

Palynology of species of Anthemideae, Eupatorieae, Inuleae, Mutiseae and Senecianeae tribes occurring in the region of Campos Gerais, Paraná State, Brazil

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ABSTRACT - We analyzed the pollen morphology of ten species belonging to nine genera of the *Asteraceae* family, with one species belonging to the Anthemideae tribe (*Matricaria chamomilla* L.), three belonging to the Eupatorieae [*Chromolaena laevigata* (Lam.) R. M. King & H. Rob., *Mikania micrantha* Kunth and *Vittetia orbiculata* (DC.) R.M.King & H.Rob.], two to the Inuleae (*Achillea millefolium* L. and *Gnaphalium purpureum* L.), two to the Mutiseae [*Chaptalia integerrima* (Vell.) Burkart and *Chaptalia graminifolia* (Dusén ex Malme) Cabrera], and two species belonging to the Senecioneae tribe [*Emilia sonchifolia* (L.) DC. and *Senecio brasiliensis* (Spreng.) Less.]. We used acetolysis to carry out the palynological analysis and pollen grains were studied under light and scanning electron microscopy. The tribes were considered eurypalynous, whose morphological detail allowed the subdivision of species in four groups related to pollen types *Anthemis*, *Eupatorium*, *Centaurea* and *Senecio*, previously defined in the literature.

Keywords: eurypalynous, palynotaxonomy, pollen grains

RESUMO – Palinologia de espécies das Tribos Anthemideae, Eupatorieae, Inuleae, Mutiseae e Senecianeae ocorrentes na região dos Campos Gerais, Paraná, Brasil. Foi analisada a morfologia polínica de dez espécies pertencentes a nove gêneros da família Asteraceae, sendo uma espécie pertencente à tribo Anthemideae [*Matricaria chamomilla* L.], três à Eupatorieae [*Chromolaena laevigata* (Lam.) R. M. King & H. Rob., *Mikania micrantha* Kunth e *Vittetia orbiculata* (DC.) R. M. King & H. Rob.], duas à Inuleae [*Achillea millefolium* L. e *Gnaphalium purpureum* L.], duas à Mutiseae [*Chaptalia integerrima* (Vell.) Burkart e *Chaptalia graminifolia* (Dusén ex Malme) Cabrera], e duas espécies à tribo Senecioneae [*Emilia sonchifolia* (L.) DC. e *Senecio brasiliensis* (Spreng.) Less.]. Para a análise dos grãos de pólen realizou-se acetólise e estes foram observados em microscopia óptica e eletrônica de varredura. As tribos foram consideradas euripolínicas e o detalhamento morfológico permitiu a subdivisão das espécies em cinco grupos relacionados aos tipos polínicos *Anthemis*, *Eupatorium*, *Centaurea* e *Senecio* previamente definidos na literatura.

Palavras-chave: euripolínico, grãos de pólen, palinotaxonomia

INTRODUCTION

The Asteraceae has the largest number of taxa within Angiosperms, with about 23,000 species and 17 tribes. According to APG III (2009), the *Asteraceae* family is inserted in the Asterids clade of the Asterales order. In Brazil, there are 14 tribes and according to Mondin (2006), the Eupatorieae tribe covers most generic richness in Brazil with 85 genera. The Mutiseae tribe is in fourth place having 25 genera, the Senecioneae with seven genera, and the *Anthemideae* with only two genera.

The pollen grains of Asteraceae (Anthemideae, Eupatorieae, Inuleae, Mutiseae and Senecioneae) here

were previously described by Cancelli (2008) and Cancelli *et al.* (2005, 2010) from specimens collected in the state of Rio Grande do Sul (including *Chaptalia integerrima*, *Chromolaena laevigata* (*Eupatorium laevigatum*), *Mikania micrantha* and *Senecio brasiliensis*). *Chaptalia integerrima* was also described by Pastana (1988) in a palynological study with 14 Brazilian species from the Mutiseae tribe, belonging to the National Museum Herbarium – RJ. The pollen grains of *Mikania micrantha* were also studied by Mendonça & Gonçalves-Esteves (2000) in a study with species from the Eupatorieae occurring at Restinga de Carapebus, Carapebus - RJ. While studying medicinal plants from Ilha Grande in Rio de Janeiro, Galvão *et al.*

(2009) described *Matricaria chamomilla* (*Chamomilla recutita*) pollen grains and their medicinal use. Meo & Khan (2003), described pollen characteristics of some *Achillea* genus specimens in Pakistan, including *Achillea millefolium*. The pollen grains of *Emilia sonchifolia* were studied for structural morphology by Adedeji (2005) in a study developed in Nigeria with three species of the genus *Emilia* Cass.

In the first stage of the studies on pollen grains in the Campos Gerais region, the pollen morphology of ten species belonging to five genera and two tribes were analyzed (Stanski *et al.* 2013). This study focuses on the morphological characterization of pollen grains from species of the Asteraceae family within Anthemideae, Eupatorieae, Inuleae, Mutiseae and Senecioneae, occurring in the Campos Gerais region, Paraná, Brazil, contributing to palynological and paleoecological studies.

MATERIALS AND METHODS

The plant material used was obtained from flower buds removed from vouchers held at the herbarium at the Universidade Estadual de Ponta Grossa (HUPG), Paraná, Brazil. The species most representative of the Campos Gerais region were selected based on a list carried out by the HUPG herbarium and floristic survey of the region. For each species, the pollen of at least three flower buds was removed to standardize size variations of pollen grains (Salgado-Labouriau 1973).

