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Palynotaxonomy of Brazilian *Viguiera* (Asteraceae) Species

MARA ANGELINA GALVÃO MAGENTA[1], ALESSANDRA DE DEUS NUNES[2],
CLÁUDIA BARBIERI FERREIRA MENDONÇA[2] y VÂNIA GONÇALVES-ESTEVES[2]

Summary: With the aim of improving inter-specific delimitation of the genus *Viguiera* Kunth in Brazil, a palynological analysis was undertaken with 27 taxa, representing around 77% of all occurring species. Samples were obtained from herbarium specimens and the pollen grains were analyzed through light and scanning electron microscopy. Characteristics, including the shape of the pollen grains, polar and equatorial diameter, aperture measurements, surface ornamentation and exine thickness, were registered and compared. The pollen grains were medium-sized (25 - 50 mm), isopolar and oblate spheroidal (prolate spheroidal in *V. aspilioides* Baker). The observed values corroborated the synonymity of some species and also highlighted certain micro-morphological differences, such as polar diameter and the dimensions of the colpus and endoaperture, thus confirming the groups of species delimitation obtained by morphologic and phylogenetic analyses.

Kew words: Asteraceae, Brazil, Compositae, pollen, taxonomy, *Viguiera*

Resumo: Palinotaxonomia de espécies brasileiras de *Viguiera* (Asteraceae-Heliantheae). Com a finalidade de obter subsídios para a delimitação interespecífica do gênero *Viguiera* Kunth no Brasil, foi efetuado um estudo palinológico com 27 táxons, representando cerca de 77% das espécies ocorrentes. As amostras foram obtidas de material herborizado e o grão de pólen foi analisado sob microscopias óptica e eletrônica de varredura. Os grãos de pólen foram caracterizados quanto à forma, às dimensões e tipo da abertura, à ornamentação da superfície e à espessura da exina. Os grãos de pólen são médios (25 - 50mm), isopolares, oblato-esferoidais (prolato-esferoidais em *V. aspilioides* Baker), tricolporados, endoabertura lalongada, sexina espinhosa. Os valores obtidos corroboraram a sinonimização de algumas espécies e diferenças micro-morfológicas, tais como os valores do diâmetro polar, as dimensões do colpo e da endoabertura confirmaram algumas delimitações de grupos de espécies obtidas em análises filogenéticas de morfologia.

Palavras-chave: Asteraceae, Compositae, pólen, taxonomia, *Viguiera*

Introduction

Viguiera Kunth *sensu* Blake (1918) is a Neotropical genus with more than 140 species belonging to the subtribe Helianthinae (Heliantheae - Asteraceae), with representatives occurring in an area stretching from southeastern North America to southern South America. In South America, the species of *Viguiera* are distributed from the equator to about 40º. In Brazil, the genus occurs mainly in the Brazilian Cerrado.

Over recent decades, several proposals to divide the genus have been put forward, mainly based on molecular data (Schilling & Jansen, 1989; Schilling & Panero, 1996a, b; Schilling *et al*., 2002). These authors agree that the Brazilian species form a sole cohesive group together with certain representatives from other South American countries. Nevertheless, problems in specific and even generic delimitation are still prevalent. Panero (2007) stated that without

1 Universidade Santa Cecília, R. Oswaldo Cruz 266, Boqueirão, Santos, 11045-907, Brazil, maramagenta@unisanta.br
2 Museu Nacional, Quinta da Boa Vista, São Cristóvão, Rio de Janeiro, 20940-040, Brazil.

a doubt, when there were more available data, the classification of *Viguiera* will undergo modifications regarding delimitation and phylogenetic relations with other genera. The proposed sinonimization of *Viguiera* in *Rhysolepis*, by Robinson & Moore (2004) has no significant base (Magenta, 2006; Magenta *et al*., 2010).

Palynological analysis represents an efficient tool in taxonomical studies of the family Asteraceae (Wodehouse, 1926, 1928; Wells, 1971; Tomb *et al*., 1974; Feuer & Tomb, 1977; Vezey *et al*., 1994; Perveen, 1999; Qreshi *et al*., 2002; Wortley *et al*., 2007). In the tribe Heliantheae, the pollen grains present a structural pattern that has been named Helianthoid (Skvarla & Larson, 1965a, b; Skvarla & Turner, 1966, 1969; Skvarla *et al*., 1977). Such a pattern is also found in the tribes Eupatorieae, Astereae, Helenieae and Calenduleae, as well as in certain taxa from Inuleae *senso lato*, Senecioneae and Anthemideae, and is characterized by caveate exines and an internal foramen. Several works on pollinic morphology have presented relevant results for Heliantheae taxonomy (Wodehouse, 1928; Felippe & Salgado-Labouriau, 1964; Wells, 1971; Horner & Pearson, 1978; Melhem *et al*., 1979; Gonçalves-Esteves & Esteves, 1989; Meo & Khan, 2006). However, it is necessary to take into consideration which pollinic feature is best suited for use when analyzing the systematics of each group. For example, when working with spiny pollen grains, Felippe & Salgado-Labouriau (1964) proposed employing the presence or absence of perforations in the spines in order to differentiate species. Nevertheless, as the authors themselves noted, *Bidens gardneri* Baker is capable of presenting multiple types, as outlined in the work: some pollen grains have only one cavity, and others have two, whereas several have solid spines. Because of this, Skvarla *et al*. (1977) and Bolick *et al*. (1984) rejected the use of this feature in the Heliantheae tribe. Even though it is necessary to undertake isolated studies for the diverse taxa, palynological characteristics not only furnish additional information but also further systematic analyses (Qreshi *et al*., 2002).

The only analysis of groups of Brazilian species of *Viguiera* was done by Gonçalves-Esteves & Esteves (1989), who examined 10 species. The authors situated the pollen in the 'Aspilia' type, a term that was adopted by Salgado-Labouriau (1973) to designate the morphology of the pollen of *V. robusta* Gardner, which is similar to that of *Aspilia* Thouars (Ecliptinae). The pattern is defined by pollen grains that are oblate spheroidal to prolate spheroidal and tricolporate, with nexine and sexine that are separated by a cavity and joined only at the edges of the openings, and sexine tegillate.

The aim of this study is to establish the palynological differences between species and the similarities between groups of species belonging to the genus *Viguiera* in Brazil.

Material and Methods

A palynological analysis was undertaken with 27 taxa (Appendix), which represent around 77% of all *Viguiera* species occurring in Brazil. The other species present in Brazil were not analyzed because it was not possible to find samples with mature anthers. Fertile anthers from flowers in anthesis and/or well developed flower-buds were removed from sheets deposited in the following herbaria (acronyms according to Holmgren *et al*.,1990): BR, CPAP, D, HAS, HASU, HEPH, HUEPG, HUFU, IBGE, ICN, IPA, K, MBM, P, R, RB, SP, SPF, SPFR, SPSF, TENN, TEX, UB, UEC, US.

