Palynology of species in the *Astereae* and *Heliantheae* tribes occurring in the region of Campos Gerais, Paraná State, Brazil

Carin Stanski[1], Cynthia Fernandes Pinto Luz[2], Alessandro Nogueira[3] & Melissa Koch Fernandes de Souza Nogueira[4*]

[1] Universidade Estadual de Ponta Grossa, Laboratório de Palinologia, Ponta Grossa, Paraná, Brasil. carin.stansk@gmail.com
[2]Instituto de Botânica de São Paulo, Núcleo de Pesquisa em Palinologia, Av. Miguel Estéfano, 3687, Água Funda – CEP 04301-012, São Paulo, São Paulo, Brasil . cynthialuz@gmail.com
[3] Universidade Estadual de Ponta Grossa, Departamento de Engenharia de Alimentos. Av. General Carlos Cavalcanti, 4748 – CEP 84030-900, Ponta Grossa, Paraná, Brasil. alessandronog@yahoo.com.br
[4] Universidade Estadual de Ponta Grossa, Departamento de Biologia Geral, Laboratório de Palinologia. Av. General Carlos Cavalcanti, 4748 – CEP 84030-900, Ponta Grossa, Paraná, Brasil.melissaknog@yahoo.com.br

ABSTRACT – We analyzed the pollen morphology of twelve species belonging to five genera of the *Asteraceae* family; four species belonging to the *Astereae* tribe [*Aster haplopappus* (Remy) Kuntze, *Baccharis axillaris* DC, *Baccharis semiserrata* DC and *Baccharis trimera* (Less.) DC.], and eight to the *Heliantheae* tribe [*Aspilia montevidensis* (Spreng) Kuntze, *Aspilia reflexa* Baker, *Bidens alba* (L) DC, *Bidens pilosa* L, *Calea cymosa* Less, *Calea hispida* (DC) Baker, *Calea parvifolia* (DC) Baker and *Calea pinnatifida* (R. Br.) Less] occurring in the region of Campos Gerais, Paraná State, Brazil. We used acetolysis to carry out the palynological analysis and pollen grains were studied under light and scanning electron microscopy. The pollen grains of the two tribes had common morphological features such as 3-colporus, lalongate endoapertures, echinate exine type, a sexine of one stratum and with cavea, however, they differed regarding the structure size and appearance of the spine. The two tribes were considered stenopalynous.

Key words: grassland, palynotaxonomy, pollen grain, stenopalynous

RESUMO – **Palinologia de espécies das Tribos *Astereae* e *Heliantheae* ocorrentes na região dos Campos Gerais, Paraná, Brasil.** Foi analisada a morfologia polínica de doze espécies pertencentes a cinco gêneros da família *Asteraceae*, sendo quatro espécies pertencentes à tribo Astereae [*Aster haplopappus* (Remy) Kuntze, *Baccharis axillaris* DC, *Baccharis semiserrata* DC e *Baccharis trimera* (Less.) DC.] e oito à tribo Heliantheae [*Aspilia montevidensis* (Spreng) Kuntze, *Aspilia reflexa* Baker, *Bidens alba* (L) DC, *Bidens pilosa* L, *Calea cymosa* Less, *Calea hispida* (DC) Baker, *Calea parvifolia* (DC) Baker e *Calea pinnatifida* (R. Br.) Less], ocorrentes na região dos Campos Gerais, Ponta Grossa, Paraná, Brasil. Para a análise dos grãos de pólen realizou-se a acetólise e estes foram observados em microscopia óptica e eletrônica de varredura. Posteriormente ás análises, as duas tribos foram consideradas estenopolínicas, entretanto, o detalhamento morfopolínico permitiu a subdivisão das espécies em três grupos relacionados aos tipos polínicos *Baccharis*, *Helianthus* e *Senecio* previamente definidos na literatura.

Palavras-chave: Campos Gerais, estenopolínicas, grãos de pólen, palinotaxonomia

INTRODUCTION

The *Asteraceae* family is the most numerous group within the Angiosperms, with about 23,000 species and 17 tribes. According to APG III (2009), the *Asteraceae* family is classified in the Asterideas clade and Asterales order. In Brazil, there are 14 tribes, and Heliantheae is in third position featuring 41 genera, while the Astereae tribe comprises 18 genera (Mondin, 2006).

Plants in the *Asteraceae* family vary greatly in appearance, mainly including herbs or shrubs, though rarely trees. The family has a cosmopolitan distribution and in Brazil, is common in open areas, mainly in the Cerrado and grasslands (Souza & Lorenzi, 2005).

The asterological flora of southern Brazil is considered dense, with high species diversity and better represented by the tribes Astereae, Inuleae, Helenieae, and Mutiseae with microthermal species, especially in cold temperate climate (Matzenbacher, 2003). The high density in this region may be attributed to the evolutionary history of the family being linked to the southern portion of South America, south of latitude 30° (Bremer & Gustafsson, 1997).

According to the Brazilian vegetation classification system (IBGE, 1992), the Campos Gerais region, in southern Brazil, is called a grassland ecosystem, also known as native fields (Leite & Klein, 1990). The *Asteraceae* family in the Campos Gerais region, Ponta Grossa, Paraná State is among the richest families in species and individuals. According to Kozera *et al.* (2008), a floristic survey of the grassland determined 49 taxa belonging to the *Asteraceae* family. Dalazoana (2010), on the other hand, identified 91 taxa in the same vegetation.

Some pollen grains of *Asteraceae* (Astereae and Heliantheae tribes) studied here were previously described by Cancelli (2008) and Cancelli *et al.* (2005, 2007, 2010) from specimens collected in the state of Rio Grande do Sul (Brazil) (including *Aspilia montevidensis* (Spreng.) Kuntze, *Baccharis trimera* (Less.) DC., *Bidens alba* (L.) DC., *Bidens pilosa* L., and *Calea pinnatifida* (R. Br.) Less.). The pollen grains of *Bidens pilosa* were also described by Ikuse (1956) for the flora of Japan, Huang (1972) for the flora of Taiwan, Galvão *et al.* (2009) in their study on medicinal plants of Ilha Grande in Rio de Janeiro (Brazil), Sanchez & Lupo (2009) in their study on *Asteraceae* for apiculture in Argentina, and Melhem *et al.* (1979) in their study on pollen morphology of alergogens *Asteraceae* of Brazil. Melhem *et al.* (2003) studied the pollen grains of *Baccharis trimera* and *Calea pinnatifida* of Campos do Jordão in São Paulo State, Brazil. Felippe & Salgado-Labouriau (1964) studied pollen of the Heliantheae tribe of the Cerrado and described *Aspilia reflexa*. Souza *et al.* (2008) studied the pollen grains of nine other species of *Baccharis* from southern Brazil, and Gonçalves (1977) studied other *Aspilia* species from Brazil.

