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Pollen morphology of species of *Graphistylis* B. Nord. (Asteraceae) of Brazil

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ABSTRACT

This paper presents the results of a palynological study of the species of *Graphistylis* that were segregated from *Senecio* (*s.l.*) (*Senecio* sect. *Dichroa*) and that are all endemic of Brazil. These species are found in mountainous regions of the states of Espirito Santo, Minas Gerais, Paraná, Rio de Janeiro, São Paulo and Santa Catarina. The pollen grains of eight species were acetolyzed and analyzed and photographed using light microscopy. Unacetolyzed pollen grains were analyzed and photomicrographed using scanning electron microscopy. The current study undertook a palynologically analysis of *Graphistylis* in order to obtain information useful for making a better ranking of these genus. The results show medium-sized pollen grains that were oblate spheroidal or prolate spheroidal and subtriangular amb and tricolporate. The pollen grains possessed lalongate endoapertures with a median constriction and the presence of costa in only two species. The exine is echinate with a varying number of spines in the apocolpium region around the central spine in polar view. The spines are short with basal perforations at varying distances. Although the morphology of the pollen of species of *Graphistylis* is very similar, multivariate analysis highlights the importance of quantitative traits in distinguishing species.

Keywords: cluster analysis, ordination analysis, palynology, pollen grains, Senecioneae

Introduction

The genus *Graphistylis* is endemic to Brazil where it is found in the mountainous regions of the states of Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Nordenstam 1978; Jeffrey 1992). It is in the tribe Senecioneae (Cassini 1818), which is considered the largest tribe of Asteraceae with approximately 3500 species distributed among 150 genera (Nordenstam 2007).

Graphitylis was created by Nordenstam (1978) for all six of the species of *Senecio* sect. *Dichroa*. Zardini (1992) described a new species of *Senecio* sect. *Dichroa*, *Senecio serranus*, endemic to Paraná. Hind (1993) placed *Cacalia dichroa* in *Graphistylis* (*G. dichroa*) and, a year later, Nordenstam (1994) proposed the combination *Graphistylis serrana* for *Senecio serranus*. Teles & Nordenstam (2008) described a new species endemic to Minas Gerais, *Graphistylis riopretensis,* resulting in a total of nine species in the genus.

The forms of the style-arm apices are useful for distinguishing species within the genera of Senecioneae. In *Senecio,* the style-arm apices may be truncated, convex, circular or rhombic, with papillae varying in length, form, and distribution (Jeffrey *et al.* 1975).

It was mainly based on the characteristics of the style arms that *Graphistylis* was segregated from *Senecio s.l.* In *Graphistylis,* species are easily recognized by having style arms that have a conspicuous tuft of trichomes emerging from the apex of the central arm, surrounded by a crown of shorter trichomes.

The morphology and ultrastructure of pollen grains have been useful in the systematics of Asteraceae, and although part of the family has been well studied palyno-

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logically, there have been few such studies of Brazilian species (Mendonça & Gonçalves-Esteves 2000).

Several authors have studied the palynology of the tribe Senecioneae and the genus *Senecio*, such as Skvarla & Turner (1966), Moore & Webb (1978), Vincent & Norris (1989), Bolick (1991), Otieno & Tadesse (1992), Hodolová & Mártonfi (1995), Melhem *et al.* (2003), Cancelli *et al.* (2006, 2007), Medeanic *et al.* (2008) and Cancelli (2008), although no study has investigated the pollen grains of the species of *Graphistylis*.

Bibliographical surveys found that the only taxonomic studies addressing species of *Graphistylis* (previously subordinate to *Senecio* sect. *Dichroa*) were those conducted by Cabrera (1957), Nordenstam (1978) and Teles (2008).

Therefore, the present study aims to fill this gap in knowledge by palynologically analyzing the species of *Graphistylis* and, consequently, provide information that will aid in better classifying this genus.

Materials and methods

Pollen grains of all species of *Graphistylis* B. Nord. were analyzed. Pollen material was obtained from fertile anthers of flowers in anthesis and/or from buds in pre-anthesis, derived from samples deposited in herbaria in southeastern and southern Brazil: MBM, R, RB and RFA. Acronyms are in accordance with Thiers (continuously updated).

Examined material

Graphistylis argyrotricha (Dusén) B. Nord.: Brazil. Rio de Janeiro: Itatiaia, Itatiaia National Park, 19.IV.1957, *Luiz Emygidio 1477** (R); Itatiaia, Itatiaia National Park, X.1964, *Segadas-Vianna et al. s/n*. R158027; Itatiaia, Itatiaia National Park, 16.II.1958, *Fuad Atala s/n*. R158030; Itatiaia, Itatiaia National Park, 08.III.1980, *Soares Nunes 236* (R); Passa Quatro, Itaguaí, 29.VI.2006, *Brade 19022* (RB).