Wherever possible, an attempt was made to analyze pollen grains from three specimens of the same species. One of these was chosen as the standard (material that has withstood the process of acetolysis and provided sufficient quantity in the histological slide - indicated in the examined material by means of an asterisk) for measurements, pollinic descriptions and illustrations. The remaining specimens were used for comparing results. Pollen grains were prepared according to the Erdtman (1952) acetolythic method. Acetolyzed pollinic material was used for obtaining photomicrographs. For scanning electron microscopy (SEM) studies, non-acetolysed and acetolysed (see legends) pollen samples were mounted on stubs and coated with gold-platinum. The analysis was done by using a JSM-5310 microscope at the Hertha Meyer Cell Ultrastructure Laboratory at the Institute of Biophysics (Universidade Federal do Rio de Janeiro).

For most species, twenty-five pollen grains from standard materials were divided into equatorial sections (polar diameter = PD and equatorial diameter = ED) and subjected to measurements. The results were analyzed statistically, using a calculator, in order to calculate the arithmetic mean ($\bar{x}$), standard deviation of the sample (s), standard deviation of the mean (s_x), coefficient of variability (CV%) and confidence interval (CI) to 95%. For measurements of the remaining characteristics, such as the equatorial diameter in polar view (EDPV), the apocolpium side (AS), the polar area index (PAI), the openings and the exine, the arithmetic means of 10 measurements were calculated. This was also performed for measurements of the diameters of pollen grains of the comparison material.

Adopted terminology and pollinic descriptions were applied according to criteria defined by Barth & Melhem (1988) and Punt *et al*. (2007) to take into consideration size, shape, number of openings, and sexine ornamental pattern. Denomination and size of the polar area and the size of the aperture are in accordance with the classification established by Faegri & Iversen (1966) for the polar area index.

The decision to keep the name *Viguiera* instead *Rhysolepis*, as proposed by Robinson & Moore (2004) for the species in Brazil, is based on the results of cladistic analysis using morphological features of Magenta (2006). In these analisys, *Rhysolepis* and *Viguiera* species from Brazil emerged in different clades.

The species full names are cited in the appendix.

Results

The pollen grains were medium-sized (25 - 50 mm), isopolar, oblate spheroidal, (prolate spheroidal in the case of *Viguiera aspilioides*) (Table 1), and tricolporate with a small (0.26 - 0.32 mm) to very small (0.21 - 0.25 mm) polar area (Table 2), subcircular amb and spiny sexine.

The lowest values of the confidence interval of the polar diameter in equatorial section (Table 1) were found in *Viguiera veredensis* (32.3 - 33.1 mm), and the highest values were found in *V. macrorhiza* and *V. oblongifolia* (41.1 - 42.0 mm).

The colpi were long to very long, narrow with acute extremities and had a notably elongated endoape. The extremities were acute (Fig. 2 D, M) and obtuse only in *V. aspilioides* (Fig. 1 K), *V. bracteata* (Fig. 1 P) and *V. pilosa* (Fig. 3 B). The longest colpus (ca. 18.0 mm) was found in *V. filifolia,* and the shortest (ca. 11.3 mm) was found in *V. aspilioides* (Table 3). The presence of a granulated membrane was observed in colpi in SEM (Figs. 1 C, 2 N, 3 N).

The exine were thick, contained a cavity [the widest cavity (ca. 1.5 mm) was found in *Viguiera nudibasilaris* and the narrowest (ca. 0.8 mm) in *V. grandiflora*], simplicolumellate, and had long and narrow spines presenting perforations at the base. The distance between the spines was ca. 8.0 mm (Fig. 2 H, Q, T). Both the layer of columellae and the cavity were pronounced. The sexine was always thicker than the nexine.

The spines were conical (Figs. 1 L, 2 E, 3 I) or present as projections at the base (Figs. 1 F, 2 A, 3 G). The longest, widest (ca. 11.0 x 6.1 mm) and farthest apart (ca. 10.4 mm) were encountered in *Viguiera oblongifolia* (Table 3). The shortest spines (ca. 5.0 mm) were found in *V. veredensis*. The narrowest (ca. 2.5 mm) was found in *V. rubra,* and *V. amphychlora* had spines that were the closest together (ca. 7.1 mm) (Table 3).

When analyzing the results obtained for comparison to the respective standard material (Table 4), it was evident that both the shape and size of the pollen grains were constant except in the case of *Viguiera aspilioides*, which presented a variation in form from prolate spheroidal (standard material) to oblate spheroidal (comparison material).

By using pollinic characteristics, it was possible to separate species into four groups when applying confidence interval values to 95% of the polar diameter. Most species are included in the fourth group and this one was divided into sub-groups as shown below.

Identification key for groups of species

I - CI 95% polar diameter = 41.1 - 42.0 mm: *V. macrorhiza*, *V. oblongifolia*
  II- CI. 95% polar diameter = 39.3 - 40.6 mm: *V. aspilioides*, *V. paranensis*, *V. pilosa*, *V. santacatarinensis*
    III-CI 95% polar diameter = 32.3 - 33.1 mm: *V. veredensis*
    IV - CI 95% polar diameter = 34.0 - 39.2 mm
    IV.1- length of colpus = 11.3 - 14.9 mm
      a- width of endoaperture = 5.5 mm: *V. discolor*
      b- width of endoaperture = 10.3 - 11.7 mm

b.1- small polar area: *V. bracteata* (0.27), *V. gardneri* (0.28), *V. nudibasilaris* (0.25), *V. tuberosa* (0.26)

b.2-very small polar area: *V. bakeriana* (0.21), *V. megapotamica* (0.24)

c- width of endoaperture = 12.0 - 14.0 mm

c.1- small polar area: *V. corumbensis* (0.28), *V. tenuifolia* (0.28)

c.2- very small polar area: *V. arenaria* (0.23) *V. rubra* (0.24)

IV.2- length of colpus = 15.8 - 18.0 mm

a- width of endoaperture = 11.3 - 11.7 mm

a.1- length of spine = 6.0 mm: *V. grandiflora*

a.2- length of spine = 7.4 mm: *V. trichophylla*

b- width of endoaperture = 12.0 – 13.2 mm: *V. amphychlora* (0.26), *V. anchusifolia* (0.29), *V. filifolia* (0.29), *V. hispida* (0.25), *V. kunthiana* (0.32), *V. robusta* (0.30), *V. squalida* (0.29)

Discussion and Conclusions

Pollinic data on *Viguiera* are sparse in the literature. Based on the results obtained in the present study, the genus can be characterized by the presence of medium-sized, oblate-spheroidal and tricolporate pollen grains with a small to very small polar area, long to very long colpi, notably elongated endoapertures in most species and spiny exine with a pronounced cavity. Even though there were discrepancies between the groups that were obtained based on their pollinic characteristics and those based on similarities in the external phenotype, variations in the size of the pollen grains (expressed in confidence interval values), polar area and aperture sizes (colpus and endoaperture) were found to contribute to our understanding of the taxonomy of the genus and have proved to be extremely useful in the delimitation of various species.

Viguiera hilairei was originally regarded as a valid species (Blake, 1918). In a taxonomic study undertaken by Magenta (2006) for re-delimitation of Brazilian species, it was noted that *V. hilairei* should be considered synonymous with *V. bracteata*. The pollinic analysis elaborated herein corroborated this position.