The present study aims to characterize the pollen morphology of twelve species within the *Asteraceae* family, Astereae and Heliantheae tribes, occurring in the region of Campos Gerais, Paraná State, Brazil, contributing to palynological, ecological, and paleoecological studies.

MATERIALS AND METHODS

The plant material used was obtained from flower buds removed from mounted specimens in the herbarium of the Universidade Estadual de Ponta Grossa (HUPG), Paraná State. 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 pollen was removed from at least three flower buds to standardize size variations (Salgado-Labouriau, 1973).

Pollen grains were acetolyzed following Erdtman (1952), with modifications proposed by Melhem *et al.* (2003) for light microscopy observation.

Measurements were conducted using an OLYMPUS OSM micrometer drum coupled to the microscope eyepiece of an OLYMPUS BX 50. Mean pollen dimension were derived from measurements of 25 pollen grains per sample. Measurements of morphological structures were based upon ten pollen grains per sample. Subsequently, a statistical analysis was performed by calculating the arithmetic mean (x), standard deviation (s_x), sample standard deviation (s), coefficient of variation (CV), with the significance interval at 95 % and variation range.

To better define the ornamentation aspect of pollen grains, we measured the base width and spine height yielding indices that define classeswhich vary between species as in Coutinho & Dinis (2007) for *Asteraceae* species and Barth *et al.* (2005) for Cucurbitaceae species. An index below 1.03 was obtained for spines with a base width proportional to the height; an index equal to 1.11 for spines slightly longer than wide at the base, and from 1.16 to 1.22 for spines longer than wide at the base, i.e., long spines. We also characterized the apex/tip appearance (straight or curved) as well as the columella and cavities at the spine bases, mainly under scanning electron microscopy (SEM), differing in number and height between the species.

The terminology used for the palynological descriptions followed Barth & Melhem (1988) based on updates by Punt *et al.* (2007) and Punt & Hoen (2009) for the *Asteraceae* pollen grains. 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 electronic microscopy, non-acetolysed pollen grains were spread on the surface of double-sided carbon tape wrapped on aluminum brackets ("stubs"), properly numbered. The samples were transferred to a vacuum pump and coated with a thin layer of gold palladium (ca. 150 angstroms thick) and were subsequently analyzed under Shimadzu SSX-550 model belonging to the Laboratory of Scanning Electron Microscopy, Department of Material Engineering at the Universidade Estadual de Ponta Grossa.

RESULTS

Following are the pollen morphology descriptions of the twelve tribes of two grassland species of *Asteraceae* occurring in the Campos Gerais, Paraná State, Brazil. The general palynological characteristics that each tribe shares are presented under the tribe name. They were omitted from specific descriptions to avoid duplication of information.

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

Astereae tribe

(Figs. 1-12)

Pollen grains of small size, isopolar, radial symmetry, oblate spheroidal or prolate spheroidal, amb subcircular, 3-zonocolporate, echinate.

Aperture: longiaperturate colpi, broad colpi at the equator or very narrow colpi, colpi with acute ends or obtuse ends, no central constriction. Endoaperture lalongate, regular type (convex sides, straight and identical ends), with acute ends, with or without central constriction.

Exine: two distinct layers (sexine and nexine) with cavea (indistinct or well delimited). 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 dome shaped (swollen at the base) and usually with acute and straight spine apex. Basis of echinae distinctly filled with at least 4 columellae. Sexine thicker than the nexine.

Aster Linnaeus

Aster haplopappus (Remy) Kuntze

(Figs. 1-3)

Prolate spheroidal pollen grains.

Aperture: narrow colpi with acute ends, endoaperture without central constriction.

Exine: well-delimited cavea, spines as long as wide at the base (index 0,94), spines dome shaped. Around eighteen spines in polar view. Both sexines 1 and 2 are thicker than the nexine.

Baccharis Linnaeus

Baccharis axillaris DC.

(Figs. 4-6)

Oblate spheroidal pollen grains.

Aperture: very narrow colpi with obtuse ends, endoaperture with central constriction.

Exine: well-delimited cavea, spines slightly longer than wide at the base (index 1,11), spines conical shaped. Around twelve spines in polar view. Both sexines 1 and 2 are twice as thick as the nexine.

Baccharis semiserrata DC.

(Figs. 7-9)

Oblate spheroidal pollen grains.

Aperture: narrow colpi with acute ends, endoaperture with central constriction.

Exine: well-delimited cavea, spines as long as wide at the base (index 0,97), spines conical shaped. Around twelve spines in polar view. Both sexines 1 and 2 are twice as thick as the nexine.

Baccharis trimera (Less.) DC.

(Figs.10-12)

Prolate spheroidal pollen grains.

Aperture: broad colpi at the equator with acute ends, endoaperture with central constriction.