Graphistylis cuneifolia (Gardner) B. Nord.: Brazil. Rio de Janeiro: Teresópolis, Serra dos Órgãos National Park, II/1953, *J. Vidal s/n* R157909*; Rio de Janeiro: Teresópolis, Serra dos Órgãos, Pedra da Baleia, III/1952, *J. Vidal 5734* (R); Rio de Janeiro: Serra dos Órgãos, Teresópolis, s/d, *J. Vidal s/n*, R158136. Teresópolis, Serra dos Órgãos National Park, 28.II.1931, *A.C. Brade 10934*(R).

Graphistylis dichroa (Bong.) D.J.N. Hind.: Brazil. Goiás: Niquelândia, 15.IV.1996, *R. Marquete 2532** (RB).

Graphistylis itatiaiae (Dusén) B. Nord.: Brazil. Minas Gerais: Serra do Caparão, 13.IX.1941, *A.C. Brade 16929** (R). Rio de Janeiro: Itatiaia, Itatiaia National Park, 21.VI.1930, *A.C. Brade 10096* (R); Itatiaia, Itatiaia National Park, Brejo da Lapa, 22.X.1998, *R.L. Esteves 967* (R); Itatiaia, Itatiaia National Park, Prateleiras, VI.1943, *Fernando Vianna s/n* (RFA23077).

Graphistylis oreophila (Dusén) B. Nord.: Brazil. Minas Gerais: Campos dos Caparaós, bordering Espírito Santo, 21.XII.1957, *Adolpho Lutz 1203** (R); Serra de Ibitipoca, I. 1895, *H. Magalhães s/n* (R156855). Rio de Janeiro: Itatiaia, Itatiaia National Park, 25.V.1961, *Edmundo Pereira et al. 5697* (R); Itatiaia, Itatiaia National Park, s/d, *A.J. Sampaio 4730* (R).

Graphistylis organensis (Casar.) B. Nord.: Brazil. Minas Gerais: São João D´el Rei, IV.1921, *Bertha Lutz s/n** (R44825). Rio de Janeiro: Teresópolis, Serra dos Órgãos National Park, Pedra do Sino, V.1953, *J. Vidal 6691* (R); Teresópolis, Serra dos Órgãos National Park, Campo das Antas, III.1942, *Luiz Emygdio et al. s/n* R37291; Pedra da Tijuca, *Ernani de Almeida Anselmo s/n* (R156857).

Graphistylis serrana (Zardini) B. Nord.: Brazil. Minas Gerais: Serra do Ibitipoca, 13.V.1970, *P.L. Krieger 8640** (R). Rio de Janeiro: Itatiaia, Itatiaia National Park, 19.IV.1957, *Luiz Emygdio 1478* (R). Paraná: Serra do Ibitiraquire, trail to Caratuva peak, 16.V.2004, *J.M. Silva et al. 4082* (MBM). São Paulo: Serra da Bocaína, 16.I.2004, *O.S. Ribas et al. 5789* (MBM).

Graphistylis toledoi (Cabr.) B. Nord.: Brazil. São Paulo: Morro da Boa Vista, s/d, *Segadas-Vianna 2583** (R); Serra da Bocaína, Morro da Boa Vista, 7.V.1951, *A.C. Brade 20894* (RB); Serra da Bocaína, Morro da Boa Vista, 25.III.1964, *Segadas-Vianna et al. 2533* (R); Serra da Bocaína, Morro da Boa Vista, III.1951, *Segadas-Vianna 2573* (R).

For light microscopy, pollen material was processed according to the method of acetolysis established by Erdtman (1952) with the modifications proposed by Melhem *et al.* (2003).

Slides were deposited in the Palynology Collection of Alvaro Xavier Moreira Palynology Laboratory, in the Botany Department at National Museum of Federal University of Rio de Janeiro.

For scanning electron microscopy (SEM), anthers were macerated and the pollen grains, non-acetolyzed, were pulverized over stubs covered in carbon tape (Melhem *et al.* 2003). The pollen was metallized with a layer of pure gold for approximately 3 minutes and subsequently analyzed using a JSM-5310 SEM of the Hertha Meyer Ultrastructure Laboratory of the Biophysics Institute at the Federal University of Rio de Janeiro and a JEOL JSM 6390 LV SEM at the Electronic Microscopy Laboratory of Invertebrates of the National Museum, Federal University of Rio de Janeiro.

For descriptions and illustrations a specimen considered as "standard" was selected for each studied species and is indicated by an asterisk (*) after the name of the collector. In order to document morphometric variation we studied, when possible, up to four specimens for each species as "material for comparison" (Abreu *et al.* 2014; Mezzonato-Pires *et al.* 2015; Vignoli-Silva *et al.* 2015).

The polar and equatorial views of pollen grains from the standard material were measured. Random measurments included twenty-five measurements of polar (PD) and equatorial (ED) diameters in equatorial view, 10 meas-

urements of equatorial diameter in polar view (EDPV) and 10 measurments of the apocolpium side (AS).