Viguiera anchusifolia can present a vegetative portion similar in shape to that of *V. pilosa*. Furthermore, it is apparent that both species form hybrids, thereby complicating their delimitation. Based on the results found here, we have demonstrated that the taxa are pollinically different, with *V. anchusifolia* (R.I. 95% of polar diameter = 34.8 - 35.2 mm) remaining in the IV.2.b.2 group, and *V. pilosa* (R.I. 95% of polar diameter = 39.3 - 39.7 μm) can be placed in group II (pollinic groups herein established).

Certain species are very similar morphologically and their separation only becomes possible after detailed observation of reproductive characteristics. In herbaria, *Viguiera arenaria, V. gardneri* and *V. rubra* are often confused with *V. robusta* but can also be distinguished by taking into consideration pollinic characteristics related to polar diameter and width of the endoaperture. The width of the endoaperture of pollen grains has provided a new measure for delimiting several of these taxa, as shown in the following cases.

In group IV.1 (length of the colpus = 11.3 - 14.9 mm), *Viguiera discolor* is placed in subgroup **a** (width of the endoaperture = 5.5 mm) and *V. bakeriana* (width of the endoaperture within the range 10.3 - 11.7 mm) in subgroup **b**.

In group IV.2 (length of the colpus = 15.8 - 18.0 mm), *Viguiera grandiflora* is allocated to subgroup **a** (width of the endoaperture = 11.3 - 11.7 mm) and *V. squalida* to subgroup **b** (width of the endoaperture = 12.0 - 13.2 mm). In this same group, *V. trichophylla* is placed in subgroup **a,** and *V. filifolia* in subgroup **b**. Each of these pairs of species is very similar morphologically.

Other species of difficult delimitation are: *Viguiera tenuifolia* (group IV.1) and *V. kunthiana* (group IV.2); *V. megapotamica* (group IV.1) and *V. anchusifolia* (group IV.2); *V. aspilioides* (group II) and *V. tuberosa* (group IV.1). As revealed in the present study, these taxa present marked morpho-pollinic characteristics.

Felippe & Salgado-Labouriau (1964) and Salgado-Labouriau (1973) dealt with Heliantheae (Asteraceae) species from the Cerrado, including *Viguiera arenaria* and *V. robusta*, which fell into the ‘Aspilia’ type. According to the authors, this type is common throughout the Heliantheae tribe and is

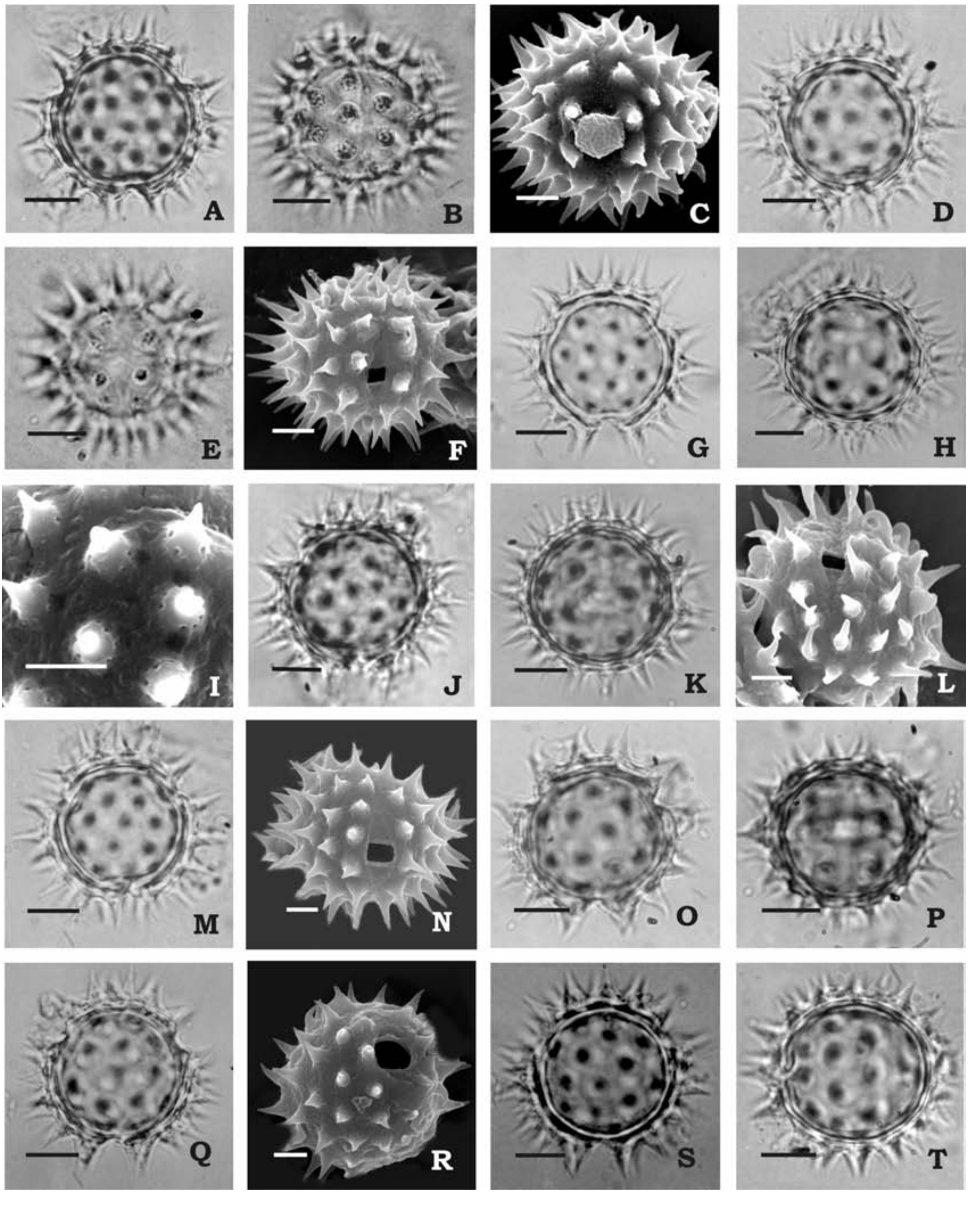

Fig. 1. Photomicrographs and electromicrographs of *Viguiera* species. **A-C:** *V. amphychlora*. **A:** Polar view, optical section. **B, C:** Equatorial view, aperture and surface. **D-F:** *V. anchusifolia* (*). **D:** Polar view, optical section. **E, F:** Equatorial view, aperture and surface. **G-I:** *V. arenaria*. **G:** Polar view, optical section. **H:** Equatorial view, optical section. **I:** Equatorial view, surface detail. **J-L:** *V. aspilioides* (*). **J:** Polar view, optical section. **K:** Equatorial view, optical section. **L:** Equatorial view, aperture and surface. **M, N:** *V. bakeriana* (*). **M:** Polar view, optical section. **N:** Equatorial view, aperture and surface. **O, P:** *V. bracteata*. **O:** Polar view, optical section. **P:** Equatorial view, optical section. **Q, R:** *V. corumbensis* (*). **Q:** Polar view, optical section. **R:** Equatorial view, aperture and surface. **S, T:** *V. gardneri*. **S:** Polar view, optical section. **T: E**quatorial view, optical section. Scale bars. Photomicrographs = 10 μm; electromicrographs = 5 μm. (*) acetolized for SEM.