Pollen grains were prepared according to the acetolysis method by Erdtman (1952), with modifications proposed by Melhem *et al.* (2003), for light microscopy observation. Measurements were made using the Olympus OSM micrometric drum coupled to the microscope eyepiece of an Olympus BX 50 microscope. The measurements of the polar and equatorial axes were made in an equatorial view in 25 pollen grains distributed in five slides for each species, aiming at the uniformity of the sample. The measurements of the apertures, thickness and dimensions of the morphological structures of the exine were performed randomly in 10 pollen grains and only the arithmetic means was calculated (Salgado-Labouriau 1973). Measurements of the polar and equatorial axes in the equatorial view were performed using a magnification of 1.200 X and for the apertures, thickness and exine ornamentation, a magnification of 2000 X was used. Subsequently, a statistical analysis was performed by calculating the arithmetic mean (\bar{x}), standard deviation (s_x), sample standard deviation (s), coefficient of variation (CV), with the significance interval at 95 % and variation range.

To better characterize the pollen grain ornamentation, the width of the base and height of the spines were obtained, yielding indices that define classes which varied between species as in Coutinho & Dinis (2007) for Asteraceae and Barth *et al.* (2005) for Cucurbitaceae.

An index equal to 1.01 was obtained for spines with a base width proportional to the height; indices below 0.89 to 0.98 slightly wider at the base than tall and below 0.64 spines much wider at the base than long, *i.e.*, short spines. It was also possible to characterize the apex/tip appearance (straight or curved), as well as the columellae and cavities at the spine base, mainly under scanning electron microscopy (SEM), differing in number and height among the species.

The terminology adopted was proposed by Punt *et al.* (2007) and Punt & Hoen (2009), considering the size, shape, number of apertures and the sexine ornamentation patterns. The species and their respective authors were consulted on the botanical site (Tropicos®) of the Missouri Botanical Garden.

The acetolysed pollen grains prepared for light microscopy were digitally photographed with the photomicroscope Leica DM 2500 coupled to a video camera and a computer using the LAS EZ 1.6.0 software. For the analysis under scanning electron microscopy, non-acetolysed pollen grains were spread under the surface of double-sided carbon tape wrapped in aluminum brackets ("stubs"), properly numbered. The samples were transferred to a vacuum pump and sputter coated with a thin layer of gold palladium (ca. 150 angstroms thick). The samples were then analyzed using a Shimadzu SSX-550 model belonging to the Scanning Electron Microscopy Laboratory at the Materials Engineering Department at the Universidade Estadual de Ponta Grossa.

The pollen characteristics shared among the species of each tribe are stated below. The general palynological characteristics that each tribe shares are presented under the tribe name. They were omitted from specific descriptions to avoid information being duplicated.

RESULTS

In the following section, we will describe the pollen morphology descriptions of ten species belonging to nine genera of the *Asteraceae*, occurring in the Campos Gerais, Paraná State, Brazil. One species belongs to the *Anthemideae* (*Matricaria chamomilla* L.), three species belong to the Eupatorieae [*Chromolaena laevigata* (Lam.) R. M. King & H. Rob., *Vittetia orbiculata* (DC.) R. M. King & H. Rob. and *Mikania micrantha* Kunth], two species belong to the Inuleae tribe (*Achillea millefolium* L. and *Gnaphalium purpureum* L.), two species belong to the Mutiseae tribe [*Chaptalia integerrima* (Vell.) Burkart and *Chaptalia graminifolia* (Dusén ex Malme) Cabrera] and two species belong to the Senecioneae tribe [*Emilia sonchifolia* (L.) DC. and *Senecio brasiliensis* (Spreng.) Less.] occurring in Campos Gerais, Paraná State, Brazil.

The general morphological characterization and measurements of pollen grains of the studied species are shown in Tables 1-3.

Table 1. General morphological characterization of pollen grains of the *Asteraceae* family in the Anthemideae, Eupatorieae, Inuleae, Mutiseae, and Senecioneae tribes occurring in Campos Gerais, Paraná state, Brazil.

Species	Pollen size	Aperture	Colpus	Endoaperture
<i>Achillea millefolium</i>	medium	3-colporate	long size and narrow	lalongate
<i>Chaptalia graminifolia</i>	medium	3-colporate	long size and narrow	lalongate
<i>Chaptalia integerrima</i>	medium	3-colporate	long size and narrow	lalongate
<i>Chromolaena laevigata</i>	small	3-colporate	long size and narrow	circular
<i>Emilia sonchifolia</i>	medium	3-colporate	long size and narrow	circular
<i>Gnaphalium purpureum</i>	small	3-colporate	long size and narrow	lalongate
<i>Matricaria chamomilla</i>	small	3-colporate	long size and narrow	lalongate
<i>Mikania micrantha</i>	small	3-colporate	long size and narrow	lalongate
<i>Senecio brasiliensis</i>	small	3-colporate	long size and narrow	lalongate
<i>Vittetia orbiculata</i>	small	3-colporate	long size and narrow	lalongate

Table 2. Measurements (µm) in equatorial view of the diameters (n=25) and arithmetic means of measurements of apertures (n=10) of pollen grains of the species of *Asteraceae* from the tribes Anthemideae, Eupatorieae, Inuleae, Mutiseae and Senecioneae occurring in Campos Gerais, Paraná state, Brazil. P = Polar Diameter, E = equatorial diameter, x = arithmetic mean, s = sample standard deviation, sx = mean standard deviation, CI = confidence interval 95%, L = length, W = width.