Additionally, for each species 10 measurements of the aperture and endoaperture (length and width) were taken, as well as exine thickness (sexine and nexine), length, and width and the distance between the spines.

The arithmetic mean ($\bar{x}$), standard deviation (s), the standard deviation of the mean (s_x) and the confidence interval of 95% (CI) were calculated for each sample. The statistical results are shown in tables. The standard deviation and the coefficient of variation of the sample were calculated to confirm the results, but are not included in the tables.

Terminology for size, shape, number of apertures and the pattern of sexine ornamentation follows Punt *et al.* (2007). Descriptions of the polar area and the aperture size follow the classification established by Faegri & Iversen (1966) for the polar area index.

Electron micrographs and photomicrographs were transformed into shades of gray and modified for contrast and brightness using Corel Draw X5.

Exploratory data analysis was carried out using the PC-ORD version 5.31 (McCune & Mefford 2011) program. Pollen features of the analyzed species were organized in a matrix with 17 variables. In the graphs species names were abbreviated with the first three letters and pollen features (variables) were expressed as numbers.

Variables included in the multivariate analysis with their respective numbers in parentheses were: PD (1), ED (2), P/E (3), length (4) and width of colpo (5), length (6) and width of endoaperture (7), EDPV (8), AS (9), IPA (10), exine (11), sexine (12), nexine (13), costa (14), seven spines in the apocolpium (15), six spines in the apocolpium (16), five spines in the apocolpium (17).,Qualitative characters were presented in the matrix as (1) for present or (0) for absent. In order to avoid ambiguities regarding characters and to minimize possible erros, the categorical variables of shape and size of pollen grains and polar area were removed from the matrix since corresponding measured variables were already included (P/E, PD, ED and IPA).

Measured data used in the multivariate analyses (PCA and Cluster Analysis) were standardize by ½ power square root transformation.

Principal component analysis (PCA) was conducted to determine whether the pollen features could cluster species. The variance and covariance matrix (var-cov) was obtained from the means of the morphometric data of the palynological analysis, and the coordinates in the biplot graph are based on Euclidian distances and show the first and second principal components. The matrix of characters, the values of the vectors in each axis and the total of accumulative variance are presented in tables.

The cluster analysis (AHC Clustering) was conducted with the aim of classifying the analyzed species into groups based on shared pollen variables (similarity). Two factors were considered in forming the groups from the set of analyzed variables: the percentage of information (variables) necessary to arrive at the groups related to the final number of groups formed. A dendrogram was made using Euclidian distances (Caccavari *et al.* 2008) and using Ward's linkage method.

Results

Dispersion and unit size

Graphistylis pollen grains (Figs. 1, 2) are monads of medium size, ranging from 36.6 to 49.3 µm in polar diameter and 37.3 to 49.7 µm in equatorial diameter (Tab. 1).

Polarity, shape and polar area

All the species examined have isopolar pollen grains that are radially symmetrical, oblate spheroidal or prolate spheroidal, and subtriangular in shape. The polar area ranges from very small in *G. argyrotricha*, *G. itatiaiae* and *G. serrana,* to small in the other species (Tab. 2).

The smallest confidence intervals for polar and equatorial diameters in equatorial view were for *G. toledoi* (PD=36.0-37.2 µm; ED= 36.7-37.9 µm), and the largest for *G. cuneifolia* (PD= 48.9-49.7 µm; ED= 49.1-50.3 µm).

Pollen grain apertures

The pollen grains are tricolporate; the ectoaperture is restricted to an ornamented aperture area (Fig. 2L, bracket) of varying dimensions and whose limits are usually poorly define their limits within the ectoaperture. The ectoaperture range from being long to very long, and are narrow with acute ends and lalongate endoaperture (Tab. 3), and possess a median constriction (Figs. 1B, 2F).

The longest aperture area was found in *G. dichroa* (ca. 28.8 µm) and the shortest was in *G. toledoi* (ca. 23.7 µm). The longest colpus was found in *G. organensis* (ca. 19.6 µm) and the shortest in *G. toledoi* (ca. 13.4 µm); the longest endoaperture was found in *G. serrana* (7.7 µm), and the shortest in *G. argyrotricha* (3.1 µm); the smallest width was found in *G. toledoi* (ca. 14.7 µm) and the largest in *G. dichroa* (18.2 µm). When seen in equatorial view, a row with five to seven pairs of spines can be seen bordering the aperture (Figs. 1F, G, 2B, L). *Graphistylis oreophila* (Fig. 2A, arrow) and *G. organensis* (Fig. 2D, arrow) were the only two species with costa.