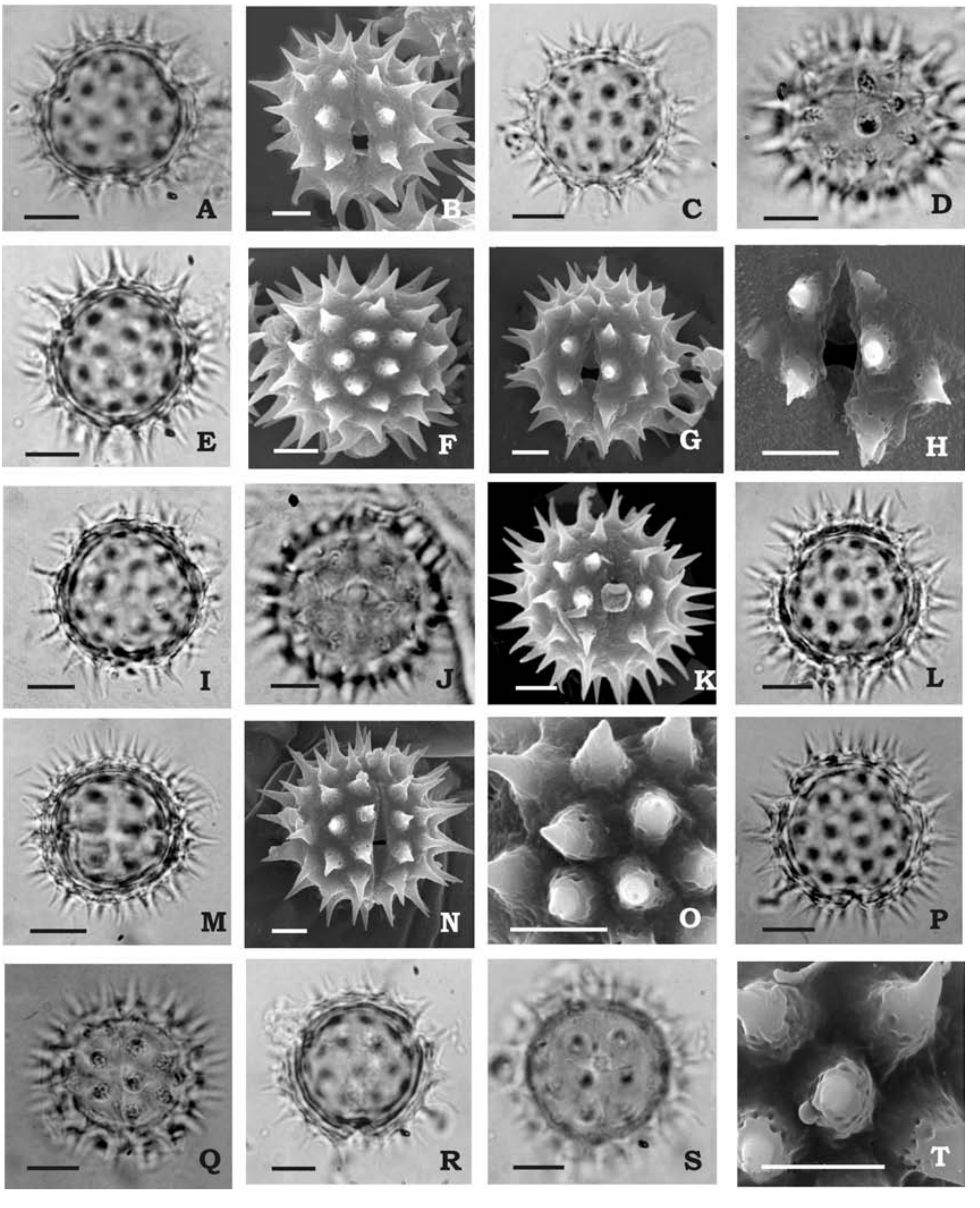

Fig. 2. Photomicrographs and electromicrographs of *Viguiera* species. **A, B:** *V. grandiflora* (*). **A:** Polar view, optical section. **B:** Equatorial view, aperture and surface. **C, D:** *V. hispida*. **C:** Polar view, optical section. **D:** Equatorial view, aperture. **E-H:** *V. kunthiana* (*). **E:** Polar view, optical section. **F:** Polar view, surface at the region of the apocolpium. **G:** Equatorial view, aperture. **H:** Equatorial view, detail of the surface and aperture. **I-K:** *V. macrorhiza*. **I:** Polar view, optical section. **J:** Equatorial view, aperture. **K:** Equatorial view, aperture and surface. **L-O:** *V. megapotamica*. **L:** Polar view, optical section. **M:** Equatorial view, aperture. **N:** Equatorial view, aperture and surface. **O:** Equatorial view, detail of the surface. **P, Q:** *V. nudibasilaris*. **P:** Polar view, optical section. **Q:** Equatorial view, aperture. **R-T:** *V. paranensis*. **R:** Polar view, optical section. **S:** Equatorial view, aperture. **T:** Equatorial view, detail of the surface. Scale bars. Photomicrographs = 10 μm; electromicrographs = 5 μm. (*) acetolized for SEM.