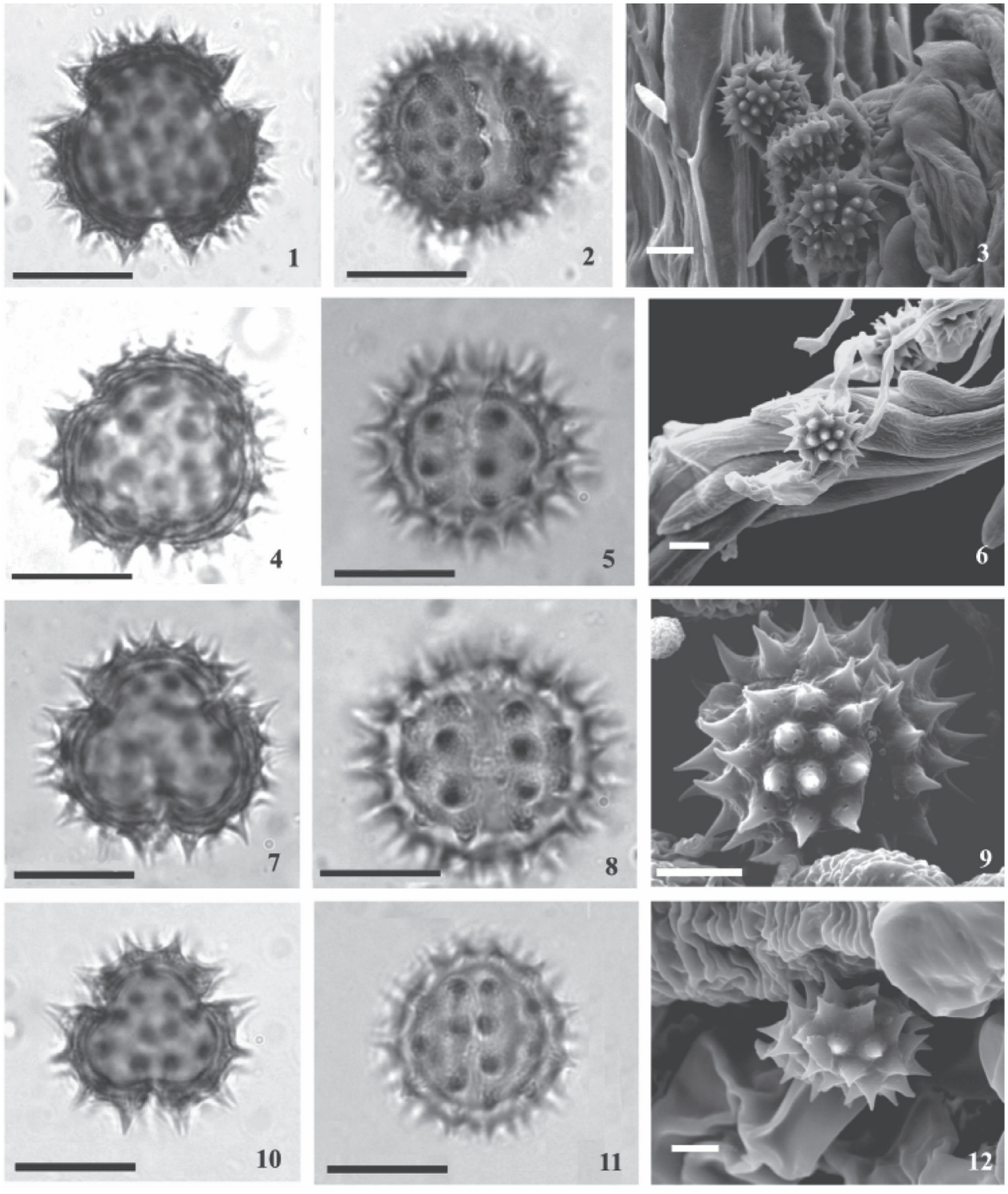
Exine: indistinct cavea, spines as long as wide at the base (index 1,03), spines conical shaped. Around twelve spines in polar view. Sexines 1 and 2 are thicker than tnexine.

Heliantheae tribe

(Figs. 13-15)

Pollen grains of small to medium size, isopolar, radial symmetry, oblate spheroidal or prolate spheroidal, amb subcircular, amb subtriangular or with square amb, 3-4-zonocolporate, echinate.

Aperture: medium size to longiaperturate colpi, broad colpi at the equator, slightly sunken colpi or very narrow colpi, colpi with acute or obtuse ends, central constriction. Endoaperture lalongate, presenting two forms: regular type (convex sides, straight and identical ends) or sigmoid type (double curved in opposite directions like an S), with acute ends, with or without central constriction.



Figs. 1-12. Pollen grain of *Asteraceae*, tribe Astereae. **1-3** *Aster haplopappus*. **1.** Polar view (LM). **2.** Equatorial view (LM). **3.** General aspect of three pollen grains (SEM). **4-6.** *Baccharis axillaris*. **4.** Polar view (LM). **5.** Equatorial view (LM). **6.** General aspect of one pollen grain (SEM). **7-9.** *Baccharis semiserrata*. **7.** Polar view (LM). **8.** Equatorial view (LM). **9.** General aspect of one pollen grain (SEM). **10-12.** *Baccharis trimera*. **10.** Polar view (LM). **11.** Equatorial view (LM). **12.** General aspect of one pollen grain (SEM). Bars: **Figs. 1-8, 10, 11** = 10 μm; **Figs. 9, 12** = 5 μm.

Exine: two distinct layers (sexine and nexine) with cavea (indistinct or well-delimited). Simplicolumellate exine with sexine 1 (columellae), sexine 2 (tectum) and sexine 3 (spines, sculpture elements). The structure of the spines is columellar and tegillum type, meaning they have a perforation at the base. The spinular columellae are longer than the inter-spinular columellae. 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. Basis of echinae distinctly filled with at least 4 columellae. The sexine is thicker than the nexine or it is the same thickness of the nexine.

Aspilia Thouars

Aspilia montevidensis (Spreng.) Kuntze

(Figs. 13-15)

Pollen grains of small size, oblate spheroidal, amb subcircular.

Aperture: 3-colporate, longiaperturate, very narrow colpi, with acute ends. Endoaperture of regular type, with or without central constriction.

Exine: indistinct cavea, spines longer than wide at the base (index 1.16), spines conical shaped, with acute and straight apex or with curved apex. Around fifteen spines in polar view. Sexine 1 and 2 are twice as thick as the nexine.

Aspilia reflexa Baker

(Figs.16-18)

Pollen grains of medium size, oblate spheroidal, amb subcircular.

Aperture: 3-colporate, longiaperturate, broad colpi at the equator, with acute ends. Endoaperture of regular type, with central constriction.

Exine: indistinct cavea, spines longer than wide at the base (index 1,22), spines slightly dome shaped, with acute and straight apex or with curved apex. Around fifteen spines in polar view. Sexines 1 and 2 are thicker than the nexine.

Bidens Linnaeus

Bidens alba (L.) DC.

(Figs.19-21)

Pollen grains of small size, prolate spheroidal, amb subcircular.

Aperture: 3-colporate, longiaperturate, very narrow colpi, with obtuse ends. Endoaperture of regular type (40 %) or sigmoid type (60 %), with central constriction (50 %) or without central constriction (50 %).

Exine: well delimited cavea, spines longer than wide at the base (index 0.96), spines conical shaped, with acute and straight apex. Around fifteen spines in polar view. Sexines 1 and 2 are as thick as the nexine.

Bidens pilosa L.

(Figs. 22-24)

Pollen grains of small size, prolate spheroidal, square amb or rarely amb subtriangular.

Aperture: (3)-4 colporate (90 % of 4-colporate), longiaperturate, very narrow colpi, with acute ends. Endoaperture of sigmoid type, with central constriction (50 %) or without central constriction (50 %).