Exine stratification and ornamentation

The exine is thick, caveate (Fig. 1A, D), and with a sexine of two layers of columellae separated by an infratectum, both of which are difficult to measure (Fig. 1A, D). The widest cavea was found in *G. cuneifolia* (ca. 2.5 µm), and

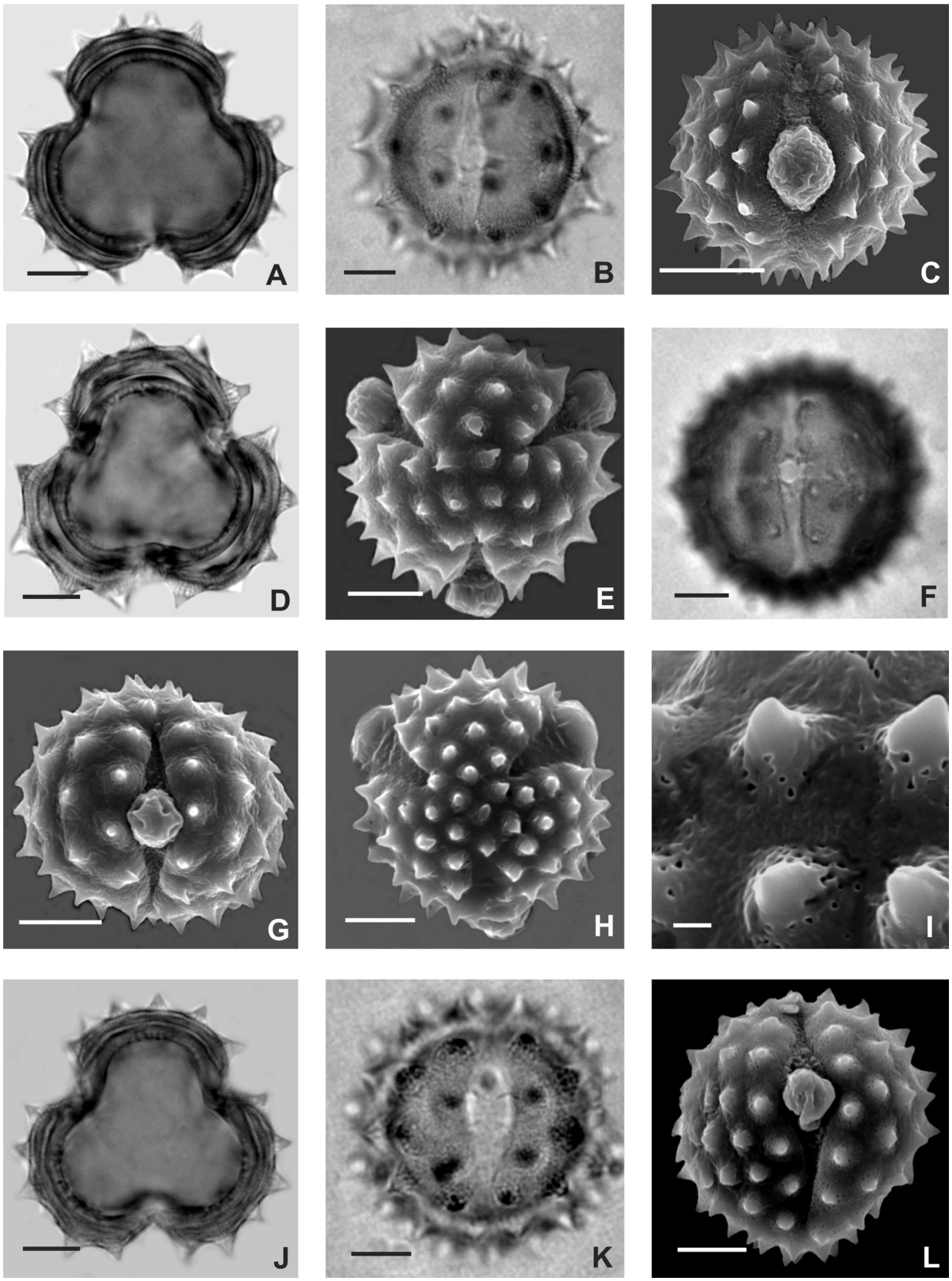

Figure 1. Photomicrographs and eletromicrographs of species of *Graphistylis* (Asteraceae). *G. argyrotricha* A. polar view showing the layers of the exine (arrow) (LM); B. equatorial view (LM); C. equatorial view (SEM). *G. cuneifolia* D. polar view (LM); E. polar view (SEM); F. equatorial view (LM); G. equatorial view (SEM). *G. dichroa* H. polar view (SEM); I. surface detail (SEM). *G. itatiaiae* J. polar view (LM); K. equatorial view (LM); I equatorial view (SEM). Scales bar: 1-8, 10-12= 10μm; 9 =1μm.