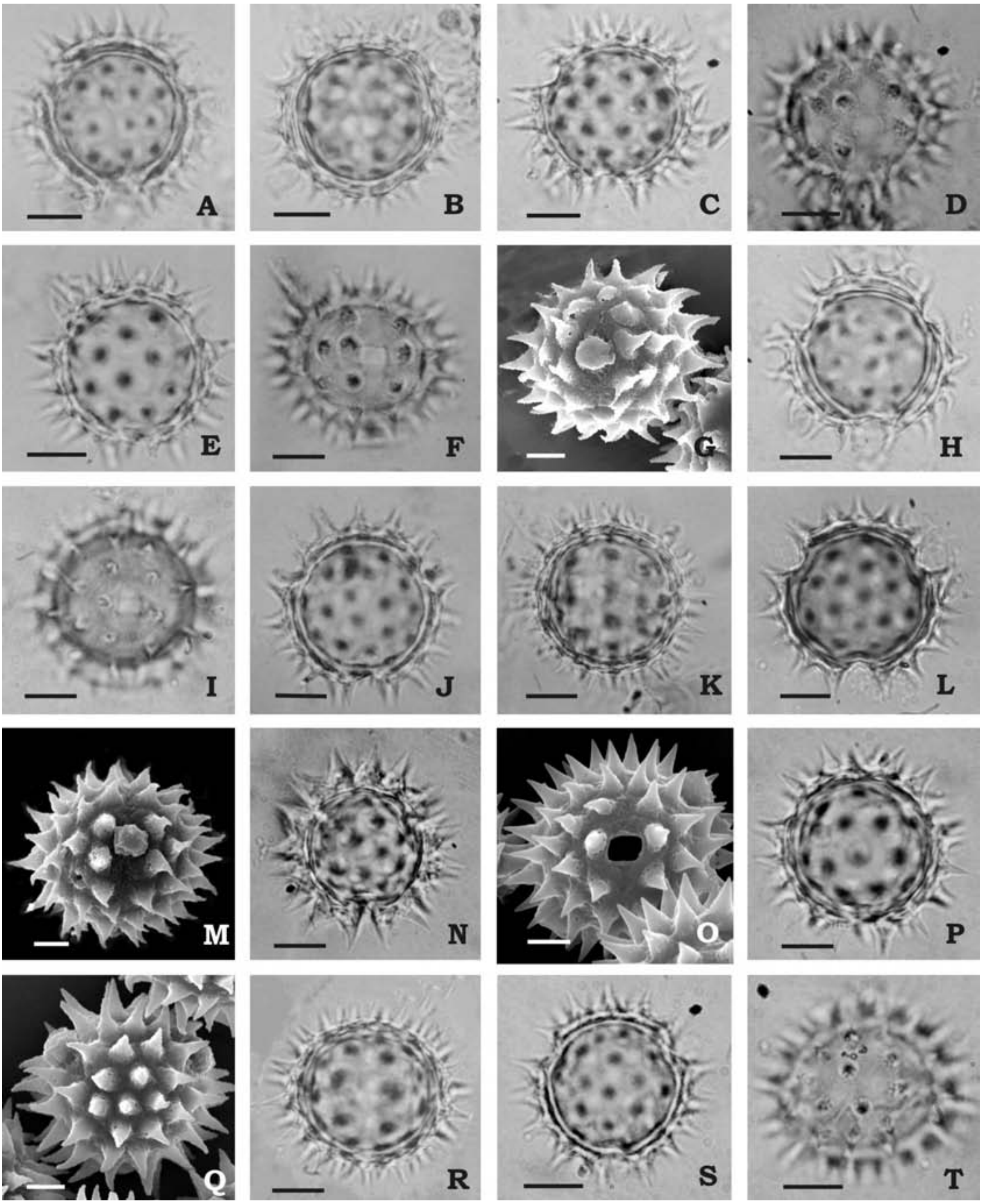

Fig. 3. Photomicrographs and electromicrographs of *Viguiera* species. **A, B:** *V. pilosa*. **A:** Polar view, optical section. **B:** Equatorial view, optical section and aperture. **C, D:** *V. robusta*. **C:** Polar view, optical section. **D:** Equatorial view, aperture. **E-G:** *V. rubra*. **E:** Polar view, optical section. **F:** Equatorial view, aperture. **G:** Equatorial view, aperture and surface. **H, I:** I. *V. santacatarinensis*. **H:** Polar view, optical section. **I:** Equatorial view, aperture. **J, K:** *V. squalida*. **J:** Polar view, optical section. **K:** Equatorial view, optical section. **L, M:** *V. tenuifolia*. **L:** Polar view, optical section. **M:** Equatorial view, aperture and surface. **N, O:** *V. trichophylla* (*). **N:** Polar view, optical section. **O:** Equatorial view, aperture and surface. **P-R:** *V. tuberosa*. **P:** Polar view, optical section. **Q:** Polar view, surface at the region of the apocolpium. **R:** Equatorial view, optical section. **S, T:** *V. veredensi*s. **S:** Polar view, optical section. **T:** Equatorial view, aperture. Scale bars. Photomicrographs = 10 μm; electromicrographs = 5 μm. (*) acetolized for SEM.

Table 1: Measurements (in m) of pollen grains of Viguiera, in equatorial section (n = 25)[1]

Species	Polar diameter (PD)				Equatorial diameter (ED)				P/E
	variation rate	$\bar{x} \pm s_x$	CI 95%	CV%	variation rate	$\bar{x} \pm s_x$	CI 95%	CV%	
V. amphychlora	36.3-38.8	37.3+0.2	36.9-37.7	2.7	38.8-41.3	39.9+0.1	39.7-40.1	1.2	0.93
V. anchusifolia	33.8-36.2	35.0+0.1	34.8-35.2	1.4	37.5-40.0	38.2+0.2	37.8-38.6	2.6	0.91
V. arenaria	37.5-40.0	38.5+0.2	38.1-38.9	2.6	40.0-43.8	42.2+0.2	41.8-42.6	2.4	0.91
V. aspilioides	38.8-41.2	40.2+0.2	39.8-40.6	2.5	37.5-41.2	39.8+0.3	39.1-40.3	3.8	1.01
V. bakeriana	35.0-38.0	36.9+0.2	36.5-37.3	2.2	38.8-41.2	39.8+0.1	39.6-40.0	1.3	0.92
V. bracteata	35.0-37.5	36.2+0.2	35.8-36.6	2.8	37.5-41.2	39.4+0.2	39.0-39.8	2.5	0.91
V. corumbensis	36.2-40.0	38.4+0.2	38.0-38.8	2.6	40.0-42.5	41.0+0.2	40.6-41.4	2.4	0.93
V. discolor	37.5-38.8	38.1+0.1	37.9-38.3	1.3	37.5-42.5	39.9+0.2	39.5-40.3	2.5	0.95
V. filifolia	35.5-38.8	36.9+0.2	36.5-37.3	2.7	38.0-41.2	39.7+0.2	39.3-40.1	2.5	0.92
V. gardneri	37.5-41.2	38.4+0.2	38.0-38.8	2.6	40.5-42.5	41.1+0.2	40.7-41.5	2.4	0.93
V. grandiflora	35.0-37.5	36.5+0.2	36.1-36.9	2.7	38.0-41.2	40.0+0.1	39.8-40.2	1.2	0.91
V. hispida	35.0-40.0	37.5+0.2	37.1-37.9	2.7	37.5-43.0	40.5+0.2	40.1-40.9	2.5	0.92
V. kunthiana	32.5-36.2	34.4+0.2	34.0-34.8	2.9	35.0-38.8	36.8+0.2	36.4-37.2	2.7	0.94
V. macrorhiza	40.0-42.5	41.5+0.2	41.1-41.9	2.4	42.5-45.0	43.6+0.2	43.2-44.0	2.3	0.95
V. megapotamica	37.5-40.0	38.8+0.2	38.4-39.2	2.6	38.8-42.5	41.0+0.2	40.6-41.4	2.4	0.94
V. nudibasilaris	35.0-37.5	36.5+0.2	36.1-36.9	2.7	37.5-42.5	40.8+0.3	40.2-41.4	3.7	0.89
V. oblongifolia	41.2-42.0	41.6+0.2	41.2-42.0	2.4	42.5-46.2	44.7+0.1	44.5-44.9	1.1	0.93
V. paranensis	37.5-41.3	39.8+0.2	39.4-40.2	2,5	42.5-47.5	43.4+0.2	43.0-43.8	2.3	0.91
V. pilosa	38.8-40.0	39.5+0.1	39.3-39.7	1.3	42.5-45.0	42.8+0.1	42.6-43.0	1.2	0.92
V. robusta	36.2-40.0	37.6+0.2	37.2-38.0	2.7	38.8-42.5	40.4+0.2	40.0-40.8	2.5	0.93
V. rubra	35.0-37.5	35.9+0.2	35.5-36.3	2.8	36.3-40.0	38.2+0.2	37.8-38.6	2.6	0.93
V. santacatarinensis	37.5-42.5	39.9+0.2	39.5-40.3	2.5	40.0-43.8	41.5+0.2	41.1-41.9	2.4	0.96
V. squalida	35.0-40.0	38.2+0.3	37.6-38.8	3.9	40.0-42.5	40.9+0.3	40.3-41.5	3.7	0.93
V. tenuifolia	37.5-40.0	37.9+0.2	37.5-38.3	2.6	37.9-38.7	38.7+0.2	38.3-39.1	2.6	0.91
V. trichophylla	35.0-38.8	36.8+0.3	36.2-37.4	4.1	37.5-41.2	39.2+0.2	38.8-39.6	2.6	0.93
V. tuberosa	33.8-36.2	35.3+0.1	35.1-35.5	1.4	37.5-41.2	39.1+0.2	38.7-39.5	2.6	0.90
V. veredensis	31.2-33.8	32.7+0.2	32.3-33.1	3.1	33.8-36.2	35.4+0.2	35.0-35.8	2.8	0.92