Exine: well-delimited cavea, spines longer than wide at the base (index 0.97), spines conical shaped, with acute and straight apex or with curved spine apex. Around eighteen spines in polar view. Sexines 1 and 2 are as thick as the nexine.

Calea Linnaeus

Calea cymosa Less

(Figs. 25-27)

Pollen grains of medium size, oblate spheroidal, amb subcircular.

Aperture: 3-colporate, medium size aperture, slightly sunken colpi, with acute ends. Endoaperture of regular type (80 %) or sigmoid type (20 %), with central constriction (90 %) or without central constriction (10 %).

Exine: indistinct cavea, spines longer than wide at the base (index 0.98), spines dome shaped, with acute and straight apex. Around twelve spines in polar view. Sexines 1 and 2 are thicker than the nexine.

Calea hispida (DC) Baker

(Figs. 28-30)

Pollen grains of small size, prolate spheroidal, amb subcircular.

Aperture: 3-colporate, medium size aperture, slightly sunken colpi, with acute ends. Endoaperture of regular type (50 %) or sigmoid type (50 %), with central constriction (40 %) or without central constriction (60 %).

Exine: indistinct cavea, spines longer than wide at the base (index 1.01), spines conical shaped, with acute and straight apex. Around twelve spines in polar view. Sexines 1 and 2 are slightly thicker than nexine.

Calea parvifolia (DC) Baker

(Figs. 31-33)

Pollen grains of small to medium size, prolate spheroidal, amb subcircular.

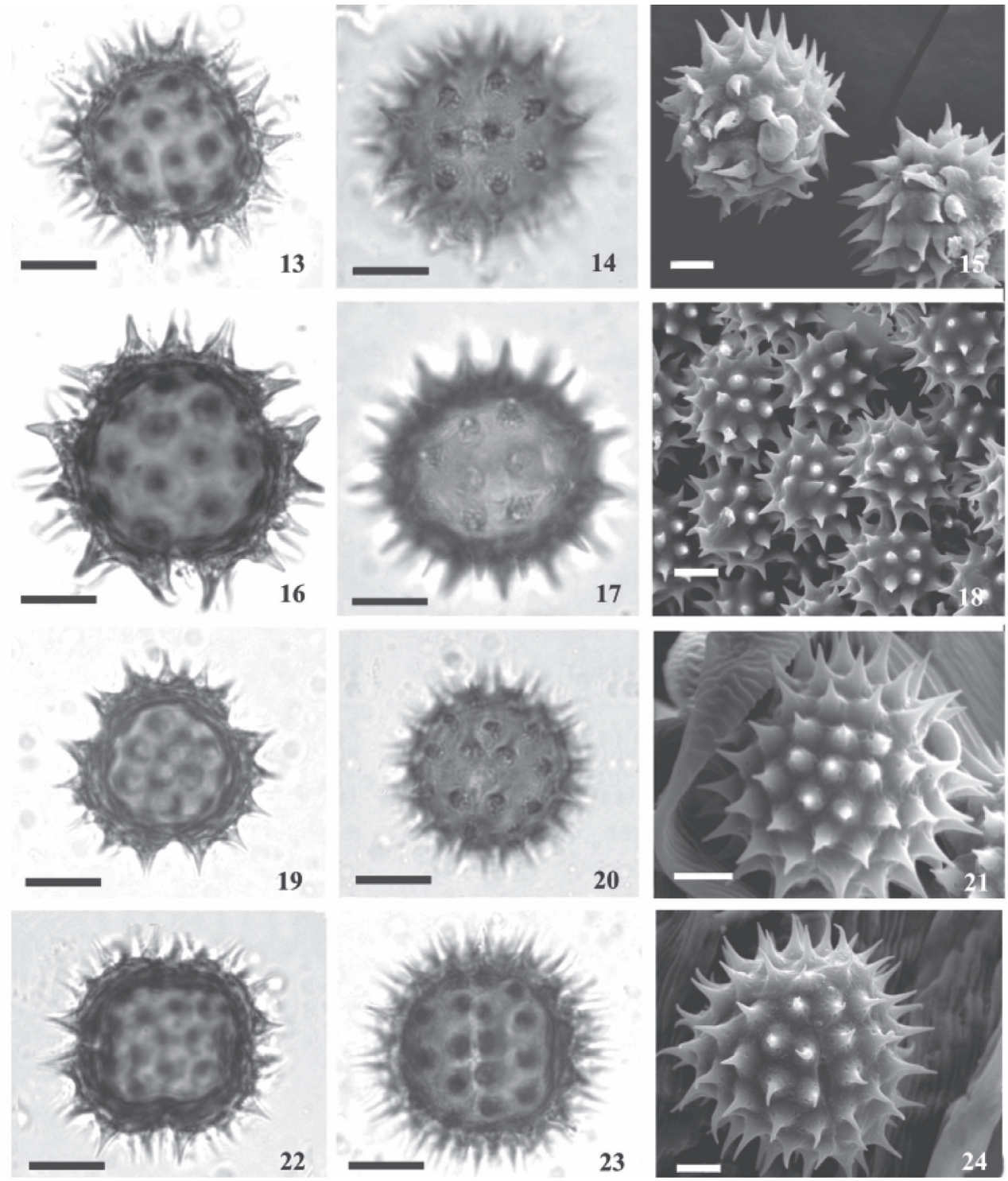
Aperture: 3-colporate, medium size aperture slightly sunken colpi, with acute ends. Endoaperture of regular type, with central constriction (80 %) or without central constriction (20 %).

Exine: well-delimited cavea, spines longer than wide at the base (index 0,94), spines dome shaped, with acute and straight apex. Around twelve spines in polar view. Sexines 1 and 2 are thicker than the nexine.

Calea pinnatifida (R. Br.) Less.

(Figs. 34-36)

Pollen grains of small size, oblate spheroidal, amb subcircular.



Figs.13-24. Pollen grains of *Asteraceae*, tribe Heliantheae. **13-15**. *Aspilia montevidensis*. **13.** Polar view (LM). **14.** Equatorial view (LM). **15.** General aspect of two pollen grains (SEM). **16-18.** *Aspilia reflexa*. **16.** Polar view (LM). **17.** Equatorial view (LM). **18.** General aspect of several pollen grains (SEM). **19-21.** *Bidens alba*. **19.** Polar view (LM). **20.** Equatorial view (LM). **21.** General aspect of one pollen grain (SEM). **22-24.** *Bidens pilosa*. **22.** Polar view (LM). **23.** Equatorial view (LM). **24.** General aspect of one pollen grain (SEM). Bars: **Figs. 13, 14, 16-20, 22, 23** = 10 μm; **Figs. 15, 21, 24** = 5 μm.