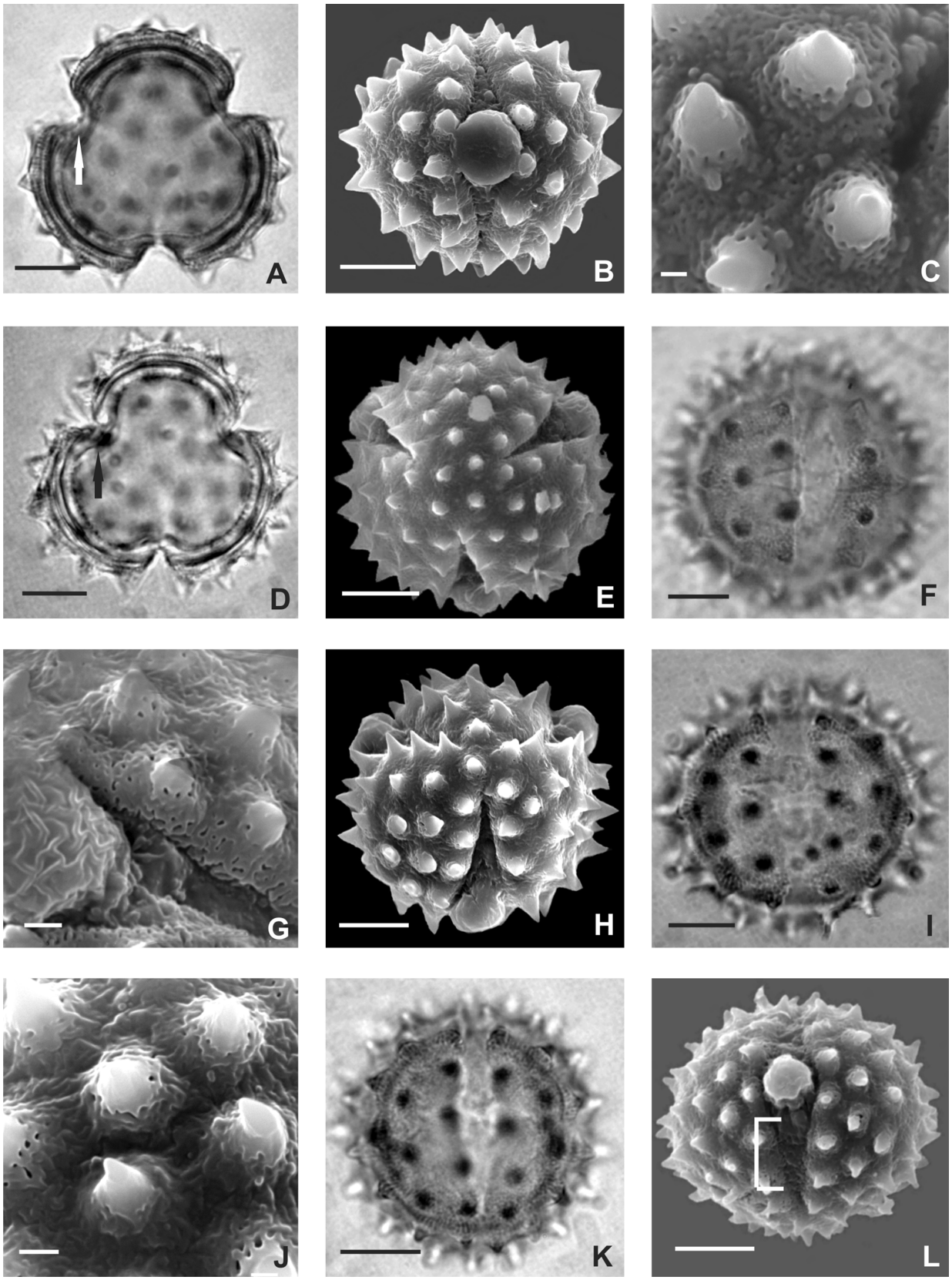

Figure 2. Photomicrographs and eletromicrographs of species of *Graphistylis* (Asteraceae). *G. oreophila* A. polar view showing the costa (arrow) (LM); B. equatorial view (SEM); C. surface detail showing spines (SEM). *G. organensis* D. polar view showing costa (arrow) (LM); E. polar view (SEM); F. equatorial view (LM); G. surface detail. *G. serrana* H. polar view (SEM); I. equatorial view (LM); J. surface detail. *G. toledoi* K. equatorial view (LM); L. equatorial view, square bracket (SEM). Scales bar: 1, 2, 4-6, 8, 9= 10μm; 3, 7, 10=1μm.

Table 1. Measurements (µm) of pollen grains in equatorial view of species of *Graphistylis* (Asteraceae) (n = 25): polar diameter (PD); equatorial diameter (ED), arithmetic mean ($\bar{x}$); standard deviation ($s_{\bar{x}}$); confidence interval (CI 95%); PD/ED = relationship between the polar and equatorial diameters.