Table 2 - Means (in m) of pollen grains from Viguiera species, in polar section (n = 10)[2]

	EDPV		SA		PAI
Species	**variation rate**	**x̄**	**variation rate**	**x̄**	
V. amphychlora	37.5 - 40.0	38.6	10.0 - 12.5	10.3	0.26
V. anchusifolia	37.5 - 40.0	38.0	10.0 - 12.5	11.2	0.29
V. arenaria	40.0 - 43.8	42.1	10.0 - 10.0	10.0	0.23
V. aspilioides	41.2 - 43.8	42.2	10.0 - 12.5	12.2	0.29
V. bakeriana	38.8 - 41.2	40.5	7.5 - 10.0	8.6	0.21
V. bracteata	38.8 - 40.0	39.6	10.0 - 12.5	11.0	0.27
V. corumbensis	38.8 - 42.5	39.9	10.0 - 12.5	11.5	0.28
V. discolor	38.0 - 42.5	40.8	7.5 - 10.0	9.6	0.23
V. filifolia	37.5 - 40.0	39.1	10.0 - 12.5	11.4	0.29
V. gardneri	38.8 - 42.5	40.5	10.0 - 12.5	11.7	0.28
V. grandiflora	37.5 - 41.2	39.9	12.5 - 13.8	12.7	0.31
V. hispida	38.8 - 41.2	39.9	8.8 - 11.2	10.0	0.25
V. kunthiana	37.5 - 40.0	38.2	11.2 - 12.5	12.2	0.32
V. macrorhiza	41.2 - 43.8	42.5	10.0 - 12.5	11.8	0.27
V. megapotamica	40.0 - 41.2	40.3	8.8 - 11.2	9.9	0.24
V. nudibasilaris	40.0 - 43.8	41.5	10.0 - 12.5	10.5	0.25
V. oblongifolia	43.8 - 45.5	44.9	10.0 - 12.5	11.2	0.24
V. paranensis	42.5 - 45.0	43.4	10.0 - 11.3	10.1	0.23
V. pilosa	42.5 - 45.0	43.6	12.5 - 13.8	12.6	0.28
V. robusta	37.5 - 41.2	39.0	10.0 - 12.5	11.9	0.30
V. rubra	36.3 - 40.5	38.5	7.5 - 10.0	9.6	0.24
V. santacatarinensis	40.0 - 42.5	41.0	10.0 - 12.5	11.2	0.27
V. squalida	37.5 - 41.2	40.1	10.0 - 12.5	11.7	0.29
V. tenuifolia	41.2 - 42.5	42.0	10.0 - 12.5	11.8	0.28
V. trichophylla	37.5 - 41.2	38.7	11.2 - 13.8	12.5	0.32
V. tuberosa	37.5 - 41.2	39.4	10.0 - 12.5	10.4	0.26
V. veredensis	32.5 - 36.2	34.0	8.8 - 10.0	9.6	0.28

basically characterized by the shape of the pollen grains (oblate spheroidal to prolate spheroidal) as well as by the amb with around 12 spines, the type of aperture (tricolporate) and by the spiny exine with a cavity. Results presented here describing these species are similar to those reported by the above-mentioned authors; the only difference is the values of the diameters of pollen grains.

When evaluating systematic implications through the use of transmission electron microscopy in studies on Asteraceae, Skvarla & Turner (1966) made use of 184 species from 11 tribes, including

Table 3 - Means (in m) of pollen grain apertures and exine of Viguiera species (n = 10); * total exine including spine

Species	colpus		endoaperture		exine thickness			spine		
	length	width	length	width	exine*	cavea	nexine	length	width	distance
V. amphychlora	15.8	4.3	4.3	12.6	7.1	1.0	1.0	5.1	3.0	7.1
V. anchusifolia	16.6	3.0	2.8	12.5	10.1	0.9	1.0	8.2	4.8	8.5
V. arenaria	14.0	4.5	3.0	14.0	9.4	1.1	0.9	7.4	4.5	8.4
V. aspilioides	11.3	3.6	3.4	10.9	9.2	1.0	1.0	7.2	4.7	9.0
V. bakeriana	11.9	3.5	3.0	10.9	8.2	1.0	1.0	6.2	3.3	9.0
V. bracteata	13.0	3.8	3.4	11.0	8.9	1.0	1.0	6.9	4.0	8.5
V. corumbensis	13.4	3.6	3.5	12.4	8.1	1.1	0.9	6.1	3.6	7.7
V. discolor	14.1	3.2	2.2	5.5	9.0	1.0	1.0	7.0	3.8	8.6
V. filifolia	18.0	3.2	2.9	13.0	8.2	1.0	1.0	6.2	3.2	8.0
V. gardneri	14.5	4.0	3.6	10.9	6.0	1.0	0.9	6.3	4.3	8.3
V. grandiflora	17.2	2.3	2.4	11.3	7.7	0.8	0.9	6.0	3.0	8.0
V. hispida	16.5	3.5	3.2	12.0	7.7	1.0	0.9	5.8	2.9	9.2
V. kunthiana	16.1	3.9	3.4	12.5	9.1	1.0	1.0	7.1	4.9	8.0
V. macrorhiza	12.9	4.4	4.4	12.0	8.0	0.9	1.0	6.1	3.4	8.8
V. megapotamica	14.2	3.5	2.8	11.3	7.7	1.1	0.9	5.7	3.2	8.7
V. nudibasilaris	13.2	4.2	3.3	10.3	8.5	1.5	0.8	6.1	2.7	7.9
V. oblongifolia	14.8	2.9	3.1	11.5	13.0	1.0	1.0	11.0	6.1	10.4
V. paranensis	16.5	4.0	3.5	10.7	10.0	1.0	1.0	8.0	3.3	7.3
V. pilosa	16.8	2.9	2.9	14.5	10.4	1.0	0.9	8.5	6.0	9.7
V. robusta	15.8	6.3	4.8	13.2	8.3	1.0	0.9	6.4	3.3	8.6
V. rubra	14.3	5.0	4.5	12.2	7.6	1.0	1.0	5.6	2.5	7.7
V. santacatarinensis	14.5	4.8	3.8	12.0	8.2	1.0	1.0	6.2	3.0	8.2
V. squalida	17.0	4.1	2.9	12.2	7.0	1.1	0.9	5.1	2.7	8.1
V. tenuifolia	14.2	4.0	3.2	12.6	8.1	1.0	1.0	6.1	4.3	9.0
V. trichophylla	16.2	3.3	3.0	11.7	9.4	1.0	1.0	7.4	5.5	8.3
V. tuberosa	13.7	3.4	2.9	11.4	8.0	1.0	0.9	6.1	3.0	10.0
V. veredensis	14.2	3.8	3.0	12.0	6.9	0.9	0.9	5.0	3.0	9.0

