



Ultrastructure and pollen morphology of Bromeliaceae species from the Atlantic Rainforest in Southeastern Brazil

VANESSA J.D. SILVA, ESTER M. RIBEIRO, ANDREA P. LUIZI-PONZO and ANA PAULA G. FARIA

Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, s/n, Campus Universitário, Bairro São Pedro, 36036-900 Juiz de Fora, MG, Brasil

Manuscript received on December 22, 2014; accepted for publication on January 30, 2015

ABSTRACT

Pollen grain morphology of Bromeliaceae species collected in areas of the Atlantic Rainforest of southeastern Brazil was studied. The following species were analyzed: *Aechmea bambusoides* L.B.Sm. & Reitz, *A. nudicaulis* (L.) Griseb., *A. ramosa* Mart. ex Schult.f., *Ananas bracteatus* (Lindl.) Schult.f., *Billbergia distachia* (Vell.) Mez, *B. euphemiae* E. Morren, *B. horrida* Regel, *B. zebrina* (Herb.) Lindl., *Portea petropolitana* (Wawra) Mez, *Pitcairnia flammea* Lindl., *Quesnelia indecora* Mez, *Tillandsia polystachia* (L.) L., *T. stricta* Sol., *T. gardneri* Lindl., *T. geminiflora* Brongn. and *Vriesea grandiflora* Leme. Light and scanning electron microscopy were used and the species were grouped into three pollen types, organized according to aperture characteristics: Type I – pantoporate pollen grains observed in *P. petropolitana*, Type II – 2-porate pollen grains, observed in the genera *Ananas*, *Aechmea* and *Quesnelia*, and Type III – 1-colpate pollen grains, observed in the genera *Billbergia*, *Pitcairnia*, *Tillandsia* and *Vriesea*. Pollen data led to the construction of an identification key. The results showed that the species analyzed can be distinguished using mainly aperture features and exine ornamentation, and that these characteristics may assist in taxonomic studies of the family.

Key words: epiphytes, morphology, pollen grains, taxonomy.

INTRODUCTION

Bromeliaceae Juss comprises nearly 3.172 species, distributed in 58 genera (Luther 2008). It is considered the fourth-richest Angiosperm family in terms of the number of species in the Atlantic Forest (Stehmann et al. 2009), and the southeastern of Brazil represents a center of endemism for many of its taxa.

The traditional infrafamilial taxonomy of Bromeliaceae (Smith and Downs 1974, 1977, 1979) relies on the characteristics of the position of the ovary, as well as types of habit, fruits and seeds in order to recognize the subfamilies Bromelioideae, Pitcairnoideae and Tillandsioideae. However, phylogenetic studies based on morphological and molecular data only consider Bromelioideae and Tillandsioideae to be monophyletic, while Pitcairnoideae is considered to be polyphyletic (Terry et al. 1997, Givnish et al. 2007, 2011).

Taxonomic disagreements regarding Bromeliaceae at the generic and infrageneric levels are widely spread among the three subfamilies and are worsened by the limited knowledge of many morphological

Correspondence to: Ana Paula Gelli de Faria
E-mail: ana.gelli@ufjf.edu.br

structures (Faria et al. 2004). The floral morphology is usually poorly preserved in herbarium collections, and therefore, it is also poorly studied. This limits the utilization of many characteristics that could potentially be diagnostic. Benzing (1994) and Schulte and Zizka (2008) mentioned the importance and the necessity of more studies focusing on the detailed analysis of underemployed characteristics, such as the morphological variation of stigmas and petal appendages, as well as the pollen morphology. In this context, pollen morphology has provided important information for the taxonomy of Bromeliaceae, aiding in a better definition of the generic and specific delimitation (Moreira et al. 2005).

Mez (1891-1894, 1896, 1934-1935) made the first attempt to apply pollen morphology to the systematics of Bromeliaceae. Based on the types of aperture, the author classified the subfamilies Pitcairnoideae and Tillandsioideae as stenopalynous, due to having pollen grains that were predominantly monocolpate, while Bromelioideae was considered euripalynous and had different types of aperture (including the tribes Poratae and Sulcatae), in addition to inaperturate grains (tribe Integrae). Other important palynological studies concerning Bromeliaceae include the studies by Ehler and Schill (1973), Erdtman and Praglowski (1974), Wanderley and Melhem (1991), Halbritter (1992), Sousa et al. (1997), Melhem et al. (2003), Souza et al. (2004) and Moreira et al. (2005). However, knowledge regarding the palynology of Bromeliaceae is still scarce, especially when considering the species' richness within the family.

The present work describes the pollen morphology of bromeliad species from the Atlantic Rainforest in southeastern Brazil, with the aim to provide additional morphological characteristics to support the taxonomy and systematic of the family.

MATERIALS AND METHODS

Pollen grains were obtained from herbarium specimens deposited in the CESJ herbarium (acronyms follow Index Herbariorum; Thiers 2014 continuously updated). The species analyzed were gathered from portions of the Atlantic Rainforest in the states of Minas Gerais and Espírito Santo, southeast of Brazil. Whenever possible, more than one exsiccate was examined, in order to compare all possible variations within a single species. The best quality material was indicated as "standard" (marked with an asterisk in the examined material list below), and the other materials from the same species were referred to as "comparison" materials.