Species	P			E			P/E	Shape	Colpus		Endoaperture	
	s	x ± sx	CI 95%	s	x ± sx	CI 95%			L	W	L	W
<i>Achillea millefolium</i>	1.8	19.3±0.4	18.6-20.0	2.4	24.3±0.5	23.3-25.3	0.79	suboblate	16.98	2.95	8.96	1.86
<i>Chaptalia graminifolia</i>	1.9	32.4±0.4	31.6-33.2	1.6	30.2±0.3	29.6-31.0	1.06	oblate-spheroidal	28.6	2.87	11.06	2.28
<i>Chaptalia integerrima</i>	2.7	42.8±0.5	41.7-43.9	2.1	31.2±0.4	30.0-32.1	1.37	prolate	32.68	3.40	13.4	3.42
<i>Chromolaena laevigata</i>	2.4	19.2±0.2	18.7-19.7	1.5	17.5±0.1	17.1-17.8	1.06	prolate-spheroidal	13.96	3.39	6.41	1.65
<i>Emilia sonchifolia</i>	1.8	26.9±0.4	26.1-27.6	2.1	25.9±0.4	25.1-26.1	1.03	prolate-spheroidal	19.64	4.15	5.46	5.44
<i>Gnaphalium purpureum</i>	0.6	16.4±0.1	16.2-16.7	0.9	16.0±0.2	15.6-16.4	1.02	prolate-spheroidal	13.96	3.39	6.41	1.65
<i>Matricaria chamomilla</i>	2.2	16.6±0.4	15.6-17.5	1.7	16.6±0.3	15.9-17.3	1.00	prolate-spheroidal	12.00	2.52	10.57	3.47
<i>Mikania micrantha</i>	1.0	15.6±0.2	15.1-16.0	1.0	15.2±0.3	14.6-15.7	1.02	prolate-spheroidal	13.92	1.91	5.30	1.07
<i>Senecio brasiliensis</i>	1.5	26.9±0.3	26.2-27.5	1.3	26.6±0.3	26.0-27.1	1.01	prolate-spheroidal	21.12	4.89	11.60	3.17
<i>Vittetia orbiculata</i>	3.2	17.9±0.6	16.6-19.2	1.9	18.4±0.4	17.6-19.2	0.97	oblate-spheroidal	12.56	2.69	5.95	1.80

Table 3. Arithmetic mean of measurements (µm) of pollen grain exine layer thickness of species of *Asteraceae* family in the tribes Anthemideae, Eupatorieae, Inuleae, Mutiseae and Senecioneae, occurring in Campos Gerais, Paraná state, Brazil. (n=10). N = nexine, CA = caveae, S1 = sexine 1 (columellar layer), S2 = sexine 2 (internal tectum), Cs = number of infrategillate columellae at the base of spines, Hs = length of spine, Ws = width of spine basis, Hs/Ws = spine index, Fs = form of spine apex/tip (s = straight spine apex and sc = straight and curved spine apex).

Species	N	CA	S1	S2	Cs	Hs	Ws	Hs/Ws	Fs
<i>Achillea millefolium</i>	0.67	0.60	1.53	0.98	3 to 4	3.80	6.14	0.61	sc
<i>Chaptalia graminifolia</i>	1.99	----	1.06	0.76	----	----	----	----	----
<i>Chaptalia integerrima</i>	1.77	----	1.19	1.23	----	----	----	----	----
<i>Chromolaena laevigata</i>	0.83	1.48	1.04	0.84	4 to 5	2.93	3.23	0.90	s
<i>Emilia sonchifolia</i>	1.09	Measured with Sex1	0.96	0.96	3 to 4	3.82	3.89	0.98	s
<i>Gnaphalium purpureum</i>	1.02	0.56	0.58	0.54	3	1.91	3.01	0.63	sc
<i>Matricaria chamomilla</i>	1.02	Measured with Sex1	2.52	0.72	4 to 5	3.30	5.67	0.58	s
<i>Mikania micrantha</i>	1.16	0.63	0.75	0.71	4 to 5	2.98	2.95	1.01	sc
<i>Senecio brasiliensis</i>	0.85	Measured with Sex1	0.83	0.78	3 to 4	3.46	5.36	0.64	sc
<i>Vittetia orbiculata</i>	0.64	0.50	0.70	0.74	3	2.53	2.83	0.89	sc

Anthemideae Cass.

(Figs. 1-3)

Matricaria L.

Matricaria chamomilla L.

(Figs. 1-3)

Pollen grains of small size, isopolar, radial symmetry, amb subtriangular (Fig. 1), prolate-spheroidal, echinate. Aperture with longiaperturate colpi and narrow, acute ends, no central constriction. Endoaperture lalongate, without central constriction (Fig. 2). Exine with two distinct layers (sexine and nexine) with indistinct cavea. Simplicolumellate exine with sexine 1 (columellae), sexine 2 (tectum) and sexine 3 (spines). The structure of the spines is of columellar and tegillum type, meaning they have a perforation at the base. Short spines (with about 4 columellae), much wider at the base than high (index 0.58), and with straight acute apex (Fig. 3), dome shaped (swollen at the base), spinular columellae are longer than the inter-spinular columellae. Sexine 3 times thicker than the nexine. Around 16 spines in polar view.

Note: Herbaceous plant, annual. Exotic, widely cultivated in almost all over the world, including in the southern states and southeastern Brazil (Lorenzi & Matos 2002, Nakajima *et al.* 2015).

Eupatorieae Cass.

(Figs. 4-12)

Pollen grains of small size; isopolar; radial symmetry; amb subcircular or circular; oblate spheroidal or prolate oblate spheroidal; 3-colporate; echinate. Aperture longiaperturate and narrow, with acute ends, no central constriction (Fig. 8). Endoaperture circular or lalongate, with acute ends, without central constriction (Fig. 8).