Table 4 - Means (in m) of the diameters of pollen grains in equatorial section (polar and equatorial diameter), polar view (equatorial diameter, side of the apocolpium), relationship of polar and equatorial diameters and the shape of material for comparison from Viguiera species (n = 10)3

Specimens	Polar diameter x̄	Equatorial diameter x̄	P/E	Shape	EDPV x̄	SA x̄
V. arenaria						
M. Magenta & J.E. Magenta 383		38.8	0.94	oblate-spheroidal	39.5	8.9
V. aspilioides						
P.I. Oliveira 965	42.5	43.1	0.96	oblate-spheroidal	45.8	12.5G
Hatschbach 12847	40.3	42.5	0.95	oblate-spheroidal	43.0	10.9
V. bakeriana						
M. Magenta & J.E. Magenta 483	35.8	39.7	0.90	oblate-spheroidal	40.3	9.7
M. Magenta & J.E. Magenta 494	27.2	29.7	0.91	oblate-spheroidal	28.9	7.3
V. bracteata						
M. Magenta *et al.* 444	37.1	40.2	0.92	oblate-spheroidal	40.2	10.1
M. Magenta & J.E. Magenta 631	37.3	39.5	0.94	oblate-spheroidal	40.1	10.5
M. Magenta & J.E. Magenta 637	34.8	37.1	0.93	oblate-spheroidal	36.6	10.7
V. discolor						
M. Magenta & J.E. Magenta 320	37.5	40.7	0.92	oblate-spheroidal	40.7	9.6
M. Magenta & J.E. Magenta 279	38.1	40.9	0.93	oblate-spheroidal	41.0	10.1
V. filifolia						
M. Magenta *et al.* 447	35.9	38.1	0.94	oblate-spheroidal	37.4	11.5
M. Magenta *et al.* 452	36.8	38.9	0.94	oblate-spheroidal	39.0	12.7
V. gardneri						
M. Magenta *et al.* 455	39.5	42.2	0.93	oblate-spheroidal	42.2	11.4
M. Magenta *et al.* 456	34.9	38.0	0.91	oblate-spheroidal	37.6	10.2
V. hispida						
Wanderley *et al.* 1764	37.9	41.2	0.91	oblate-spheroidal	41.6	11.4
V. kunthiana						
M.A. Silva *et al.* 1005	35.4	38.2	0.92	oblate-spheroidal	38.9	10.0
V. macrorhiza						
M. Magenta & J.E. Magenta 476	38.2	40.1	0.95	oblate-spheroidal	39.1	11.6
V. nudibasilaris						
H.F. Leitão F° 1853	36.9	39.5	0.93	oblate-spheroidal	39.1	11.6
M.J. Robim & J.P.M. Carvalho 256	36.7	40.0	0.91	oblate-spheroidal	39.0	11.0
V. oblongifolia						
M. Magenta *et al.* 349	28.4	31.4	0.90	oblate-spheroidal	30.0	8.1
M. Magenta *et al.* 352	42.2	45.1	0.93	oblate-spheroidal	44.9	11.1
V. paranensis						
J. Vidal & E.S. Araújo III-228	39.0	40.7	0.96	oblate-spheroidal	41.2	10.6
V. pilosa						
M. Magenta & C. Mondin 511	39.5	42.8	0.92	oblate-spheroidal	43.6	12.6
V. robusta						
M. Magenta & J.E. Magenta 381	33.4	36.5	0.91	oblate-spheroidal	36.7	10.2
M. Magenta & J.E. Magenta 566	40.4	42.9	0.94	oblate-spheroidal	41.6	13.1
V. squalida						
A. Lima 461-68	33.6	37.1	0.90	oblate-spheroidal	36.9	10.6
M. Magenta & J.E. Magenta 697	40.0	42.1	0.95	oblate-spheroidal	41.4	11.4
V. tenuifolia						
M. Magenta & J.E. Magenta 287	38.4	40.9	0.93	oblate-spheroidal	41.9	12.5
M. Magenta & J.E. Magenta 584	37.6	41.0	0.91	oblate-spheroidal	39.7	12.1
V. tuberosa						
M. Magenta *et al.* 604	38,5	40,75	0,94	oblate-spheroidal	40.5	10.2
C. Mondin & A. Iob 2588	36,6	39,1	0,93	oblate-spheroidal	39.7	11.4

Viguiera dentata (Cav.) Spreng., to which the genus-type belongs. According to these authors, in the Heliantheae tribe the basal layer is always very thin and the presence of the cavity in all species is common. These characteristics were demonstrated in the current study.

Gonçalves-Esteves & Esteves (1989) considered the pollen grains of these species to be homogeneously medium to large-sized with conical spines or spines possessing base projections. The spines were described as tegillum types that are 'hollow', meaning they have a perforation in the base, or 'simple', meaning that they are solid. The present study presents different results regarding pollen grain size and the reporting of the existence of the cavity. However, there is agreement with respect to the 'Aspilia' pollinic type for all of the analyzed *Viguiera* species.

The pollen grain, when considered together with its attributes, revealed itself to be an important diagnostic feature. This was mainly true at the specific level where, besides classes of size of the confidence interval of the polar diameter and polar area type, characteristics like the shape and size of the apertures could be used to identify species and corroborate many of the results presented in the Magenta (2006) taxonomic studies.