The examined material included: *Aechmea bambusoides* L.B.Sm. & Reitz - **BRAZIL. Minas Gerais:** Juiz de Fora, Campus UFJF, IX/2010, S. G. Furtado, L. Menini Neto and A. P. G. Faria 144 (CESJ 60640*). *Aechmea nudicaulis* (L.) Griseb. - **BRAZIL. Minas Gerais:** Olaria, Serra do Cruz, Mata nebular, 16/II/2011, F. E. Alves and B. R. M. Bastos 36 (CESJ 58524). **BRAZIL. Espírito Santo:** Cachoeiro de Itapemirim, 15/XII/2012, F. C. Favoreto 121 (CESJ 59876*). *Aechmea ramosa* Mart. ex Schult.f. - **BRAZIL. Espírito Santo:** Cachoeiro de Itapemirim, Burarama, Mata do Açude, 01/XI/2007, F. C. Favoreto and M. M. Redling 16 (CESJ 55655*). *Ananas bracteatus* (Lindl.) Schult.f. - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, 26/IX/2013, V. J. Silva 02 (CESJ 63718); Juiz de Fora, Mata do Krambeck, IX/2011, L. C. D. Dias 21 (CESJ 58434*). *Billbergia distachia* (Vell.) Mez - **BRAZIL. Minas Gerais:** Alagoa, Parque Estadual da Serra do Papagaio, 12/VI/2011, F. R. Salimena 3346 et al. (CESJ 58014*); Juiz de Fora, 15/VI/2006, T. Mota et al. 16 (CESJ 47598). *Billbergia euphemiae* E. Morren - **BRAZIL. Minas Gerais:** Juiz de Fora, IX/2010, M. G. C. Nogueira 02 (CESJ 59184*). *Billbergia horrida* Regel - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, 22/X/2010, L. C. D. Dias and A. P. G. Faria 06 (CESJ 56931); Juiz de Fora, Mata do Krambeck, 12/IX/2009, C. O. Silva 16 (CESJ 54831*). *Billbergia zebrina* (Herb.) Lindl. - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, 04/IV/2013, V. J. D. Silva 01 (CESJ 62710); Juiz de Fora, Mata do Krambeck, V/2011, L. C. D. Dias (CESJ 58035*). *Pitcairnia flammea* Lindl.

- **BRAZIL. Minas Gerais:** Descoberto, 09/IV/2010, C.O. Rezende et al. 879 (CESJ 56016*); Descoberto, 03/III/2002, R. C. Forzza, V. R. Scalon and A. V. Lopes 2086 (CESJ 36350). *Portea petropolitana* (Wawra) Mez - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, 22/X/2010, L. C. D. Dias and A. P. G. Faria 08 (CESJ 56920); Juiz de Fora, Mata do Krambeck, 21/IX/2010, M. C. Tagliati 01 (56934*). *Quesnelia indecora* Mez - **BRAZIL. Minas Gerais:** Chácara, 16/VII/2011, D. E. F. Barbosa et al. 8 (CESJ 60683); Juiz de Fora, VIII/2012, A. P. G. de Faria 237 (CESJ 60802*). *Tillandsia gardneri* Lindl. - **BRAZIL. Minas Gerais:** Barroso, Mata do Baú, 30/IV/2001, L. C. S. Assis 118 (CESJ 34255*). **BRAZIL. Espírito Santo:** Cachoeiro de Itapemirim, 20/VII/2012, F. C. Favoreto et al. 183 (CESJ 60385). *Tillandsia geminiflora* Brongn. - **BRAZIL. Minas Gerais:** Olaria, Serra do Cruz, Mata nebulosa, 01/I/2011, F. E. Alves et al. 213 (CESJ 60879*); Juiz de Fora, 23/XII/2004, V. Shuchter 24 (CESJ 42819). *Tillandsia polystachia* (L.) L. - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, V. J. Silva 3, 25/X/2013 (CESJ 63719); Juiz de Fora, Mata do Krambeck, 22/X/2010, L. C. D. Dias and A. P. G. de Faria 11 (CESJ 56927*). *Tillandsia stricta* Sol. - **BRAZIL. Minas Gerais:** Chácara, 06/X/2012, D. E. F. Barbosa 139 et al. (CESJ 60887*); Caldas, 14/IX/2008, T. M. Machado et al. 86 (CESJ 52078). *Vriesea grandiflora* Leme - **BRAZIL. Minas Gerais:** Juiz de Fora, Mata do Krambeck, 26/IX/2012, A. P. G. de Faria 238 (CESJ 60803*).

Pollen grains were prepared using acetolysis (Erdtman 1960) and in addition to the Wodehouse (1935) modified methodology. Fragile pollen grains were also prepared by lactic acetolysis (ACLAC 40%) following Raynal and Raynal's (1971) protocol. They were studied under light microscopy (LM). For scanning electron microscopy (SEM), the untreated pollen grains were mounted on a stub with adhesive carbon double face tape and sputtered with a 20 nm thick gold coat. Three slides containing acetolyzed pollen grains were prepared for each specimen. Whenever possible, the largest diameter of 25 pollen grains on polar view of each specimen was measured, as well as the equatorial and polar diameters on equatorial view; a micrometer ocular and a BX-41 Olympus microscope was used. To evaluate the measurements, Microsoft Excel software was applied in order to calculate the arithmetic mean (\bar{X}), size range ($X_{\min} - X_{\max}$), standard error (Sx), standard deviation (S), variability (V), and confidence interval. If it was not possible to take 25 measurements for a specimen, only the arithmetic mean was calculated.