Exine with two distinct layers (sexine and nexine) with indistinct cavea. Simplicolumellate exine with sexine 1 (columellae), sexine 2 (tectum), and sexine 3 (spines). The structure of the spines is of columellar and tegillum type, meaning they have a perforation at the base. Spines columellate, slightly larger at the base (base of spines swollen) then long or slightly longer than wide at the base, with acute and straight spine apex (Fig. 12); columellae infrategillate wider at the base of the spines and shorter between the spines. Sexine are twice as thick as the nexine.

Chromolaena DC.*Chromolaena laevigata* (Lam.) R. M. King & H. Rob.

(Figs. 4-6)

Pollen prolate-spheroidal, amb circular. Aperture longiaperturate and narrow, endoaperture circular. Exine well-delimited cavea, spines longer (with about 4 columellae) than wide at the base (index 0.90), and with

straight acute apex. Around 15 spines in polar view. Sexine 2 times thicker than nexine.

Note: Shrubby plant, perennial. Native, found throughout Brazil (Lorenzi 2000, Oliveira 2015).

Mikania Willd.*Mikania micrantha* Kunth

(Figs. 10-12)

Pollen grains prolate-spheroidal, amb subcircular. Aperture longiaperturate and narrow, with acute ends. Endoaperture lalongate, without central constriction (Fig. 11).

Exine with well-delimited cavea. Spines longer (with around 4 columellae) than wide at the base (index 1.01), with an acute straight apex. Around 14 spines in polar view. Sexine 2 times thicker than nexine.

Note: Climber plant, perennial. Native, found throughout Brazil (Cabrera & Klein 1989, Ritter *et al.* 2015).

Vittetia R. M. King & H. Rob.*Vittetia orbiculata* (DC.) R. M. King & H. Rob.

(Figs. 7-9)

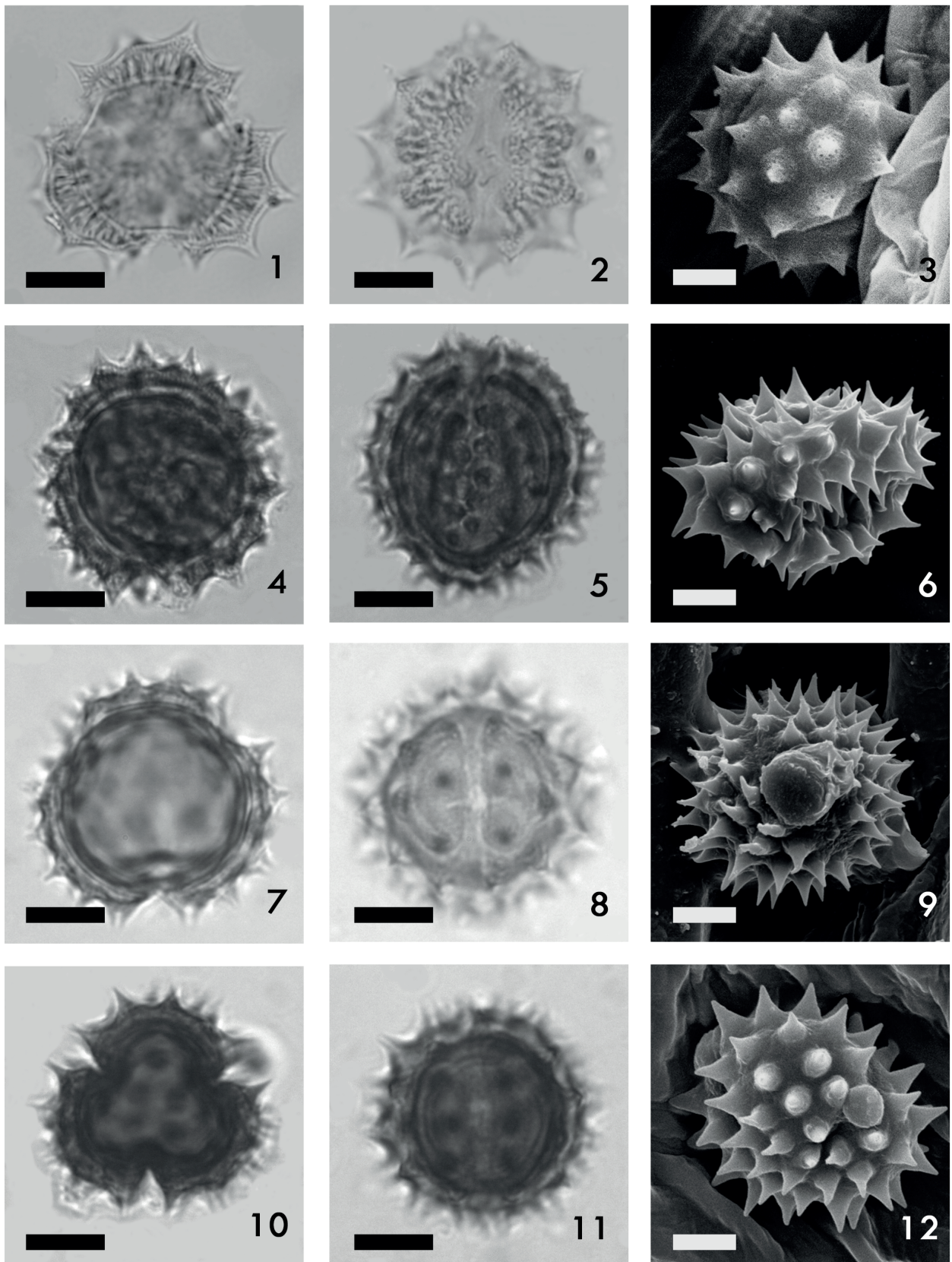
Pollen grains oblate-spheroidal, amb subcircular. Aperture longiaperturate and narrow, with acute ends. Endoaperture lalongate without central constriction (Fig. 8). Exine with well-delimited cavea. Spines with about 4 to 5 columellae, slightly larger at the base then long (index 0.89), and with straight acute apex. Around 14 spines in polar view. Sexine 2 times thicker than nexine.