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Appendix

V. amphychlora S.F. Blake(Fig. 1 A-C)	**Paraná**: *Sengés*, V.1991, N. Silveira 9786* (HAS)
V. anchusifolia (DC.) Baker(Fig. 1 D-F)	**Rio Grande do Sul:** Santiago, I.2003, M. Magenta & C. Mondin 527* (SPF)
V. arenaria Baker(Fig. 1 G-I)	**São Paulo**: *Itirapina*, II.2002, M. Magenta & J.E. Magenta Neto 383 (SPF); II.2002, M. Magenta & J.E. Magenta Neto 384* (SPF)
V. aspilioides Baker (Fig. 1 J-L)	**Paraná**: *Palmeira*, X.1985, P.I. Oliveira 965 (BR, MBM); *Ponta Grossa*, IX.2002, M. Magenta & J.E. Magenta Neto 479* (SPF); X.1965, G. Hatschbach 12847 (MBM, P, UB)
V. bakeriana S.F. Blake (Fig. 1 M, N)	**Minas Gerais**: *Piumhi*, X.2002, M. Magenta & J.E. Magenta Neto 483 (SPF); *Lagoa Dourada*, X.2002, M. Magenta & J.E. Magenta Neto 494 (SPF); *Santana do Riacho*, X.2002, M. Magenta & J.E. Magenta Neto 488* (SPF)
V. bracteata Gardner(Fig. 1 O, P)	**Distrito Federal**: *Brasília*, XI.2001, M. Magenta & J.E. Magenta Neto 312* (SPF); **Goiás:** *Alto Paraíso de Goiás*, V.2002, M. Magenta *et al.* 444 (SPF); **Minas Gerais:** *São Roque de Minas*, IV.2003, M. Magenta & J.E. Magenta Neto 631 (SPF); *Tapira*, IV.2003, M. Magenta & J.E. Magenta Neto 637 (SPF)
V. corumbensis Malme(Fig. 1 Q, R)	**Mato Grosso do Sul**: *Miranda*, III.1995, A. Pott *et al.* 7026* (CPAP, SPF)
V. discolor Baker	**Minas Gerais**: *Santa Juliana,* XI.2001, M. Magenta & J.E. Magenta Neto 320 (SPF); *São Sebastião do Paraíso*, X.2001, M. Magenta & J.E. Magenta Neto 279 (SPF); **São Paulo**: *Cristais Paulista*, XI.2001, M. Magenta & J.E. Magenta Neto 294* (HUFU, K, SPF, TEX)
V. filifolia Sch. Bip.	**Goiás**: *Alto Paraíso de Goiás*, I.2002, M. Magenta & J.E. Magenta Neto 376* (SPF); V.2002, M. Magenta *et al.* 447 (K, SPF, TEX); V.2002, M. Magenta *et al.* 452 (HUFU, K, SPF)
V. gardneri Baker(Fig. 1 S, T)	**Goiás**: *Ipameri*, V.2002, M. Magenta *et al. 441** (SPF); *Santo Antônio do Descoberto*, V.2002, M. Magenta *et al.* 455 (SPF); V.2002, M. Magenta *et al.* 456 (SPF).
V. grandiflora Gardner(Fig. 2 A, B)	**Distrito Federal**: *Sobradinho*, I.2002, M. Magenta & J.E. Magenta Neto 371* (SPF)
V. hispida Baker (Fig. 2 C, D)	**Goiás**: *Alto Paraíso de Goiás*, XII.1988, M.G.L. Wanderley *et al.* 1764 (SP, SPF); **São Paulo**: *Mogi Guaçu*, X.1955, M. Kuhlmann 3695* (SP, TENN)
V. kunthiana Gardner(Fig 2 E-H)	**Distrito Federal**: Brasília, VIII.1990, M.A. Silva *et al.* 1005 (HEPH, IBGE, RB, US); **Goiás**: *Campo Alegre de Goiás*, XI.2001, M. Magenta & J.E. Magenta Neto 316* (SPF)
V. macrorhiza Baker(Fig. 2 I- K)	**Minas Gerais**: *Paracatu*, X.1978, G. Hatschbach & A. Kasper 41675* (MBM, UEC); **São Paulo**: *Mogi Guaçu*, X.2002, M. Magenta & J.E. Magenta Neto 476 (SPF)
V. megapotamica Malme(Fig. 2 M-O)	**Rio Grande do Sul**: *Arroio dos Ratos*, I.2003, M. Magenta & C. Mondin 502* (SPF)

Appendix - (cont)

V. nudibasilaris Baker(Fig. 2 P, Q)	**Minas Gerais**: *Camanducaia*, III.1976, H.F. Leitão F° 1853 (K, UEC); *Pouso Alegre*, IV.2002, M. Magenta & J.E. Magenta Neto 430* (SPF); **São Paulo**: *Campos de Jordão*, III.1985, M.J. Robim & J.P.M. Carvalho 256 (D, MBM, SPSF, UEC)
V. oblongifolia Gardner	**Goiás**: *Piranhas*, XII.2001, M. Magenta *et al.* 349 (SPF); XII.2001, M. Magenta *et al.* 352 (K, SPF); **Mato Grosso**: *Barra do Garças*, XII.2001, M. Magenta *et al.* 340* (HUFU, K, SPF, SPSF, TEX)
V. paranensis (Malme) Santos (Fig. 2 R-T)	**Paraná**: *Ponta Grossa*, XI.1987, P.M. Araki s.n.* (HUEPG 3498); IX.1950, J. Vidal & E.S. Araújo III-228 (R)
V. pilosa Baker(Fig. 3 A, B)	**Rio Grande do Sul**: Campestre da Serra, III.2002, M. Magenta & J.E. Magenta Neto 406* (K, SPF, SPFR); *Santana do Livramento*, I.2003, M. Magenta & C. Mondin 511 (SPF).
V. robusta Gardner(Fig. 3 C, D)	**Goiás**: *Anápolis*, I.2002, M. Magenta & J.E. Magenta Neto 381 (K, SPF); **Minas Gerais**: *Camanducaia*, IV.2002, M. Magenta & J.E. Magenta Neto 428* (K, SPF); **São Paulo**: *Capão Bonito*, III.2003, M. Magenta & J.E. Magenta Neto 566 (SPF)
V. rubra Magenta(Fig. 3 E-G)	**São Paulo**: *Pirassununga*, III.2002, M. Magenta & J.E. Magenta Neto 388* (K, SPF)
V. santacatarinensis (H. Rob. & A.J. Moore) Magenta & Mondin(Fig. 3 H, I)	**Rio Grande do Sul**: *São José dos Ausentes*, 2002, M. Sobral *et al.* 9495* (ICN, SPF).
V. squalida S. Moore(Fig. 3 J, K)	**Mato Grosso**: *Lago Leo*, X.1960, A. Lima 461-68 (IPA); **Mato Grosso do Sul**: *Ribas do Rio Pardo*, XI.2003, M. Magenta & J.E. Magenta Neto 695* (SPF); XI.2003, M. Magenta & J.E. Magenta Neto 697 (SPF).
V. tenuifolia Gardner(Fig. 3 L, M)	**Minas Gerais**: *Capitólio*, X.2001, M. Magenta & J.E. Magenta Neto 287 (SPF); *Itabirito*, X.2002, M. Magenta & J.E. Magenta Neto 491* (SPF); *São Roque de Minas*, III.2003, M. Magenta & J.E. Magenta Neto 584 (SPF)
V. trichophylla Dusén(Fig. 3 N, O)	**Paraná**: *Lapa*, I.2003, M. Magenta & J.E. Magenta Neto 537* (SPF)
V. tuberosa Hassler(Fig. 3 P-R)	**Rio Grande do Sul**: *Quaraí*, IV.2003, M. Magenta *et al.* 603* (SPF); *Santana do Livramento*, IV.2003, M. Magenta *et al.* 604 (SPF); III.2002, C. Mondin & A. Iob 2588 (HASU, SPF)
V. veredensis Magenta(Fig. 3 S, T)	**Bahia**: *Cocos*, V.2001, R.C. Mendonça *et al.* 4277* (HEPH, IBGE, US)