Species	Polar diameter (PD)			Equatorial diameter (ED)			PD/ED
	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%	
G. argyrotricha	45.0-48.8	46.3±0.3	45.7-46.9	42.5-50.0	45.9±0.3	45.3-46.5	1.01
G. cuneifolia	47.5-50.0	49.3±0.2	48.9-49.7	47.5-52.5	49.7±0.3	49.1-50.3	0.99
G. dichroa	40.0-47.5	45.3±0.5	44.3-46.3	38.6-47.5	44.8±0.5	43.8-45.8	1.01
G. itatiaiae	45.0-50.0	46.0±0.3	45.4-46.6	42.5-50.0	46.2±0.4	45.4-47.0	1.00
G. oreophila	40.0-47.5	42.6±0.4	41.8-43.4	40.0-45.0	42.2±0.3	41.6-42.8	1.01
G. organensis	45.0-47.5	46.0±0.3	45.4-46.6	42.5-47.5	45.2±0.2	44.8-45.6	1.02
G. serrana	42.5-45.0	43.4±0.2	43.0-43.8	42.5-47.5	44.8±0.3	44.2-45.4	0.97
G. toledoi	35.0-40.0	36.6±0.3	36.0-37.2	35.0-40.0	37.3±0.3	36.7-37.9	0.98

Table 2. Measurements (µm) of pollen grains in polar view of species of *Graphistylis* (Asteraceae) (n=10): equatorial diameter (EDPV); apocolpus side (AS) AS - apocolpium side; PAI – polar area index; ($\bar{x}$) range.

Species	EDPV		AS		PAI
	Range	$\bar{x}$	Range	$\bar{x}$	
G. argyrotricha	42.5-47.5	45.0	10.0-12.5	10.8	0.24
G. cuneifolia	45.0-50.0	49.3	10.0-15.0	13.3	0.26
G. dichroa	37.5-47.5	44.7	10.0-12.5	11.8	0.26
G. itatiaiae	42.5-47.5	45.5	10.0-12.5	11.0	0.24
G. oreophila	40.0-45.0	43.4	12.5-17.5	13.5	0.31
G. organensis	42.5-47.5	45.3	10.0-15.0	12.3	0.27
G. serrana	42.5-45.0	44.4	5.0-7.5	7.1	0.16
G. toledoi	37.5-38.0	37.6	7.5-10.0	9.8	0.26

the narrowest in *G. argyrotricha* (ca. 0.5 µm). SEM reveals that the surface between the spines is striate (Figs. 1I, 2C, G, J) with more prominent grooves in *G. oreophila* (Fig. 2C). The sexine is always much thicker than the nexine.

The spines are short (3.0-4.8 µm), wide (3.4-6.6 µm), and with perforations at the base and at varying distances; *G. argyrotricha*, *G. cuneifolia*, *G. oreophila* and *G. organensis* having the greatest distance between spines (Tab. 3).

The spines possess projections formed by the layers of columellae at their base, with the width of the base of the spines being less than the area between spines (Figs. 1D, J, 2A, D). *G. cuneifolia* had the widest (ca. 6.6 µm) and furthest apart spines (ca. 12.6 µm). *G. argyrotricha*, *G. serrana* and *G. toledoi* had the shortest spines (ca. 3.0 µm), while *G. argyrotricha*, *G. dichroa* and *G. oreophila* had the most narrow spines (ca. 3,4 µm). The smallest distance between spines was found in *G. dichroa* (ca. 7.6 µm).

The species of *Graphistylis* vary in the number of spines, in a polar view, in the apocolpium region and in their arrangement around the central spines: *G. argyrotricha*, *G. dichroa*, *G. oreophila*, *G. serrana* and *G. toledoi* have six spines (Fig, 1H); *G. cuneifolia* has seven spines (Fig. 1E); *G. organensis* (Fig. 2E) and *G. itatiaiae* have a single central spine surrounded by five spines.

Hierarchical cluster analysis (HCA)

The cluster analysis of species of *Graphistylis* produced a dendrogram with a linkage value of 9.09 for explaining the data. Taking into account the percentage of information (variables) and the final number of groups, three groups were recognized when 50% of the information was analyzed. The species of Group 1 were *G. argyrotricha*, *G. dichroa*, *G. oreophila*, *G. serrana* and *G. toledoi*. Group 2 included only *G. cuneifolia*, and Group 3 contained *G. itatiaiae* and *G. organensis*. When 75% of the information was analyzed, four groups were produced consisting of the previous three groups, but with *G. serrana* and *G. toledoi* being removed from Group 1 to form a new group (Fig. 3).

Principal component analysis (PCA)

The first two axes of the PCA explain 64.64% of the total variance, with first and second axes explaining 43.46% and 21.18% of the variance, respectively. The most significant variables of the first principal component are: polar diameter/PD (1), EDPV (8), equatorial diameter/ED

Table 3. Measurements (μm) of the aperture and layers of the exine of pollen grains of species of *Graphistylis* (Asteraceae) (n = 10): Sexine - including spine; DBS - distance between spines.