Note: Sub-shrubby plant, perennial. Native, found in the southern states of Brazil (Cabrera & Klein 1989, Ferreira 2015).

Inuleae Cass.

(Figs. 13-18)

Pollen grains of small to medium size; isopolar; radial symmetry; amb subtriangular or subcircular (Fig. 16); suboblate or prolate-spheroidal; 3-colporate; echinate. Apertures longiaperturate colpi and narrow, with indistinct ends no central constriction (Fig. 17). Endoaperture lalongate, with narrowed ends, with or without central constriction (Fig. 17). Exine with two distinct layers (sexine and nexine) with cavea (well-delimited) and united only at the colpi. Simplicolumellate exine with sexine 1 (columellae), sexine 2 (tectum), and sexine 3 (spines, sculpture elements). The structure of the spines is of columellar and tegillum type, meaning they have a perforation at the base. The spinular columellae are longer than the inter-spinular columellae. Spines are slightly longer than wide at the base or as long as wide at the base, conical or curved (Fig. 13) (swollen at the base) and usually with acute and straight spine apex. Sexine 3 times thicker than the nexine.



Figs. 1-12. Pollen grains of Asteraceae, tribes Anthemideae and Eupatorieae. **1-3.** *Matricaria chamomilla*. **1.** Polar view (MO); **2.** Equatorial view (MO); **3.** General aspect (MEV). **4-6.** *Chromolaena laevigata*. **4.** Polar view (MO); **5.** Equatorial view (MO); **6.** General aspect (MEV). **7-9.** *Vittetia orbiculata*. **7.** Polar view (MO); **8.** Equatorial view (MO); **9.** General aspect (MEV). **10-12.** *Mikania micrantha*. **10.** Polar view (MO); **11.** Equatorial view (MO); **12.** General aspect (MEV). Bars: **Figs. 1, 2, 4, 5, 7, 8, 10, 11** = 10 μm ; **Figs. 3, 6, 9, 12** = 5 μm .

Achillea Linnaeus

Achillea millefolium L.

(Figs. 13-15)

Pollen grains of medium size, suboblate, amb subtriangular, 3-colporate, longiaperturate colpi and narrow. Endoaperture lalongate, without central constriction (Fig. 14). Exine with well-delimited cavea, short spines (with about 4 columellae) much wider at the base than long (index 0.61), spines conical shaped. Around 11 spines in polar view.

Note: Herbaceous plant, perennial. Exotic, cultivated in almost the whole of Brazil (Lorenzi & Matos 2002).

Gnaphalium Linnaeus

Gnaphalium purpureum L.

(Figs. 16-18)

Pollen grains of small size, prolate-spheroidal, amb subcircular. Aperture longiaperturate colpi and narrow, endoaperture lalongate, with central constriction (30%) or without central constriction (70%). Exine with well-delimited cavea, short spines (with about 3 columellae) much wider at the base than long (index 0.63). Around 12 spines in polar view.

Note: Herbaceous plant, annual. Exotic, found in shaded and not very dry soils (Lorenzi 2000).

Mutiseae Cass.

(Figs. 19-24)

Pollen grains of medium size; isopolar; radial symmetry; amb triangular or subtriangular (Fig. 19); oblate-spheroidal or prolate; 3-colporate; microechinate. Aperture longiaperturate colpi, and narrow, with indistinct tips, with or without central constriction (Figs. 23, 20). Endoaperture lalongate, without central constriction (Figs. 20, 23). Exine with two distinct layers (sexine and nexine) without cavea. Sexine bi-stratified, with sexine 3 (columellae), sexine 4 (tectum), sexine 1 (columellae) and sexine 2 (tectum with spicules) (on the tectum rests a new line of smaller columellae, also united by a tectum). Spicules sparsely distributed on the surface of the exine which makes it slightly waved (Fig. 23). The sexine is thicker than the nexine.

Chaptalia graminifolia (Dusén ex Malme) Cabrera

(Figs. 19-21)

Pollen grains oblate-spheroidal, amb subtriangular, 3-colporate, with a slight central constriction, endoaperture lalongate, without central constriction (Fig. 20). Exine marked thickening of the exine at the mesocolpium (mean 3.81 µm) in relation to the pole (mean 2.74 µm).

Note: Herbaceous plant, perennial. Native, occurs in dry and stony fields in the southern states of Brazil (Cabrera & Klein 1973, Roque 2015).

Chaptalia integerrima (Vell.) Burkart

(Figs. 22-24)

Pollen grains prolate, amb triangular, 3-colporate, with a slight central constriction (80%) or without central constriction (20%), endoaperture lalongate, without central constriction (Fig. 23). Exine marked thickening of the exine at the mesocolpium (mean 4.19 µm) in relation to the pole (mean 2.62 µm).

Note: Herbaceous plant, perennial. Native, occurring mainly in lean or stony fields or in coastal forests (Cabrera & Klein 1973, Roque 2015).

Senecioneae Cass.