Aperture: 3-colporate, medium size aperture, slightly sunken colpi, with acute ends. Endoaperture of regular type, with central constriction (30 %) or without central constriction (70 %).

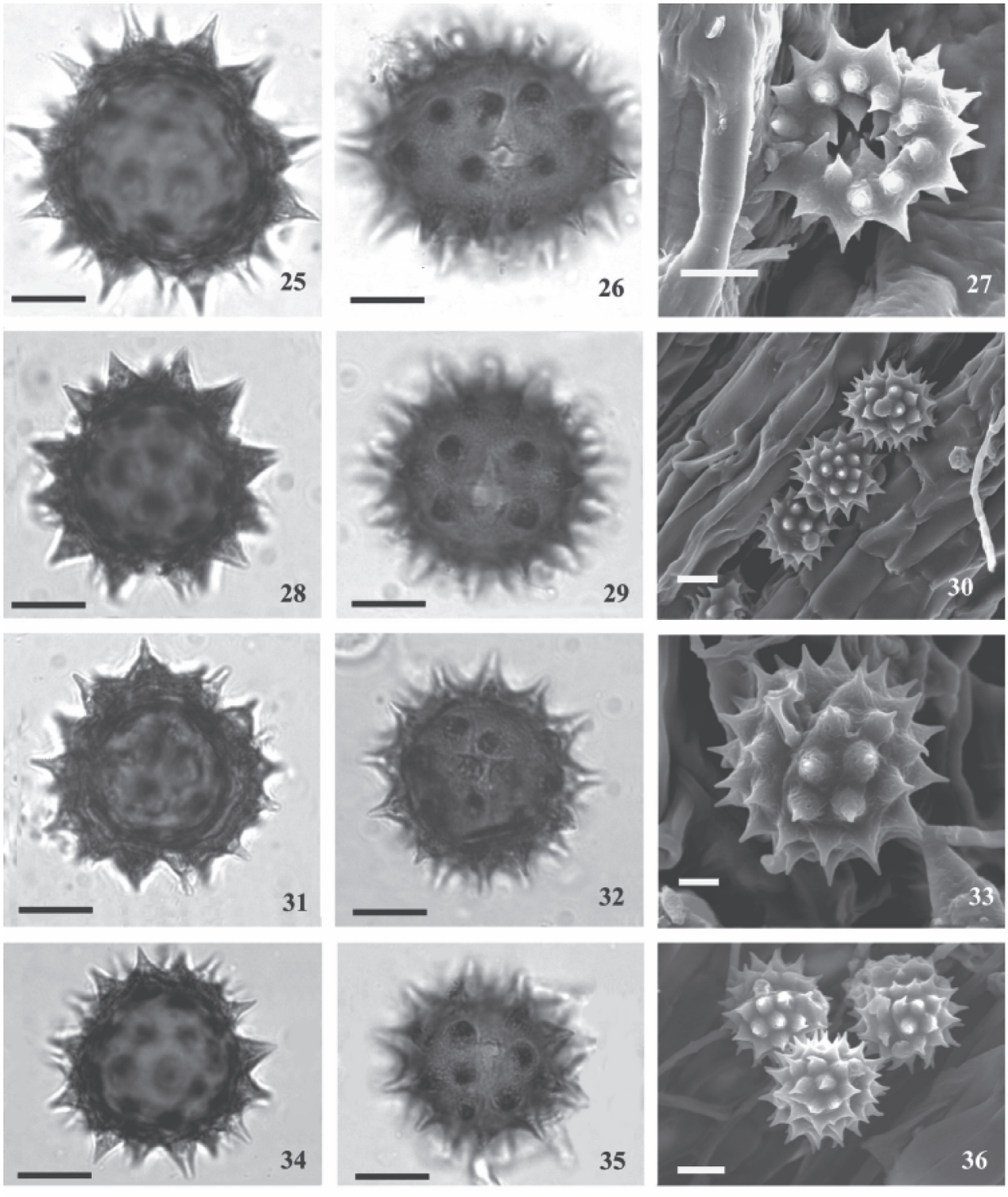
Exine: indistinct cavea, spines longer than wide at the base (index 0.94), spines slightly dome shaped, with acute and straight apex. Around fifteen spines in polar view. Sexines 1 and 2 are thicker than the nexine.

Table 1. General morphological characterization of pollen grains of the *Asteraceae* family in the Astereae and Heliantheae tribes occurring in the region of Campos Gerais, Paraná state, Brazil.

Species	Pollen size	Aperture	Colpus	Endoaperture
Aster haplopappus	small	3-zonocolporate	long size, narrow	regular type
Baccharis axillaris	small	3-zonocolporate	long size, very narrow	regular type
Baccharis semiserrata	small	3-zonocolporate	long size, narrow	regular type
Baccharis trimera	small	3-zonocolporate	long size, broad	regular type
Aspilia montevidensis	small	3-zonocolporate	long size, very narrow	regular type
Aspilia reflexa	medium	3-zonocolporate	long size, broad	regular type
Bidens alba	small	3-zonocolporate	long size, very narrow	regular/sigmoid types
Bidens pilosa	small	(3)4-zonocolporate	long size, very narrow	sigmoid type
Calea cymosa	medium	3-zonocolporate	medium size, narrow	regular/sigmoid types
Calea hispida	small	3-zonocolporate	medium size, narrow	regular/sigmoid types
Calea parvifolia	small-medium	3-zonocolporate	medium size, narrow	regular type
Calea pinnatifida	small	3-zonocolporate	medium size, narrow	regular type