The photomicrographs were taken using a Canon camera adapted to a Zeiss Primo-Star light microscope. The terminology follows Punt et al. (2007), and Barth and Melhem (1988).

RESULTS

The pollen analyses, especially concerning apertural characterization, enabled the description of three pollen grain types:

Type I - pantoporate (Figs. 1-3)

Pollen grains heterogeneous in size; subcircular; apolar; 7-(9)-pantoporate, pores circular, operculate, sparsely distributed over the surface; semitectate, reticulate; lumina rounded and irregular in size; muri narrow; sexine thicker than nexine.

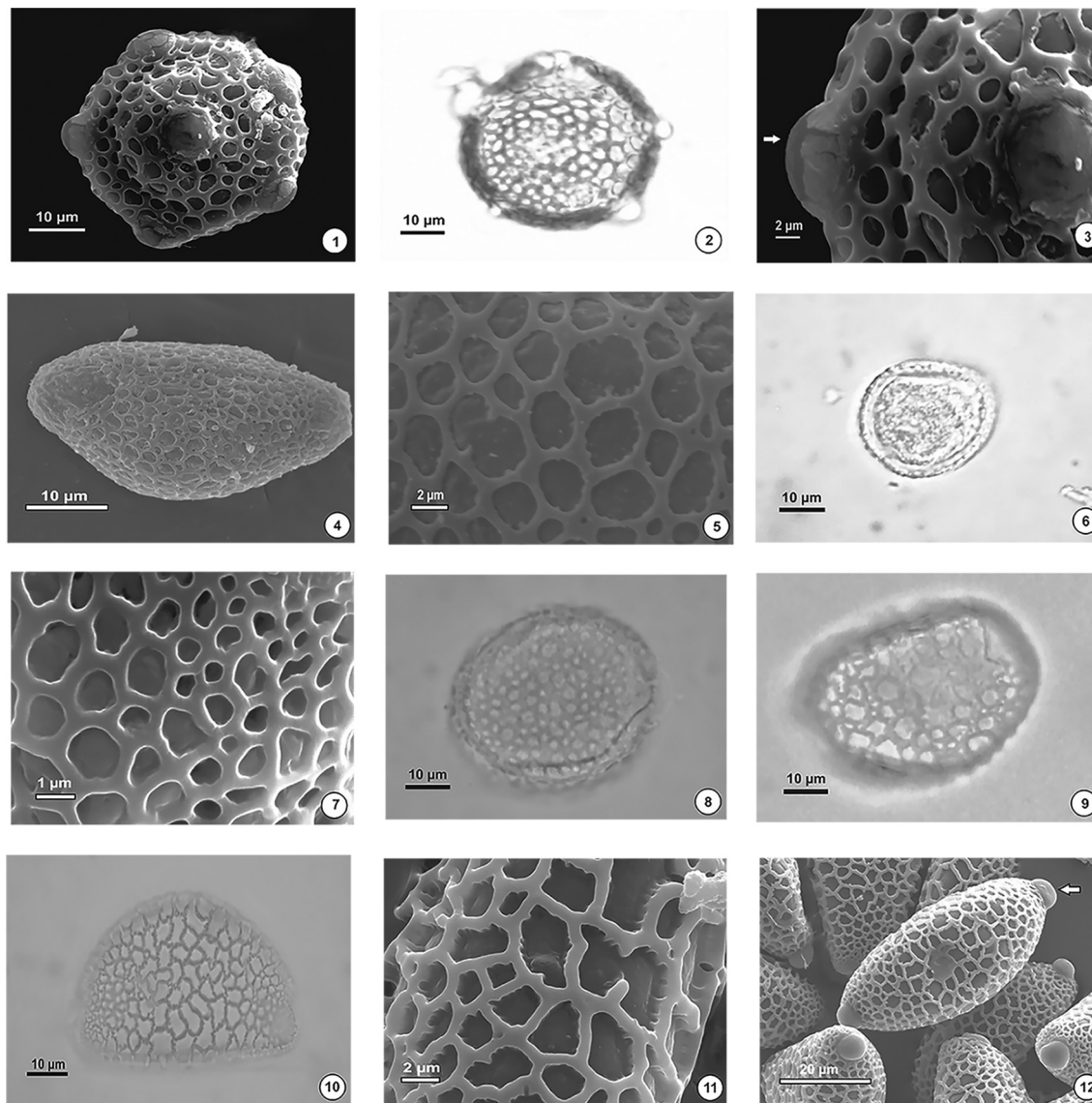
Species included: *Portea petropolitana* (Wawra) Mez.

Type II - 2-porate (Figs. 4-12)

Pollen grains homogeneous in size; elliptical to ovate; heteropolar; 2-porate, pores circular, with or without operculum; semitectate, reticulate; lumina rounded and variable in shape; muri narrow; sexine thicker than nexine.

Species included: *Aechmea bambusoides* L.B.Sm. & Reitz, *A. nudicaulis* (L.) Griseb., *A. ramosa* Mart. ex Schult.f., *Ananas bracteatus* (Lindl.) Schult.f., *Quesnelia indecora* Mez.

The pollen grains of *A. ramosa* with two or three apertures, are predominantly 2-porate (85%). Under SEM, the pollen of *A. bambusoides* shows a small reticulate pattern with granulate lumina. In *A. bracteatus*, the sexine is slightly thicker than the nexine. The pollen grains of *Q. indecora* are elliptical, with large, circular pores, the exine is heterobrochate, the lumina are smooth, polygonal. Under SEM, a decrease in the reticulum size near the apertures is observed, as well as the presence of an operculum.