Species	Ectocolpus		Endoaperture		Apertural area		Exine Layers			Spine			Cavea
	length	width	length	width	length	width	exine	nexine	sexine	length	width	DBS	length
G. argyrotricha	18.4	6.1	3.1	16.9	26.8	5.2	8.6	2.0	6.1	3.0	3.4	9.8	0.5
G. cuneifolia	17.0	6.8	5.3	17.8	26.1	5.6	12.4	2.0	7.9	4.8	6.6	12.6	2.5
G. dichroa	16.5	5.5	5.8	18.2	28.8	7.3	8.3	1.8	5.6	3.3	3.4	7.6	0.9
G. itatiaiae	15.1	5.0	5.1	16.2	25.4	6.5	8.1	2.0	5.3	3.1	4.0	8.0	0.8
G. oreophila	15.0	4.8	5.4	16.4	24.7	4.3	8.2	2.0	5.2	3.2	3.4	9.6	1.0
G. organensis	19.6	6.4	4.6	16.3	25.4	6.3	9.1	2.3	5.8	4.0	4.0	10.0	1.0
G. serrana	14.7	4.5	7.7	17.2	24.8	6.8	9.1	2.0	6.1	3.0	3.6	8.4	1.0
G. toledoi	13.4	4.6	6.5	14.7	23.7	6.5	8.3	2.0	5.7	3.0	3.5	8.4	1.0

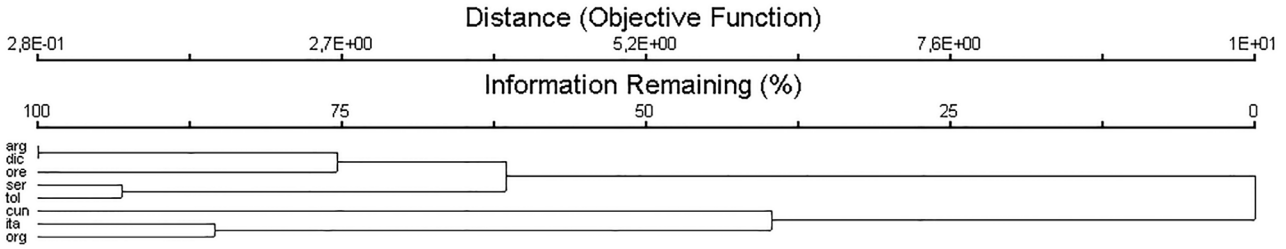

Figure 3. Cluster analysis performed with the measured variables of pollen from *Graphistylis* (Asteraceae). Abbreviations: arg = *G. argyrotricha*, cun = *G. cuneifolia*, dic = *G. dichroa*, ita= *G. itatiaiae*, ore = *G. oreophila*, org = *G. organensis*, ser = *G. serrana*, tol = *G. toledoi*.

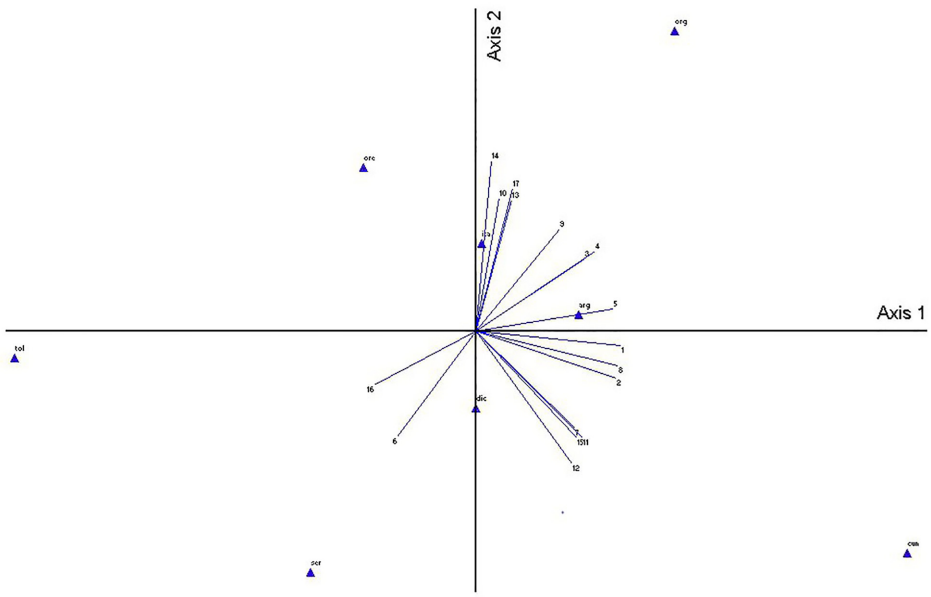

Figure 4. Principal component analysis performed with the mesured variables of pollen from species of *Graphistylis* (Asteraceae): Abbreviations: arg = *G. argyrotricha*, cun = *G. cuneifolia*, dic = *G. dichroa*, ita= *G. itatiaiae*, ore = *G. oreophila*, org = *G. organensis*, ser = *G. serrana*, tol = *G. toledoi*.