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 *Asteraceae* family in the Astereae and Heliantheae tribes occurring in the region of Campos Gerais, Paraná State, Brazil. P – Polar axis; E – Equatorial axis; *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
Aspilia montevidensis	1.3	23.4±0.3	22.9-24.0	1.5	24.1±0.3	23.5-24.8	0.97	oblate-spheroidal	16.13	3.05	10.36	2.30
Aspilia reflexa	1.9	27.8±0.4	27.0-28.5	1.9	28.9±0.4	28.1-29.7	0.96	oblate-spheroidal	15.66	5.76	10.82	3.08
Aster haplopappus	1.2	14.6±0.2	14.2-15.0	2.5	14.3±0.4	13.5-15.2	1.02	prolate-spheroidal	14.48	3.38	11.80	2.45
Baccharis axillaris	1.3	17.0±0.4	16.5-17.5	1.2	18.2±0.2	17.7-18.7	0.93	oblate-spheroidal	12.66	2.08	7.75	1.99
Baccharis semiserrata	1.6	17.6±0.3	17.0-18.3	1.8	18.6±0.4	17.8-19.3	0.94	oblate-spheroidal	12.02	2.77	8.43	2.19
Baccharis trimera	1.0	15.6±0.2	15.2-16.0	1.0	15.2±0.2	14.8-15.6	1.02	prolate-spheroidal	11.11	1.97	8.12	1.58
Bidens alba	1.5	21.4±0.3	20.7-22.0	1.4	21.2±0.3	20.6-21.8	1.00	prolate-spheroidal	11.40	1.89	9.96	1.58
Bidens pilosa	8.3	22.6±1.7	19.2-26.0	1.9	24.2±0.4	23.4-25.0	1.00	prolate-spheroidal	11.00	2.90	9.60	1.35
Calea cymosa	1.2	26.3±0.2	25.8-26.8	2.2	28.3±0.4	27.4-29.2	0.92	oblate-spheroidal	17.34	4.39	11.32	3.01
Calea hispida	1.6	24.5±0.3	23.4-24.7	1.6	23.8±0.3	23.2-24.5	1.01	prolate-spheroidal	11.27	5.16	9.58	3.97
Calea parvifolia	1.5	24.6±0.3	25.8-27.0	1.4	25.5±0.3	24.9-26.0	1.03	prolate-spheroidal	16.29	3.09	11.57	2.87
Calea pinnatifida	1.0	21.8±0.2	21.4-22.2	1.5	22.9±0.3	22.2-23.5	0.95	oblate-spheroidal	13.24	3.65	10.08	2.22



Figs. 25-36. Pollen grains of *Asteraceae*, tribe Heliantheae. **25-27.** *Calea cymosa*. **25.** Polar view (LM). **26.** Equatorial view (LM). **27.** General aspect of several pollen grains (SEM). **28-30.** *Calea hispida*. **28.** Polar view (LM). **29.** Equatorial view (LM). **30.** General aspect of several pollen grains (SEM). **31-33.** *Calea parvifolia*. **31.** Polar view (LM). **32.** Equatorial view (LM). **33.** General aspect of one pollen grain (SEM). **34-36**. *Calea pinnatifida*. **34.** Polar view (LM). **35.** Equatorial view (LM). **36.** General aspect of three pollen grains (SEM). Bars: **Figs. 25-32, 34-36** = 10 µm; **Fig. 33** = 5 µm.

Table 3. Arithmetic mean of measurements (μm) of pollen grain exine layer thickness of the *Asteraceae* family in the Astereae and Heliantheae tribes occurring in the region of Campos Gerais, Paraná State, Brazil (n=10). N – nexine; Ca – caveae; S1 – sexine 1 (columellar layer); S2 – sexine 2 (internal tectum); Cs - number of spinular columellae; Hs - length of spine; Ws – width of spine basis; Hs/Ws – spine index; Fs – form of spine apex/tip (a = acute and straight spine apex; ac = acute and straight spine apex and curved spine apex).

Species	N	Ca	S1	S2	Cs	Hs	Ws	Hs/Ws	Fs
Aspilia montevidensis	0.86	very thin cavea, measured with Sex1	0.85	0.96	4 or 5	6.38	5.47	1.16	ac
Aspilia reflexa	0.61	very thin cavea, measured with Sex1	1.01	0.96	4 or 5	6.64	5.42	1.22	ac
Aster haplopappus	0.73	0.53	0.56	0.52	4	3.66	3.88	0.94	a
Baccharis axillaris	0.51	0.45	0.51	0.49	4 or 5	3.66	3.28	1.11	a
Baccharis semiserrata	0.46	0.41	0.47	0.52	4 or 5	3.30	3.38	0.97	a
Baccharis trimera	1.11	very thin cavea, measured with Sex1	0.77	0.47	4,5,6 or 7	3.08	2.98	1.03	a
Bidens alba	1.10	0.49	0.59	0.49	6	3.88	4.04	0.96	a
Bidens pilosa	1.19	0.60	0.61	0.59	4,5,6 or 7	3.38	3.48	0.97	ac
Calea cymosa	0.88	very thin cavea, measured with Sex1	0.90	1.00	4, 5 or 6	6.24	6.32	0.98	a
Calea hispida	1.17	very thin cavea, measured with Sex1	0.67	0.63	4 or 5	5.90	5.82	1.01	a
Calea parvifola	1.20	0.77	0.66	0.61	4 or 5	5.67	5.97	0.94	a
Calea pinnatifida	0.54	very thin cavea, measured with Sex1	1.26	1.01	4, 5 or 6	5.05	5.32	0.94	a

DISCUSSION

Several authors have reported that the exine structure of *Asteraceae* is valuable for understanding the evolution, systematics, and ecology of this group. In the Heliantheae tribe, the pollen grains show a structural pattern called a Heliantoide (Skvarla & Larson, 1965a, 1965b; Skvarla & Turner, 1966, 1969; Skvarla *et al*.,1977). This pattern is also found in the Astereae tribe, as well as in others, and is characterized by a cavea and internal foramina (perforation) in the sexine. The cavea presence and a simple sexine (i.e., composed of only one extract of columellae) are synapomorphies of the *Asteroidae* subfamily to which Astereae and Heliantheae tribes belong (Bolick, 1991; Cancelli *et al.,* 2010). However, the cavea is part of the harmomegathic system and therefore, largely varying, dependent on the imbibition of liquid (Blackmore *et al*., 1984), which prevents separation of the species into pollen types based only on its thickness.

As for the material presented here, the species *Aster haplopappus* had not yet been palynologically studied. Barth (1989) referred to pollen of the *Aster* genus as a similar type to Compositae S (*Senecio*) (Stix, 1960), which was corroborated by Cancelli (2008) for *A. squamatus* Hieron of Rio Grande do Sul State, Brazil. The morphological diagnosis presented here showed its correspondence to the *Senecio* pollen type.

The three species of *Baccharis* Linnaeus (*B. axillaris*, *B. semiserrata* and *B. trimera*) analyzed here had very similar pollen grains, differing only in shape, aperture dimensions, endoapertures, and exine thickness. We classified them to the *Baccharis* pollen type (Stix, 1960) according to the pollen morphology diagnosis of these species, which had already been attributed to other *Baccharis* species in southern Brazil by Souza *et al.* (2008) and to *B. trimera* by Cancelli (2008) and Cancelli *et al.* (2005). The Compositae B pollen type presented by Barth (1989) for *Baccharis* pollen grains found in Brazilian honeys does not fit any of our species, because the author described it as not showing cavea and circular endoaperture. The description of *B. trimera* pollen presented by Melhem *et al.* (2003), however, was similar to our material.