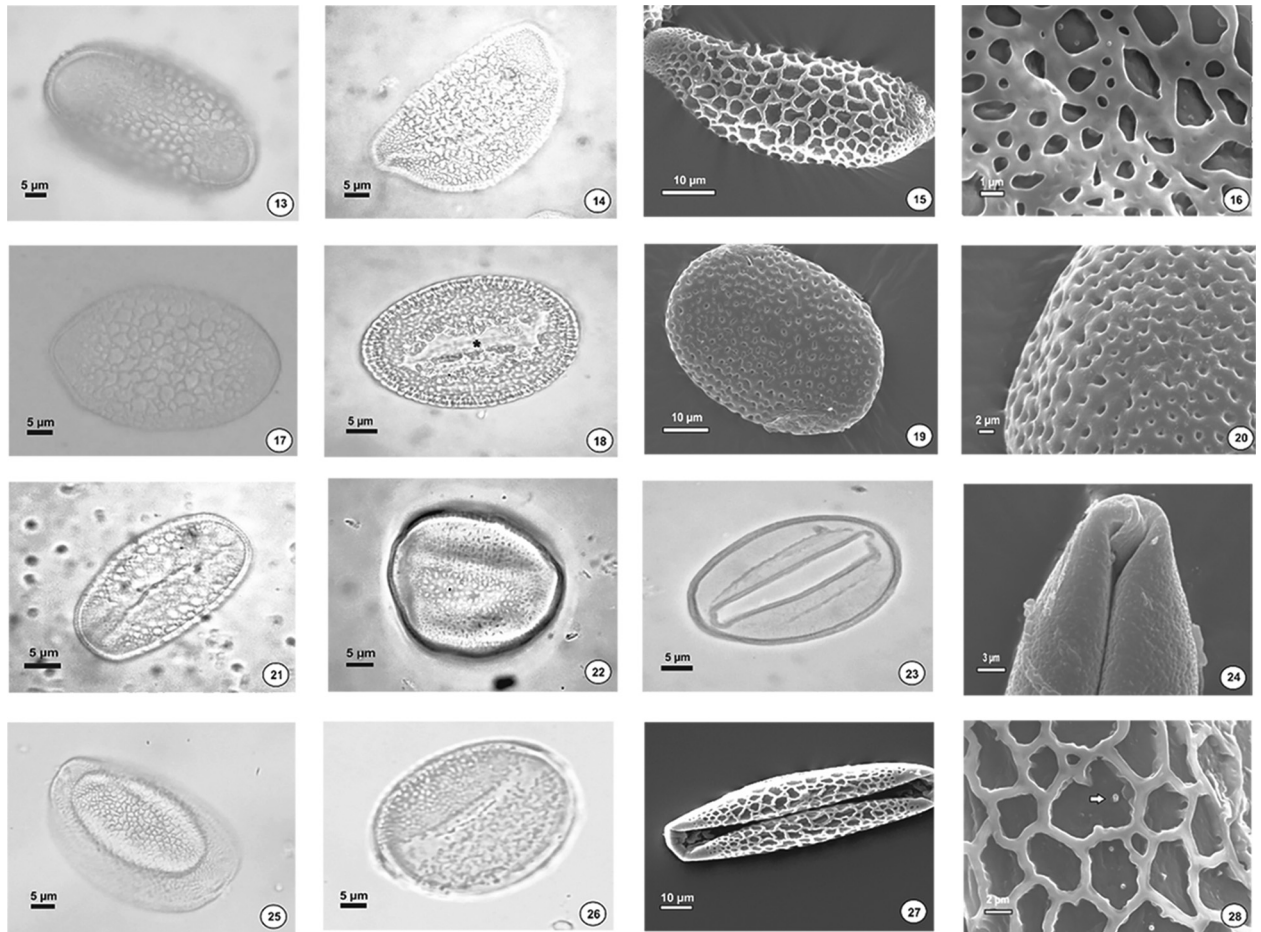
Figures 1-12: Pollen grains of Bromeliaceae. 1-3. *Portea petropolitana* (Wawra) Mez. 1. Apolar view (SEM). 2. Surface detail. 3. Operculum (arrow) (SEM). 4-5. *Aechmea bambusoides* L.B.Sm. & Reitz. 4. Overview (SEM). 5. Surface pattern with the presence of granules (SEM). 6-7. *Aechmea nudicaulis* (L.) Griseb. 6. Polar view. 7. Detail of reticulate ornamentation (SEM). 8. *Aechmea ramosa* Mart. ex Schult.f.: overview. 9. *Ananas bracteatus* (Lindl.) Schult.f.: overview, showing the ornamentation. 10-12. *Quesnelia indecora* Mez. 10. Equatorial view. 11. Detail of the exine surface. 12. Operculum detail (arrow) (SEM). See the colors in the online version.

Type III - 1-colpate (Figs. 13-28)

Pollen grains homogeneous in size; elliptical to ovate; heteropolar; 1-colpate, colpus long and irregular in shape; tectate, reticulate, heterobrochate to microreticulate; the lumina are variable in shape; muri large to narrow; sexine thicker than nexine.