(Fig. 25-30)

Pollen grains of medium size; isopolar; radial symmetry; amb subcircular (Fig. 25); prolate espheroidal; 3-colporate; echinate. Aperture longiaperturate colpi, with acute ends, no central constrictions (Figs. 26, 29). Endoaperture circular or lalongate, with acute ends, without central constriction (Figs. 26, 29). Exine with two distinct layers (sexine and nexine) indistinct cavea. Simplicolumellate exine with sexine 1 (columellae), sexine 2 (tectum) and sexine 3 (spines, sculpture elements). The structure of the spines is of columellar and tegillum type, meaning they have a perforation at the base. Spines slightly longer than wide at the base or as long as wide at the base, conical shaped, slightly dome shaped (swollen at the base), with acute and straight spine apex or with curved spine apex (Fig. 28). Sexine 2 times thicker than the nexine.

Emilia Cass.

Emilia sonchifolia (L.) DC.

(Figs. 25-27)

Pollen grains prolate-spheroidal, amb subcircular, 3-colporate, long and narrow, and without central constriction, endoaperture circular (Fig. 26). Exine with indistinct cavea, spines columellate (with about 5 columellae), isolated, a little wider at the base than tall (index 0.98). About 15 spines in polar view.

Note: Herbaceous plant, annual. Native, found throughout Brazil (Borges & Teles 2015, Lorenzi & Matos 2002).

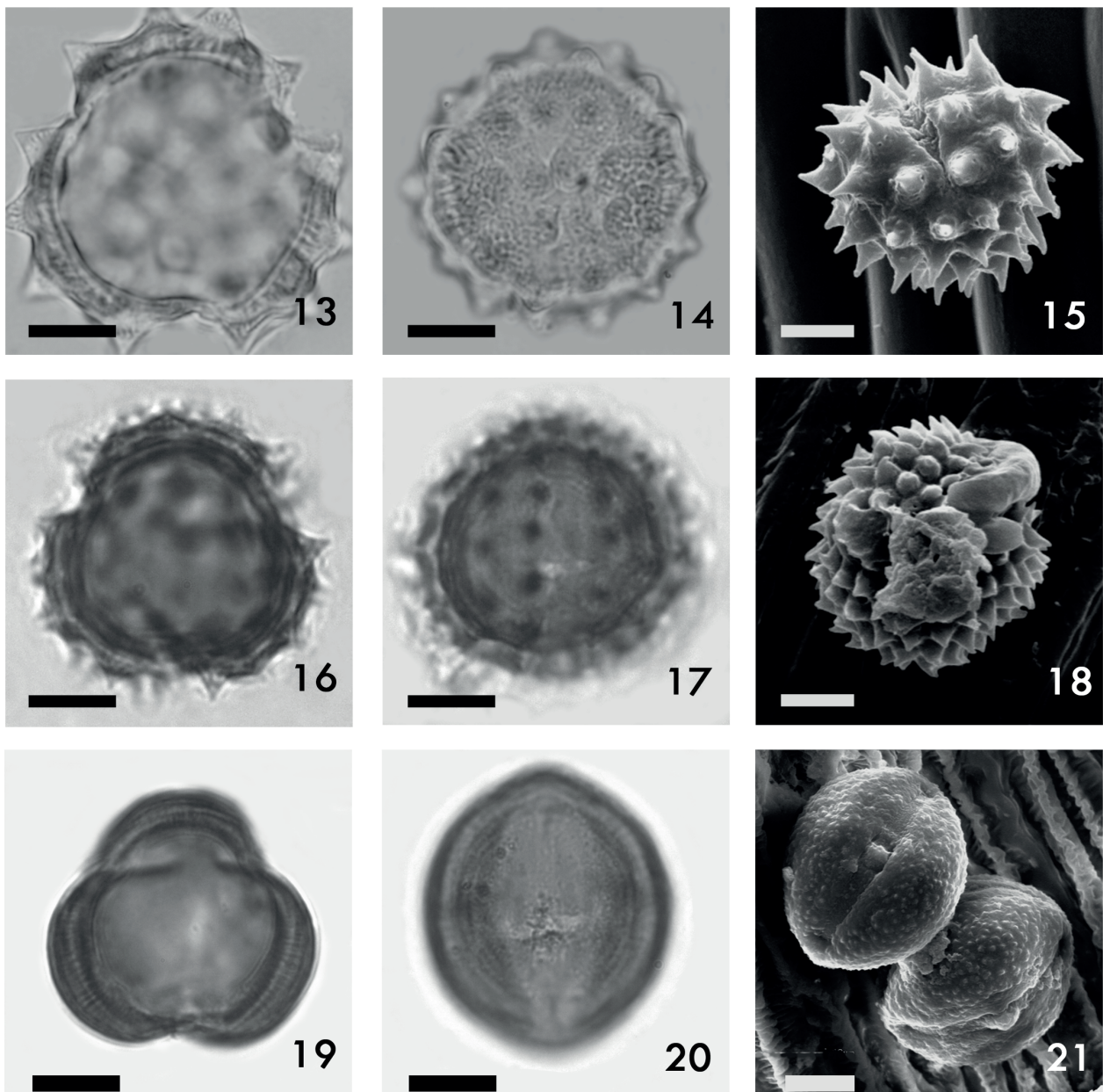
Senecio L.

Senecio brasiliensis (Spreng.) Less.

(Figs. 28-30)

Pollen grains prolate-spheroidal, amb subcircular, 3-colporate, broad colpi at the equator with acute ends, without central constriction, endoaperture lalongate, without central constriction (Fig. 29). Exine indistinct cavea, spines columellate (with about 4 columellae), isolated, spines much wider at the base than tall (index 0.64). About 14 spines in polar view.

Note: Herbaceous, sub-shrubby or shrubby plant, perennial. Native, found throughout Brazil (Lorenzi 2000, Teles 2015).