The two species of *Aspilia* treated in this study have been palynologically studied by Felippe & Salgado-Labouriau (1964), who studied *A. reflexa* of the Cerrado, and by Cancelli (2008) and Cancelli *et al*. (2005, 2010), who studied *A. montevidensis* in southern Brazil. The descriptions given by these authors were similar to ours, differing only in structure dimensions.

Gonçalves (1977) studied other *Aspilia* species from Brazil classifying most species as *Helianthus* type (Stix, 1960), however separating two species by the presence of a hollow tegillum and double layer of infratectum columellae, different features of *Helianthus* pollen types. The two species studied here differ among them in pollen grain size , colporus width, and the presence or absence of endoaperture constriction, but both fit the *Helianthus* pollen type (Stix, 1960), which has already been highlighted by Felippe & Salgado-Labouriau (1964), Cancelli (2008), and Cancelli *et al*. (2010).

The *Bidens* species (*B. alba* and *B. pilosa*) analyzed here differ in the number of apertures, spine apex type, spine number in polar view, and endoaperture types. *B. pilosa* was palynologically

studied by several authors while *B. alba* only by Cancelli (2008) and Cancelli *et al.* (2007, 2010). The prevalence of 4-colporate pollen type in the *Bidens pilosa* samples analyzed here corroborates with the results obtained by Ikuse (1956), Melhem *et al.* (1979), Sanchez & Lupo (2009) and Galvão *et al.* (2009). However, the greater representation of 3-colporate pollen grains in materials analyzed by other authors (Huang 1972, Cancelli *et al*., 2007, 2010), demonstrates the existence of morphological variability among different specimens. Despite having smaller or 4-colporate pollen grains, the *B. alba* and *B. pilosa* analyzed here fit the *Helianthus* pollen type (Stix, 1960), as well as the pollen type Compositae M (*Montanoa*) of Barth (1989).

The four species of *Calea* Linnaeus analyzed in this study have very similar characteristics. Only *C. pinnatifida* had been previously studied by Melhem *et al.* (2003) and Cancelli (2008), being classified as a *Helianthus* type (Stix, 1960), which we also confirmed here.

The studied pollen grains can provide subsidies for the reconstitution of paleovegetation through pollen stratigraphic studies. Thus, the quaternary period can be identified at a more specific level, compared with current pollen grain structures. Therefore, can be observed floristic succession and its relationship paleoclimatic and paleoecological along the geologic time.

According to Pla Jr. *et al.* (2006), paleoecological analysis is only possible due to the high resistance of the exine, which is composed by sporopollenin preserving the morphological structures of the grains, facilitating the reconstruction of paleoenvironments and paleovegetation.

CONCLUSIONS

After analyzing the morphological differences in pollen grains of twelve species of the grassland tribes Astereae and Heliantheae, we found that there is uniformity in most characteristics measured. Although the pollen grain sizes varied from small to medium and shape from oblate-spheroidal to prolate-spheroidal, there was a predominance of the 3-colporate pollen type, with a lalongate endoaperture, echinate exine with caveae, and a simple sexine. Exine variations in pollen grains of the two tribes occurred only in the dimension of the caveae and the tectum, in the form of the spine apex and the size was characterized according to the spine index, as well as endoaperture feature. The great morphopalynological similarity allowed characterizing the species of the Astereae and Heliantheae tribes in the region of Campos Gerais, Paraná State, as stenopalynous. However, details of pollen morphology allowed separating species into three groups of related pollen types *Baccharis*, *Helianthus*, and *Senecio*, previously defined in literature.

ACKNOWLEDGEMENTS

We wish to thank the HUPG Herbarium and Palynology Laboratory and Scanning Electron Microscopy at the Universidade Estadual de Ponta Grossa, Paraná State, Brazil. We thank the Institute of Botany, Environmental Department of São Paulo, Brazil, for allowing us to use the facilities and equipment of the Center for Research in Palynology. Many thanks to Dr. José Ivanildo de Souza from the Institute of Botany for lending the LEICA DM 2500 microscope. We thank the Araucaria Foundation for financial support (BEC-PROEX-UEPG) granted to CS. We give thanks to CNPq for the fellowship of "Produtividade em Pesquisa" (process number 301220/2009-3) to CFPL. We are grateful to Dr. H.D. Laughinghouse IV and R.M. Fischer for reviewing the English text.

REFERENCES

Angiosperm Phylogeny Group. 2009. An update of the phylogeny group classification for the orders and families of flowering plants: APG III**.** Botanical Journal of the Linnean Society, 161: 105-21.

Barth, O.M., Melhem, T.S. 1988. Glossário ilustrado de Palinologia. Editora Unicamp, Campinas.

75 p.

Barth, O.M. 1989. O pólen no mel brasileiro. Gráfica Luxor, Rio de Janeiro. 151 p.

Barth, O.M, Luz, C.F.P. & Gomes-Klein, V.L. 2005. Pollen morphology of Brazilian species of *Cayaponia* Silva Manso (Cucurbitaceae, Cucurbiteae). Grana, 44: 129-136.

Blackmore, S., Van Helvoort, H.A.M. & Punt, W. 1984. On the terminology, origins and functions of caveate pollen in Compositae. Review of Palaeobotany and Palynology, 43: 293-301.

Bolick, M. 1991. Pollen diameter, exine thickness, and ultrastructure type in the tribes of the Compositae. Compositae Newslett**,** 19: 17-21.

Bremer, K. & Gustafsson, M.H.G. 1997. East Gondwana of the sunflower alliance of families. Proceedings of the National Academy of Sciences of the United States of America, 94: 9188-9190.

Cancelli, R.R., Macedo, R.B., Guerreiro, C.T. & Bauermann, S.G. 2005. Diversidade Polínica em Asteraceae Martinov da Fazenda São Maximiniano, Guaíba, RS. Pesquisas. Série Botânica, 56: 209-228.

Cancelli, R.R., Evadt, A.C.P. & Bauermann, S.G. 2007. Contribuição à morfologia polínica da família Asteraceae Martinov. no Rio Grande do Sul - Parte I. Pesquisas. Série Botânica, 58: 347-374.