Species included: *Billbergia distachia* (Vell.) Mez., *B. euphemiae* E. Morren, *B. horrida* Regel, *B. zebrina* (Herb.) Lindl., *Pitcairnia flammea* Lindl., *Tillandsia gardneri* Lindl., *T. geminiflora* Brong., *T. polystachia* (L.) L., *T. stricta* Sol., *Vriesea grandiflora* Leme.

The pollen grains of *B. zebrina* show wide colpus, with an ornamented margin. Under SEM, an undulate tectum is observed, mixed with a small reticulum. *Tillandsia geminiflora* has folded apertures with fused ends. *Vriesea grandiflora* has polygonal lumina that are variable in size, containing pila. Under SEM, the reticulum decreases towards the poles and the colpus exhibit a median constriction.



Figures 13-28: Pollen grains of Bromeliaceae. 13. *Billbergia distachia* (Vell.) Mez.: polar view. 14-15. *Billbergia euphemiae* E. Morren. 14. Equatorial view. 15. Detail of heterobrochate ornamentation (SEM). 16-17. *Billbergia horrida* Regel. 16. Surface pattern (SEM). 17. Polar view. 18-20. *Billbergia zebrina* (Herb.) Lindl. 18. Colpus detail (asterisk). 19. Overview (SEM). 20. Surface pattern (SEM). 21. *Pitcairnia flammea* Lindl.: distal polar view. 22. *Tillandsia gardneri* Lindl.: overview. 23-24. *Tillandsia geminiflora* Brongn. 23. Overview. 24. Polar view, showing aperture (SEM). 25. *Tillandsia polystachia* (L.) L.: distal polar view. 26. *Tillandsia stricta* Sol.: overview. 27-28. *Vriesea grandiflora* Leme. 27. Distal polar view, with invagination of the aperture (SEM). 28. Surface pattern (arrow = pila) (SEM). See the colors in the online version.

The pollen morphometric data are presented in Tables I and II. *Billbergia distachia* and *V. grandiflora* have fragile pollen grains that do not resist to acetolysis. The largest diameters (polar view) were observed in *B. zebrina* pollen grains (76.44 μm), while the smallest ones were observed in *P. petropolitana* (23.77 μm). However, the majority of pollen grains of the species studied presented about 45.24–75.40 μm .

TABLE I
Morphometric data of the largest diameter (in polar view) of pollen grains of the studied taxa. Arithmetic mean (\bar{X}), size range (X_{\min} – X_{\max}), standard error (S_x), standard deviation (S), variability (V) and confidence interval (IC) of 95% ($n = 25$, except for the specimens with *, which $n < 25$). **It was not possible to obtain the measures.

Species	Largest diameter				
	X_{\min} – X_{\max} (μm)	$\bar{X} \pm S_x$ (μm)	S	V (%)	IC (95 %)
<i>A. bambusoides</i>					
*CESJ 60640	49.40-62.40	56.94 \pm 0.95	----	----	----
<i>A. nudicaulis</i>					
CESJ 59876	52.00-78.00	61.67 \pm 1.62	8.14	13.18	58.31-65.03
CESJ 55680	39.00-52.00	45.24 \pm 0.98	4.92	2.16	43.20-47.47
<i>A. ramosa</i>					
CESJ 55655	57.20-85.80	74.88 \pm 1.43	7.19	1.90	71.90-77.85
<i>A. bracteatus</i>					
CESJ 58434	44.20-57.20	53.04 \pm 0.52	2.60	4.90	49.57-54.11
*CESJ 63718	46.80-57.20	52.65 \pm 0.61	----	----	----
<i>B. distachia</i>					
**CESJ 58014	----	----	----	----	----
<i>B. euphemiae</i>					
CESJ 59184	62.40-85.80	74.67 \pm 1.14	5.75	7.68	72.30-77.04
<i>B. horrida</i>					
*CESJ 54831	54.60-80.60	71.28 \pm 1.78	----	----	----
<i>B. zebrina</i>					
CESJ 58035	72.80-80.60	76.44 \pm 0.49	2.48	3.26	75.41-77.46
<i>P. flammea</i>					
*CESJ 56016	54.60-65.00	61.39 \pm 0.84	----	----	----
<i>P. petropolitana</i>					
CESJ 56934	43.40-56.42	49.21 \pm 0.88			47.38-51.04
CESJ 56920	17.55-28.08	23.77 \pm 0.42	4.412.07	8.968.70	22.91-24.63
<i>Q. indecora</i>					
CESJ 60802	65.00-85.80	74.36 \pm 0.96	4.80	1.29	72.38-76.34
<i>T. gardneri</i>					
CESJ 34255	54.60-70.20	61.25 \pm 0.88	4.44	7.25	59.42-63.09
<i>T. geminiflora</i>					
CESJ 60879	65.00-83.20	75.40 \pm 1.13	5.61	7.44	73.08-77.71
<i>T. polystachia</i>					
CESJ 56927	65.1-80.29	73.29 \pm 1.60	4.82	6.57	80.29-88.48
<i>T. stricta</i>					
CESJ 60887	49.40-65.00	55.01 \pm 0.69	3.49	6.35	53.56-56.46
CESJ 52078	52.00-80.60	65.31 \pm 1.44	7.21	11.04	62.33-68.28
<i>V. grandiflora</i>					
**CESJ 60803	----	----	----	----	----

TABLE II
 Arithmetic mean (\bar{X}), size range (X_{\min} - X_{\max}) and standard error (S_x), in μm , the equatorial and polar diameters (on equatorial view) of pollen grains of the studied taxa ($n = 10$). *It was not possible to obtain the measures.