Figs. 13-21. Pollen grains of Asteraceae, tribes Inuleae and Mutiseae. **13-15.** *Achillea millefolium*. **13.** Polar view (MO); **14.** Equatorial view (MO); **15.** General aspect, ornamentation (MEV). **16-18.** *Gnaphalium purpureum*. **16.** Polar view (MO); **17.** Equatorial view (MO); **18.** General aspect (MEV). **19-21.** *Chaptalia graminifolia*. **19.** Polar view (MO); **20.** Equatorial view (MO); **21.** General aspect (MEV). Bars: **Figs. 13, 14, 16, 17, 19, 20, 21** = 10 μm ; **Figs. 15, 18** = 5 μm .

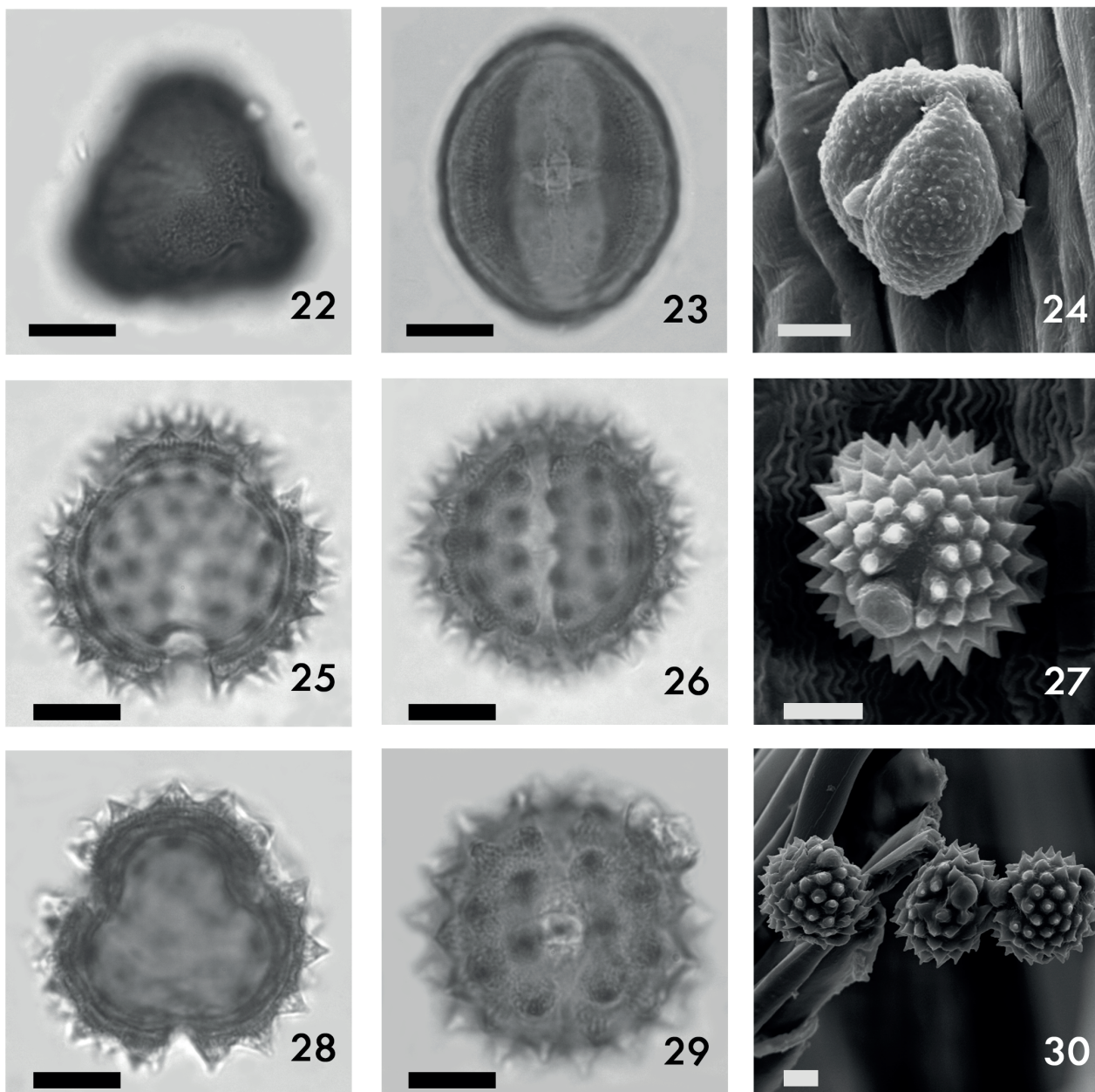
DISCUSSION

In the Eupatorieae tribe, the pollen type called *Eupatorium*, created by Stix (1960), has the characteristics of small (in size) pollen grains, oblate-spheroidal and prolate-spheroidal, lalongate endoapertures and the columellar structure of the spines of tegillum type, corroborating with the results in the analyzed taxa. Nair & Lawrence (1985) analyzed the pollen morphology of 10 taxa from the Eupatorieae tribe, including *Mikania micranta*, which have very similar characteristics to this description, and verified that pollen grains of the Eupatorieae were similar to species

of Astereae, with small pollen grains and echinate exine.

Chromolaena laevigata (Lam.) R. M. King & H. Rob. (*Eupatorium laevigatum* Lam.) was palynologically studied by Cancelli *et al.* (2010), differing from our description in relation to pollen grain dimensions, described by the author as medium in size. On the other hand, *Vitsetia orbicula*, had not yet been palynologically studied, which has oblate-spheroidal pollen grains, small and with lalongate endoaperture.