Cancelli, R.R. 2008. Palinologia de Asteraceae: morfologia polínica e suas implicações nos registros do Quaternário do Rio Grande do Sul 173 f. Dissertação de mestrado, Universidade Federal do Rio Grande do Sul, Porto Alegre.

Cancelli, R.R., Evaldt, A.C.P., Bauermann, S.G., Souza, P.A., Bordignon, S.A.L. & Matzenbacher, N.I. 2010. Catálogo palinológico de táxons da família *Asteraceae* Martinov, no Rio Grande do Sul, Brasil. Iheringia. Série Botânica, 65: 201-280.

Coutinho, A.P. & Dinis, A.M. 2007. A contribution to the ultrastructural knowledge of the pollen exine in subtribe Inulinae (Inuleae, Asteraceae). Plant Systematics and Evolution, 269: 159-170.

Dalazoana, K. 2010. Especialização dos campos nativos na escarpa devoniana do Parque Nacional dos Campos Gerais, Ponta Grossa, Paraná, Brasil 145f. Dissertação de mestrado, Universidade Estadual de Ponta Grossa, Ponta Grossa.

Erdtman, G. 1952. Pollen morphology and plant taxonomy – Angiosperms: an introduction to palynology. Alquimist & Wikseus, Stockholm. 553 p.

Felippe, G.M. & Salgado-Labouriau, M.L. 1964. Pollen Grains of the "Cerrado" - VI – Compositae – Tribus Heliantheae. Anais Acad. Brasil, 36: 85-101.

Galvão, M.N., Pereira, A.C.M., Gonçalves-Esteves, V. & Esteves, R.L. 2009. Palinologia de espécies de Asteraceae de utilidade medicinal para a comunidade da Vila Dois Rios, Ilha Grande, Angra dos Reis, RJ, Brasil. Acta Botânica Brasilica, 23: 247-258.

Gonçalves, V.B. 1977. Contribuição ao estudo palinológico da tribo Heliantheae (Compositae) II. Revista Brasileira de Biologia, 37: 399-403.

Huang, T.C. 1972. Pollen flora of Taiwan. National Taiwan University, Taiwan. 86 p.

Instituto Brasileiro de Geografia e Estatística (IBGE). 1992. Manual técnico da vegetação brasileira, Rio de Janeiro. 91 p.

Ikuse, M. 1956. Pollen grains of Japan. Hirokawa Publishing Co, Tokyo. 300 p.

Kozera, C., Kuuniyoshi, Y.S., Galvão, F. & Curcio, G.R. 2008. Composição florística de uma formação pioneira com influência fluvial em Balsa Nova, PR, BRASIL. Floresta, 39: 309-322.

Leite, P.F. & Klein, R.M. 1990. Vegetação. *In*: Fundação Instituto Brasileiro de Geografia e Estatística. Geografia do Brasil: região sul; Rio de Janeiro, Brasil. Instituto Brasileiro de Geografia e Estatística. v. 2. p. 113-150.

Matzenbacher, N.I. 2003. Diversidade florística dos campos sul-brasileiros. *In*: 54º Congresso Nacional de Botânica; Recife, Boletim de Resumos, p. 124-127.

Melhem, T.S., Silvestre, M.S.F. & Makino H. 1979. Grãos de pólen de plantas alergógenas: Compositae. Hoehnea**,** 8: 73-100.

Melhem, T.S., Cruz-Barros, M.A.V., Corrêa, A.M.S., Makino-Watanabe, H., Silvestre-Capelato, M.S. & Esteves, V.L.G. 2003. Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). Boletim do Instituto de Botânica, 16: 16-104.

Mondin, C.A. 2006. Riqueza genérica e dados biogeográficos das Asteráceas brasileiras. *In* Congresso Nacional de Botânica. Porto Alegre: 2006. p. 209-211.

Plá Junior, M.A., Corrêa, M.V.G., Cancelli, R. R., Macedo, R. B. & Bauermann, S. G. 2006. Grãos de pólen: usos e aplicações. Universidade Luterana do Brasil, Canoas. 24p.

Punt, W., Blackmore, S., Nilson, S. & Le Thomas, A. 2007. Glossary of pollen and spore terminology. Review of Paleobotany and Palynology, 143: 1-81.

Punt, W. & Hoen, P.P. 2009. The Northwest European Pollen Flora, 70: Asteraceae -Asteroideae. Review of Palaeobotany and Palynology, 157: 22-183.

Sanchez, A.C & Lupo, L.C. 2009. Asteraceae de interesse em Melissopalinologia. Bosque montano de Las Yungas (Jujuy - Argentina). Boletim da Sociedade Argentina de Botânica, 44 (1-2): 57 - 64.

Salgado-Labouriau, M.L. 1973. Contribuição à Palinologia dos Cerrados. Rio de Janeiro, Academia Brasileira de Ciências. 291 p.

Souza, V.C. & Lorenzi, H. 2005. Botânica Sistemática: guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APGII. Instituto Plantarum, Nova Odessa. 640 p.

Souza, P.C.T., Souza, F.C., Oliveira-Deble, A.O., Mendonça, C.B.F. & Gonçalves-Esteves, V. 2008. Estudo polínico de nove espécies da subtribo Baccharinae Less. (Astereae-Asteraceae) ocorrentes no Sul do Brasil. Balduinia, 12: 01-11.

Skvarla, J.J. & Larson, D.A. 1965a. An electron microscopic study of pollen morphology in the Compositae with special reference to the Ambrosiinae. Grana Palynol, 6: 210-269.

Skvarla, J.J. & Larson, D.A. 1965b. Interbedded exine components in some Compositae. Southw. Naturalist, 10: 65-68.

Skvarla, J.J. & Turner, B.L. 1966. Systematic implications from electron microscopic studies of Compositae pollen – a review. *In*: Annals of the Missouri Botanical Garden. 53: 220-257.

Skvarla, J.J. & Turner, B.L. 1969. Fine structure of Petrobinae (Compositae-Heliantheae) pollen walls. American Journal of Botany, 56: 418-419.

Skvarla, J.J., Turner, B.L., Patel, V.C. & Tomb, A.S. 1977. Pollen morphology in the Compositae and in morphologically related families. In: Heywood VH, Harborne JB, Turner BL (eds.) The biology and chemistry of Compositae; London, Academic Press, v.1. p. 141-265.

Stix, E. 1960. Pollenmorphologische Untersuchungen an Compositen. Grana Palynol, 2: 41-126.

W3 Tropicos. MOBOT. Missouri Botanical Garden. Disponível em: http://www.mobot.org. Acesso em: 20.01.2012.