Species	Equatorial diameter		Polar diameter	
	(X_{\min} - X_{\max})	$\bar{X} \pm s_x$	(X_{\min} - X_{\max})	$\bar{X} \pm s_x$
<i>A. bambusoides</i>				
CESJ 60640	52.00-65.00	56.94 \pm 1.36	31.20-44.20	37.96 \pm 1.46
<i>A. nudicaulis</i>				
CESJ 59876	41.60-52.00	45.24 \pm 1.23	26.00-39.00	32.24 \pm 1.35
<i>A. ramosa</i>				
CESJ 55655	62.40-83.20	75.02 \pm 2.39	36.40-65.00	48.10 \pm 3.33
<i>A. bracteatus</i>				
CESJ 58434	44.20-57.20	54.08 \pm 1.27	31.20-49.40	38.40 \pm 1.85
CESJ 63718	49.40-52.00	51.74 \pm 0.26	31.20-46.80	37.96 \pm 1.35
<i>B. distachia</i>				
CESJ 58014	65.00-75.40	71.50 \pm 1.35	41.60-70.20	49.66 \pm 3.36
<i>B. euphemiae</i>				
CESJ 59184	65.00-80.60	74.36 \pm 1.40	36.40-52.00	43.42 \pm 1.55
<i>B. horrida</i>				
CESJ 54831	59.80-78.00	73.06 \pm 1.96	31.20-59.80	45.50 \pm 3.31
<i>B. zebrina</i>				
CESJ 58035	70.20-80.60	76.70 \pm 1.11	36.40-67.60	53.82 \pm 2.95
<i>P. flammea</i>				
CESJ 56016	46.80-65.00	61.62 \pm 1.74	28.60-46.80	37.70 \pm 1.51
<i>Q. indecora</i>				
CESJ 60802	67.60-80.60	74.88 \pm 1.14	39.00-44.20	41.08 \pm 0.52
<i>T. gardneri</i>				
CESJ 34255	57.20-70.20	62.14 \pm 1.12	31.20-52.00	38.40 \pm 1.85
<i>T. geminiflora</i>				
CESJ 60879	59.80-83.20	75.40 \pm 2.71	41.60-49.40	44.98 \pm 0.95
<i>T. stricta</i>				
CESJ 60887	52.00-59.80	56.16 \pm 0.88	31.20-46.80	36.14 \pm 1.36
CESJ 52078	57.20-80.60	65.52 \pm 2.78	31.20-52.00	41.60 \pm 2.45
<i>T. polystachia</i>				
CESJ 56927	54.25-75.95	66.18 \pm 1.59	41.23-56.42	47.49 \pm 1.59
<i>V. grandiflora</i>				
*CESJ 60803	----	----	----	----

Pollen key for the identification of the Bromeliaceae species studied

1. Pollen grains pantoporate.....*Portea petropolitana*
1. Pollen grains with other types of aperture
 2. Pollen grains porate
 3. Pollen grains with 2 or 3 pores*Aechmea ramosa*

3. Pollen grains always have 2 pores

4. Operculate pores.....*Quesnelia indecora*

4. Non-operculate pores

5. Heterobrochate exine; pollen grains

elliptical..... *Aechmea nudicaulis*

5. Reticulate exine; pollen grains ovate

6. Lumina large, microreticulate*Ananas bracteatus*

6. Lumina small, no

microreticulate.....*Aechmea bambusoides*

2. Pollen grains 1-colpate

7. Pollen grains psilate-perforate or reticulate

8. Exine psilate-perforate.....*Tillandsia geminiflora*8. Exine reticulate*Pitcairnia flammea*

7. Pollen grains microreticulate or heterobrochate

9. Exine microreticulate

10. Colpus irregular shaped, margin thick,
ornamented.....*Billbergia zebrina*10. Colpus without thick
margin.....*Tillandsia gardneri, Tillandsia stricta*

9. Exine heterobrochate

11. Lumina with few pila.....*Vriesea grandiflora*

11. Lumina smooth

12. Muri wide.....*Billbergia horrida*12. Muri narrow.....*Billbergia distachia,*
*Billbergia euphemiae, Tillandsia polystachia***DISCUSSION**

This work describes, for the first time, the pollen morphology of *Aechmea bambusoides* and *Vriesea grandiflora*. In *Aechmea*, the largest and most morphologically diverse genus of Bromelioideae, the pollen grains embrace all types of aperture observed within the family (Faria et al. 2004). The species of *Aechmea* analyzed in this work presented porate grains and could be separated based on the number of apertures (*A. bambusoides* and *A. nudicaulis*, with two pores, and *A. ramosa*, with two to three pores). Regarding ornamentation, *A. bambusoides* differs by the reticulate exine with smaller granular lumina, while the other

Aechmea species presented heterobrochate exine with higher lumina. Sousa et al. (1997) also described differences in the type of exine (semi-tectate, tectate-perforate, rugulate, reticulate and microreticulate) for species of *Aechmea* from northeastern Brazil, strengthening the morphological diversity of the pollen grains within this genus. Wanderley and Melhem (1991) analyzed 12 species of the *Vriesea* genus and found morphological characteristics similar to those found in *V. grandiflora*, such as grains 1-colpate, heterobrochate exine and lumina with few pila.

