains are spheroidal to prolate, with spiraperturate (*Mimulus*), 3-zonocolpate (*Torenia*) and 3-zonocolporate (*Lindernia*, *Monthea*, *Melosperma*) aperture. The exine surface can be areolate (*Mimulus*, *Torenia*) or reticulate (*Mazus*, *Lindernia*). Nevertheless, these authors considered that the more common pollen type is spheroidal to prolate-spheroidal, 3-colporate and reticulate (*Bacopa*, *Lindenbergia*) to foveolate (*Stemodia viscosa*).

The pollen grains studied here were mostly spheroidal, although *Stemodia hassleriana* sometimes has prolate pollen grains. In relation to the aperture, the grains were mostly 3-colporate, although 4-colporate grains were also observed in *Stemodia lobelioides* and *Stemodia palustris*. The results obtained

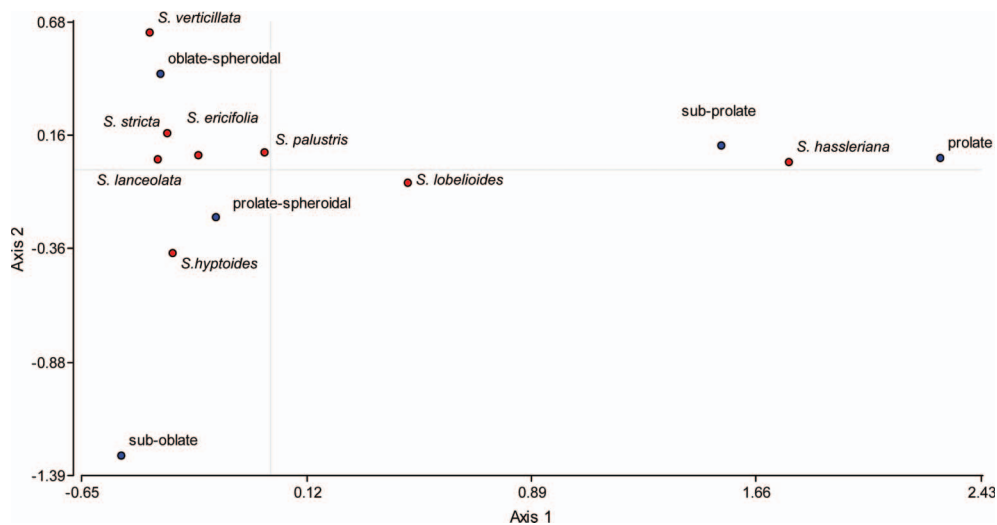


Figure 2. Correspondence analysis of eight species of *Stemodia* and pollen grain shape.

here are consistent with the features described by Varghese (1968) for the most frequent pollen type in the tribe Gratioleae.

The palynological parameters determined here indicate that *Stemodia ericifolia*, *Stemodia stricta* and *Stemodia palustris* have the largest pollen grains. *Stemodia hassleriana* and the diploid cytotype of *Stemodia hyptoides* are the species that present the smallest parameters measured. However, *Stemodia lanceolata* and *Stemodia verticillata* constitute a group with intermediate-sized pollen grains.

Some authors have found a correlation between pollen size and ploidy level (Stebbins 1971; Oliveira et al. 2004; Almada et al. 2006). The tendency of increasing the size of the pollen grains in polyploids could be attributed to the 'gigas' effect, which is characteristic of the polyploidisation process (Stebbins 1971; Oliveira et al. 2004; Almada et al. 2006; Dematteis and Pire 2008). In *Stemodia*, the increase in the pollen size of the polyploid taxa seems to be relatively clear. The pollen grains of *Stemodia hyptoides* (tetraploid and hexaploid) and *Stemodia lobelioides* (tetraploid) were comparatively larger ($>17\ \mu\text{m}$) than those observed in the diploid taxa.

Stemodia hassleriana was considered by Minod (1918) to be a monotypic genus named *Verena* based on morphological features. This species presents biternately dissected leaves and corolla irregularly campanulate, while *Stemodia* taxa generally have entire leaves and bilabiate corollas. The pollen morphology supports the segregation suggested by Minod (1918), because this is the only species of *Stemodia* with prolate pollen grains. Anatomical and cytological studies undertaken on this species (Sosa 2008; Sosa et al. 2009) have also provided evidence that *Stemodia hassleriana* can be distinguished from other species of *Stemodia*.

5. Conclusions

The results obtained here are consistent with the morphological features described for most pollen types in the tribe Gratioleae. There are differences in size, especially in the polyploid which relates to ploidy levels and is attributed to the 'gigas' effect. Species of *Stemodia* studied exhibit uniformity in pollen morphological characters, however, which suggests that the genus is stenopalynous; the only species with different characteristics is *Stemodia hassleriana*.

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Author biographies



MARIA DE LAS MERCEDES SOSA studied biology at the Universidad Nacional del Nordeste-Argentina (UNNE) where she graduated as teacher (1998) and received a doctorate (2009) degree for a botanical thesis. She currently holds a postdoctoral scholarship from CONICET. She has published five papers in national and international journals and one book chapter. Maria has held doctoral and postdoctoral scholarships. In addition she was awarded a scholarship from the Spanish Association of International Cooperation and the Myndel Botanical Foundation to visit European herbaria. Maria has attended 16 postgraduate courses and seminars and she has given 16 presentations at scientific meetings. She has been a teacher in the Department of Biology at UNNE from 2004 to the present. Maria is an active member of the Botanical Society of Argentina.



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