Two species of the genus *Chaptalia* were analyzed in which *C. integerrima* was previously studied by Cancelli *et al.* (2010) who described the pollen grain as prolate-spheroidal, and Pastana (1988), who described it



Figs. 22-30. Pollen grains of Asteraceae, tribes Mutiseae and Senecianeae. **22-24.** *Chaptalia integerrima*. **22.** Polar view (MO); **23.** Equatorial view (MO); **24.** General aspect (MEV). **25-27.** *Emilia sonchifolia*. **25.** Polar view (MO); **26.** Equatorial view (MO); **27.** General aspect (MEV). **28-30.** *Senecio brasiliensis*. **28.** Polar view (MO); **29.** Equatorial view (MO); **30.** General aspect (MEV). Bars: **Figs. 22-30 = 10µm.**

as suboblate. The other characteristics presented by the aforementioned authors were similar to our descriptions. The other characteristics were similar to the observations in this study. Melhem *et al.* (2003) also studied *C. integerrima* describing the pollen grains as prolate-spheroidal, with a lalongate endoaperture, having marked thickening at the mesocolpium. However, *C. graminifolia* had not yet been palynologically studied. In this study, it presents pollen grain oblate-spheroidal, amb triangular, colpiate, with slight median constriction and lalongate endoaperture.

The species of the Senecioneae tribe, *Emilia sonchifolia* and *Senecio brasiliensis*, although not belonging to the same genus, were similar in size, shape and amb

(medium, prolate-spheroidal, and sub circular amb). *Emilia sonchifolia* was described by Adedeji (2005) with tricolporate pollen grains, and it is the only species of the described genus in Nigeria with 4-colporate medium (in size) pollen grains. However, here it was possible to observe only tricolporate pollen grains. Cancelli *et al.* (2010) described *S. brasiliensis* presenting small in size pollen grains and prolate-spheroidal, coinciding in part with the observations of this study.

Achillea millefolium and *Matricaria chamomilla* are among the many medicinal plants included in the Asteraceae family (Souza & Lorenzi 2012). Galvão *et al.* (2009) described *Matricaria recutita*, basionym of *Matricaria*

chamomilla L., popularly known as “Camomile”, with medium in size pollen grains, larger than observed in the present study. Regarding the descriptions made by those authors, the pollen grains were described as isopolar, oblate-spheroidal, of subtriangular amb, 3-colporate and with an echinate exine, differing only in shape, described in this study as prolate-spheroidal.

Achillea millefolium pollen grains were studied by Meo & Khan (2003) in a study where two species of the *Achillea* genus were described. The analyzed specimen here shows tricolporate pollen grains medium in size, corroborating with the results described by Meo & Khan (2003).

Breitwieser & Sampson (1997) described the pollen grain in *Gnaphalium* with various types of columellae. In relation to *Gnaphalium*, some descriptions made by Perveen (1999) corroborate with the ones presented here for *Gnaphalium purpureum*, such as the small size, differing in shape, which was described by the authors as oblate-spheroidal to sub-prolate. The morphological diagnosis obtained from small (in size) pollen grains, exine with caveae, with short, sharp and few spines groups the species of the Eupatorieae tribe (*Chromolaena laevigata* (Lam.) R. M. King & H. Rob., *Vittetia orbiculata* (DC.) R. M. King & H. Rob. and *Mikania micrantha* Kunth) with *Gnaphalium purpureum* L. of the tribe Inuleae, and showed its correspondence to the *Eupatorium* pollen type described by Stix (1960), as well as the pollen type *Compositae*-H (*Helianthus*) of Zander (1935), or also of the pollen type EU (*Eupatorium*) of Barth (1989).

The two taxa analyzed from the Mutiseae, *Chaptalia integerrima* (Vell.) Burkart and *Chaptalia graminifolia* (Dusén ex Malme) Cabrera, were distinguished from the other species due to having medium size pollen grains and not having spines, but sparsely distributed spicules on the exine surface, making it slightly wavy, with two-stratum sexine. The pollen morphology of these species is related to the pollen type *Centaurea* described by Stix (1960).

On the other hand, the medium sized pollen grains with large, tall and numerous spines of *Emilia sonchifolia* (L.) DC. and *Senecio brasiliensis* (Spreng.) Less. classified them in the pollen type *Senecio* of Stix (1960), or *Compositae*-H pollen type (*Helianthus*) of Zander (1935), or also of the *Compositae* S (*Senecio*) pollen type by Barth (1989).

Two species of different tribes (*Achillea millefolium* L. - Inuleae and *Matricaria chamomilla* L.-Anthemideae) were grouped in the pollen type *Anthemis* of Stix (1960) by presenting long columellae configuring the thickest sexine 1 among the analyzed species.

The five tribes analyzed were considered eurypalynous, whose morphological detail allowed the subdivision of species in four groups related to pollen types *Anthemis*, *Eupatorium*, *Centaurea* and *Senecio* previously defined in the literature. However, the morphological similarities in pollen grains of the species of the Mutiseae and Senecioneae tribes as to the size of the openings, sexine layers and appearance of the spine or spicules, considered them as stenopalynous, *i.e.*, with a great morphopalynological

similarity that do not taxonomically separate these species. The same occurred with *Gnaphalium purpureum* (Inuleae), with all species belonging to the *Eupatorieae* tribe. They were also grouped to the *Achillea millefolium* of the Inuleae tribe and *Matricaria chamomilla* of the *Anthemideae* tribe. The groups formed by these species may be used in applied research that uses Palynology as the reconstruction of paleoenvironments and paleovegetation.

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