Portea petropolitana was distinguished from all other species by presenting pantoporate pollen grains. Smith and Dows (1979) considered this characteristic as diagnostic for the genus, and Halbritter (1992) confirmed this apertural pattern for species of *Portea*. Pantoporate grains, however, are also observed in some *Aechmea* species native to northeastern Brazil, which is related to the *Gravisia* complex (Read and Luther 1991). Mez (1891-1894, 1896, 1934-1935) first suggested the morphological proximity between *Portea* and *Aechmea* species originally described within the genus *Gravisia*, while Faria et al. (2004) confirmed this close relationship using phylogenetic studies.

This work corroborates the aperture pattern (2-porate) of *Ananas bracteatus* and *Quesnelia indecora*, as observed by Ehler and Schill (1973), Wanderley and Melhem (1991) and Halbritter (1992). However, our analysis reports differences in the exine ornamentation for both species, with *A. bracteatus* presenting a microreticulated exine vs. reticulate exine and lumina with crests and granulations (Ehler and Schill 1973, Wanderley and Melhem 1991). *Quesnelia indecora*, in turn, showed a reticulate exine, contrary to Halbritter's (1992) observations. Moreover, we also observed the presence of operculum covering the aperture of *Q. indecora*.

The *Billbergia* species have been reported in the literature to have 1-colpate pollen grains, varying from reticulate to heterobrochate (Ehler and Schill 1973, Wanderley and Melhem 1991, Halbritter 1992, Melhem et al. 2003). These characteristics were confirmed for the genus in our study. Related to the colpus, only *B. zebrina* showed ornamentation in the margins. B.A. Moreira (unpublished data), described the pollen morphology of this species, presenting a well-ornamented colpus and a psilate-perforate exine. Our study corroborates the colpus ornamentation, but indicates differences in the characterization of the exine, which is characterized here as microreticulate.

Erdtman and Praglowsky (1974) and Halbritter (1992) analyzed pollen grains of *Pitcairnia* species and observed the 1-colpate aperture pattern and exine with reticulate ornamentation in *P. flammea* pollen grains, as well as for other taxa of the genus. Our study corroborates the aperture and ornamentation pattern of this specie.

The basic 1-colpate aperture pattern of the genus *Tillandsia* is confirmed for the species analyzed in the present study. Wanderley and Melhem (1991) and Souza et al. (2004) analyzed the pollen grains of *Tillandsia* species and indicated differences in the exine structure, as the main palynological characteristic. The results obtained in this study corroborate the morphological variety of the patterns of exine ornamentation, which varied from psilate-perforate in *T. geminiflora*, and microreticulate in *T. stricta* and *T. gardneri*, to heterobrochate in *T. polystachia*.

This study revealed some useful taxonomical characteristics that could be used to identify species, to delimit groups of species, and for future taxonomic and phylogenetic studies of Bromeliaceae.

ACKNOWLEDGMENTS

Financial support was provided to Ana Paula Gelli de Faria, Ester Maiolini Ribeiro and Vanessa Joana Darc da Silva by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), through

the Programa de Capacitação em Taxonomia (PROTAX), process number 562218/2010-6. The authors would also like to thank the Bryophyte Laboratory (Universidade Federal de Juiz de Fora), the Núcleo de Microscopia e Microanálise da Universidade Federal de Viçosa and Fátima Salimena (curator of CESJ herbarium), for the logistic support.

RESUMO

A morfologia dos grãos de pólen de espécies de Bromeliaceae coletadas em áreas de Floresta Atlântica do sudeste do Brasil foi estudada. As seguintes espécies foram analisadas: *Aechmea bambusoides* L. B. Sm. & Reitz, *A. nudicaulis* (L.) Griseb., *A. ramosa* Mart. ex Schult.f., *Ananas bracteatus* (Lindl.) Schult.f., *Billbergia distachia* (Vell.) Mez, *B. euphemiae* E. Morren, *B. horrida* Regel, *B. zebrina* (Herb.) Lindl., *Portea petropolitana* (Wawra) Mez, *Pitcairnia flammae* Lindl., *Quesnelia indecora* Mez, *Tillandsia polystachia* (L.) L., *T. stricta* Sol., *T. gardneri* Lindl., *T. geminiflora* Brongn. e *Vriesea grandiflora* Leme. Microscopia de luz e microscopia eletrônica de varredura foram empregadas e as espécies foram agrupadas em três tipos polínicos organizados de acordo com as características das aberturas: Tipo I - grãos de pólen pantoporados, observados em *P. petropolitana*; Tipo II - grãos de pólen 2-porados, observados nos gêneros *Ananas*, *Aechmea* e *Quesnelia* e Tipo III - grãos de pólen 1-colpado observado nos gêneros *Billbergia*, *Pitcairnia*, *Tillandsia* e *Vriesea*. Os dados polínicos levaram à elaboração de uma chave de identificação. Os resultados mostraram que as espécies analisadas podem ser separadas utilizando-se, principalmente, o padrão de aberturas e ornamentação da exina, e que essas características podem auxiliar nos estudos taxonômicos da família.

Palavras-chave: epífitas, morfologia, grãos de pólen, taxonomia.