(2), and colpus width (7). The most significant variables of the second axis are: presence of costa (14), number of spines on the apocolpium (15, 16, 17), index of polar area (10) and nexine thickness (13).

The species of *Graphistylis* are quite dispersed between the positive and negative poles of the first two axes. *G. organensis*, *G. cuneifolia*, *G. oreophila*, *G. serrana* and *G. toledoi* are polarized with *G. oreophila* and *G. organensis* isolated from the other species towards the positive pole of the second axis due to possessing costa; *G. organensis* separates from *G. oreophila* because it has one of the longest colpi (4) and P/E (3), and from *G. cuneifolia* at the negative pole of the scond axis because it has the greatest values for PD (1) and ED (2), as well as the thickest exine (11). Variables 6 and 16, corresponding to aperture length and number of spines on the apocolpium, were most significant in grouping *G. serrana* and *G. toledoi*, with the latter distancing itself due to smaller values of PD (1) and ED (2).

Although morphologically the pollen grains of species of *Graphistylis* are very similar, the analyses conducted here highlight the importance of quantitative characters in distinguishing species.

Discussion

Pollen grain morphology is quite homogeneous among the species of *Graphistylis* (medium, isopolar, tricolporate, lalongate endoaperture with mean constriction, presence of cavea and echinate sexine). However, they do differ in shape (oblate spheroidal or prolate spheroidal), polar area (small or very small), presence of costa (only in *G. oreophila* and *G. organensis*), and size of aperture area. *Graphistylis toledoi* differed from all other species by having the smallest pollen grains.

Using transmission electron microscopy, Skvarla & Turner (1966), analyzed the pollen grains of *Blennosperma* and *Crocidium*, subordinate genera to the tribes Helenieae and Senecioneae (Asteroideae), respectively. They reached the conclusion that the ultrastructure of the pollen grain wall is a diagnostic characteristic since these genera are closely related. The three large groups of pollen types created by Skvarla & Larson (1965), and confirmed in Skvarla & Turner (1966), are: helianthoid, anthemoid and senecioid, the last being characteristic of Senecioneae and Ambrosiineae. Senecioid type of pollen is described as lacking an internal foramen but having a thick basal layer and an interrupted nexine. The pollen grains of *Graphistylis* are of the senecioid type.

Moore & Webb (1978) succinctly analyzed *Senecio* pollen grains and regarded them as not possessing a visible columella below the structure of the echinate tectum. The species of *Graphistylis* of the present study, formerly regarded as subordinate to *Senecio*, differ from what was reported by Moore & Webb (1978) for *Senecio*, specifically their observation of bacula crossing the tectum in LO-analysis and in optical sectioning.

Using scanning electron microscopy, Vincent & Norris (1989) analyzed 95 species of *Senecio* that occur predominantly in South Africa, plus 11 species of genera of the subtribe Senecioninae. These authors described the pollen grains as having a high density of spines that they classified with respect to length/height (very large to moderately large). The authors concluded by affirming that the genus *Senecio* is closely related to the subtribe Senecioninae, in agreement with the studies of Jeffrey *et al*. (1978). The results presented herein show that the spines of *Graphistylis* range from 3µm to 4.8µm, while those of *Senecio*, according to Vincent & Norris (1989), only reach up to 3µm in length. Therefore, *Graphistylis* spines would be in the range of "very large" and of high density in the opinion Vincent & Norris (1989); however, the present study considered these spines as short, thereby differing from these authors.

Bolick (1991) analyzed the size of the pollen grains and exine thickness in species belonging to 14 tribes of the subfamily Asteroideae, and concluded that pollen grains are small and have a proportionately thinner exine. These characteristics were correlated with the type of exine ultrastructure, which may be caveate (helianthoid and senecioid types) or non-caveate (anthemoid and arctoid types). According to the present study, the pollen grains of *Graphistylis* can be considered senecioid due to the presence of cavea.

In a study of pollen morphology of the family Asteraceae in Rio Grande do Sul, Cancelli *et al.* (2007) analyzed *Senecio cisplatinus* Cabrera, *S. conyzaefolius* Baker, and *S. platensis* Arechav and found their pollen grains to be medium, suboblate and prolate spheroidal in shape, tricolporate, with lalongate endoaperture, long colpia bordered by four to five pairs of spines, and caveate exine with large columellae at the base of the spines. In the polar view, there were 12-15 large, conic, columellate spines with large bases and tapering apices. The results reported herein for the species of *Graphistylis* are similar, differing only in shape, and in the number of pairs of spines in the aperture and the apocolpium. The differences in the findings of these two studies underscore the diversity of pollen grains among the species of these two genera.

Conclusion

According to the literature, the pollen grains of *Senecio* and *Graphistylis* are similar, differing mainly in the number of spines and in the appearance of the cavea, which is more apparent in *Graphistylis*. The pollen grains of the species of *Graphistylis* are quite homogenous, which was confirmed by the multivariate analysis presented herein.

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