REFERENCES

- BARTH OM AND MELHEM TS. 1988. Glossário ilustrado de Palinologia. Campinas: Editora Unicamp, 75 p.
- BENZING DH. 1994. How much is know about Bromeliaceae in 1994? Selbyana 15: 1-7.
- EHLER N AND SCHILL R. 1973. Die Pollenmorphologie der Bromeliaceae. Pollen Spores 15: 13-45.
- ERDTMAN G. 1960. The acetolysis method. A revised description. Svensk Bot Tidskr 54: 561-564.
- ERDTMAN G AND PRAGLOWSKI K. 1974. A note on pollen morphology. In: Smith LB and Downs RJ (Eds), Fl Neotropica 14(1): 28-33.
- FARIA APG, WENDT T AND BROWN GK. 2004. Cladistic relationships of *Aechmea* (Bromeliaceae, Bromelioideae) and allied genera. Ann Mo Bot Gard 91: 303-319.
- GIVNISH TJ ET AL. 2011. Phylogeny, adaptive radiation and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. Am J Bot 98: 872-895.
- GIVNISH TJ, MILLAM KC, BERRY PE AND SYTSMA KJ. 2007. Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *ndhF* sequence data. Aliso 23: 3-26.
- HALBRITTER H. 1992. Morphologie und Systematische Bedeutung des Pollens der Bromeliaceae. Grana 31: 197-212.
- LUTHER H. 2008. An alphabetical list of Bromeliad binomials. Bromeliad Society International, 11th ed., Sarasota: The Marie Selby Botanical Gardens, 110 p.
- MELHEM TS, CRUZ-BARROS MAV, CORRÊA MAS, MAKINO-WATANABE H, SILVESTRE-CAPELATO MSF AND ESTEVES VLG. 2003. Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). Bol Bot Univ São Paulo 16: 1-106.
- MEZ C. 1891-1894. Bromeliaceae. In: Martius CFP, Eichler AG and Urban I (Eds), Fl. Bras., 3, München, Wien, Leipzig, p. 173-634.
- MEZ C. 1896. Bromeliaceae. In: De Candolle APP and De Candolle ACP (Eds), Monographiae Phanerogamarum, 9, Paris, G. Masson, p. 1-990.
- MEZ C. 1934-1935. Bromeliaceae. In: Engler HGA (Ed), Das Pflanzenreich, 4, Wilhelm Engelmann, Berlin, Wilhelm Engelmann, p. 1-667.
- MOREIRA BA, CRUZ-BARROS MAV AND WANDERLEY MGL. 2005. Morfologia polínica de algumas espécies dos gêneros *Neoregelia* L.B. Sm. e *Nidularium* Lem. (Bromeliaceae) do Estado de São Paulo, Brasil. Acta Bot Bras 19: 61-70.
- PUNT W, HOEN PP, BLACKMORE S, NILSSON S AND LE THOMAS A. 2007. Glossary of pollen and spore terminology. Rev Paleobot Palyno 143: 1-81.

- RAYNAL A AND RAYNAL J. 1971. Une technique de préparation des grains de pollen fragiles. *Adansonia* 11: 77-79.
- READ RW AND LUTHER HE. 1991. The *Aechmea/Gravisia* complex (Bromeliaceae). *Selbyana* 12: 54-67.
- SCHULTE K AND ZIZKA G. 2008. Multi locus plastid phylogeny of Bromelioideae (Bromeliaceae) and the taxonomic utility of petal appendages and pollen characters. *Candollea* 63: 209-225.
- SMITH LB AND DOWNS RJ. 1974. Pitcairnioideae (Bromeliaceae). In: Wurdack JJ (Ed), *Fl Neotropica* 14(1), New York: Hafner Press, p. 1-658.
- SMITH LB AND DOWNS RJ. 1977. Tillandsioideae (Bromeliaceae). In: Rogerson CT (Ed), *Fl Neotropica* 14(2), New York: Hafner Press, p. 663-1492.
- SMITH LB AND DOWNS RJ. 1979. Bromelioideae (Bromeliaceae). In: Rogerson CT (Ed), *Fl Neotropica* 14(3), New York: Hafner Press, p. 1493-2141.
- SOUSA GM, WANDERLEY MGL AND CRUZ-BARROS MAV. 1997. Morfologia polínica de *Aechmea* Ruiz & Pav. (Bromeliaceae) de Pernambuco, Brasil. *Bol Bot Univ São Paulo* 16: 21-30.
- SOUZA FC, MENDONÇA CBF AND GONÇALVES-ESTEVEZ V. 2004. Estudo polínico de espécies de Pitcairnioideae e Tillandsioideae (Bromeliaceae Juss.) ocorrentes na Restinga de Carapebus, Estado do Rio de Janeiro. *Arq Mus Nac* 62: 15-23.
- STEHMANN JR, FORZZA RC, SALINO A, SOBRAL M, COSTA DP AND KAMINO LHY. 2009. *Plantas da Floresta Atlântica*. Rio de Janeiro: Jardim Botânico do Rio de Janeiro, 516 p.
- TERRY GR, BROWN GK AND OLMSTEAD RG. 1997. Examination of subfamilial phylogeny in Bromeliaceae using comparative sequencing of the plastid locus *ndhF*. *Am J Bot* 84: 664-670.
- THIERS B. 2014 [continuously updated]. *Index Herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available in <www.sweetgum.nybg.org/ih>. Accessed on Dec 20, 2014.
- WANDERLEY MGL AND MELHEM TS. 1991. Flora polínica da reserva do Parque Estadual das Fontes do Ipiranga. *Família: 178-Bromeliaceae*. *Hoehnea* 18: 5-42.
- WODEHOUSE RP. 1935. *Pollen Grains. Their structure, identification and significance in Science and medicine*. New York: McGraw-Hill Book Company, 574 p.