

Pollen morphology of *Fridericia* Mart. (Bignoniaceae) from Brazilian forest fragments

Cintia Neves de Souza · Eduardo Custódio Gasparino

Received: 18 October 2013 / Accepted: 3 December 2013 / Published online: 17 December 2013
© Botanical Society of Sao Paulo 2013

Abstract A pollen morphology study of 10 Brazilian native species of *Fridericia* (Bignoniaceae) from forest fragments was performed using light microscopy and scanning electron microscopy, in search of new characters that might increase knowledge of pollen morphology of the species, and also to help the taxonomic characterization of the genus. The pollen grains were acetolysed, measured, photographed, and described qualitatively. The quantitative data were analyzed by descriptive statistics and multivariate statistics. Non-acetolysed pollen grains were observed under scanning electron microscopy for further details of exine and pollen surface. The pollen grains are isopolar, medium to large, with circular to subcircular amb, oblate-spheroidal to subprolate, tricolporate, with long colpi, constricted or not, sometimes with margo, rounded or truncated at the polar ends, endoaperture lalongate, and microreticulate to reticulate exine, sexine thicker than nexine. The results indicate a stenopalynous genus, however, in some cases, it is possible to identify the studied species by the pollen morphology. Morphological considerations are also discussed.

Keywords Bignoniaceae · Palynotaxonomy · Pollen grains · Stenopalynous

Introduction

The fragmentation process of forest habitats has increased in most ecosystems particularly in the tropics, so this has caused, in general, the loss of the biodiversity (Turner 1996; Myers et al. 2000). The northwestern of São Paulo State, Brazil, is a region consisting of vegetation that includes small fragments of semideciduous forest and large areas of Cerrado (Kronka et al. 1993). This structure is a result of fragmentation in natural forest (Atlantic Forest of Brazil), which currently only take up 5 % of the original forest (Fonseca 1985).

Ranga et al. (2012) analyzed the floristic structure of eighteen remnants forest fragments in the northwest of São Paulo State, and identified about 500 angiosperm species, among them a large number of species of trees and climbers, especially the families Apocynaceae, Bignoniaceae, Malpighiaceae, and Sapindaceae. This survey contributed to the expansion of knowledge about plant species in priority conservation areas and also for the increase of available botanical material for further studies of this species (Ranga et al. 2012).

Bignoniaceae Juss. has 82 genera and 827 species (Lohmann and Ulloa Ulloa 2012), is part of Lamiales along with more 22 families (APG III 2009), and has distribution in tropical and subtropical regions, with a few species in temperate regions, being very diverse in South America (Fischer et al. 2004; Lohmann 2006). A large number of Bignoniaceae species have the climbing habit, and it is a very important feature of the family (Gentry 1980).

Recent monographs and phylogenetic studies have contributed with information about generics and tribal boundaries of the family, such as Zyhra et al. (2004) to Coleae, Chen et al. (2005) to *Incarvillea* Juss., Grose and Olmstead (2007a, b) to *Crescentieae/Tabebuia* s.l, Li

C. N. de Souza · E. C. Gasparino (✉)
Departamento de Biologia Aplicada à Agropecuária, Faculdade de Ciências Agrárias e Veterinárias (FCAV), UNESP—Univ. Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, SP 14884-900, Brazil
e-mail: ecgasparino@fcav.unesp.br

(2008) to *Catalpa* Scop., and Lohmann and Taylor (2013) in Bignoniaceae. Gentry (1980) and subsequently Spangler and Olmstead (1999) showed the monophyly of the family, based on morphological data, and *rbcL/ndhF* sequence data. Bignoniaceae was first divided into eight tribes: Bignoniaceae, Coleeae, Crescentieae, Eccremocarpeae, Oroxyleae, Schlegelieae, Tecomeae, and Tourrettieae (Gentry 1980). However, a recent classification system proposed by Fischer et al. (2004) excludes the tribe Schlegelieae because the genera *Schlegelia* Miq. and *Exarata* A.H.Gentry, previously treated in Bignoniaceae, today constitute the family Schlegeliaceae together with two other genera.

Fridericia Mart. is one of 21 Bignoniaceae genera belonging to the Bignoniaceae tribe, which has about 400 species of lianas distributed in several Neotropical ecosystems e.g., rainforests, seasonally dry woodlands, and savannas (Lohmann 2006; Lohmann et al. 2013). This genus has about 70 native species in the Cerrado, the Caatinga, and in the forests of tropical America, in Brazil are described 24 endemic species from 59 native species (Lohmann 2013). These plants are lianas or rarely shrubs, with inflorescences in thyrses, terminal or axillary, flowers with green calyx, campanulate, tubular or urceolate, corolla usually magenta, pink, red or rarely white, tubular or infundibular, and septicidal capsule (Lopes 2005; Lohmann 2003). Current nomenclatural changes recognized in *Fridericia* a large number of species previously treated with *Arrabidea* DC. (Lohmann and Ulloa Ulloa 2012; Lohmann 2013).

For Gentry and Tomb (1979), the recognition of Bignoniaceae genera can be a very difficult task and in some cases the palynology can help as an important tool in the genera delimitation in the family. Bove (1993, 1994) demonstrated that the pollen morphology of the genera in Bignoniaceae is highly variable, but nevertheless the morphological characters of Bignoniaceae pollen grains appear to be constant within the genera, with a high value on this taxonomic level (Gentry and Tomb 1979; Bove 1993, 1994; Burelo-Ramos et al. 2009). The pollen grains of different species of Bignoniaceae were described by De Sampaio (1934), Corrêa-Gomes (1951, 1955, 1957), Erdtman (1952), (Salgado-Labouriau and Barth 1962), Ferguson and Santisuk (1973), Gentry (1973), Salgado-Labouriau (1973), Suryakanta (1973), Buurman (1977), Gentry and Tomb (1979), Silvestre (1984), Silvestre and Melhem (1989), Bove and Barth (1992), Bove (1993, 1994), Gonçalves-Esteves and Crespo (1994), and Burelo-Ramos et al. (2009). These authors observed the differences in the pollen morphology, especially to aperture and exine sculpture, thus concluding that the family is euripalynous.

Small differences in *Fridericia* pollen grains were specially identified by the studies of Ferguson & Santisuk (1973), Silvestre (1984), Silvestre and Melhem (1989), and Gonçalves-Esteves and Crespo (1994). Bove (1994)

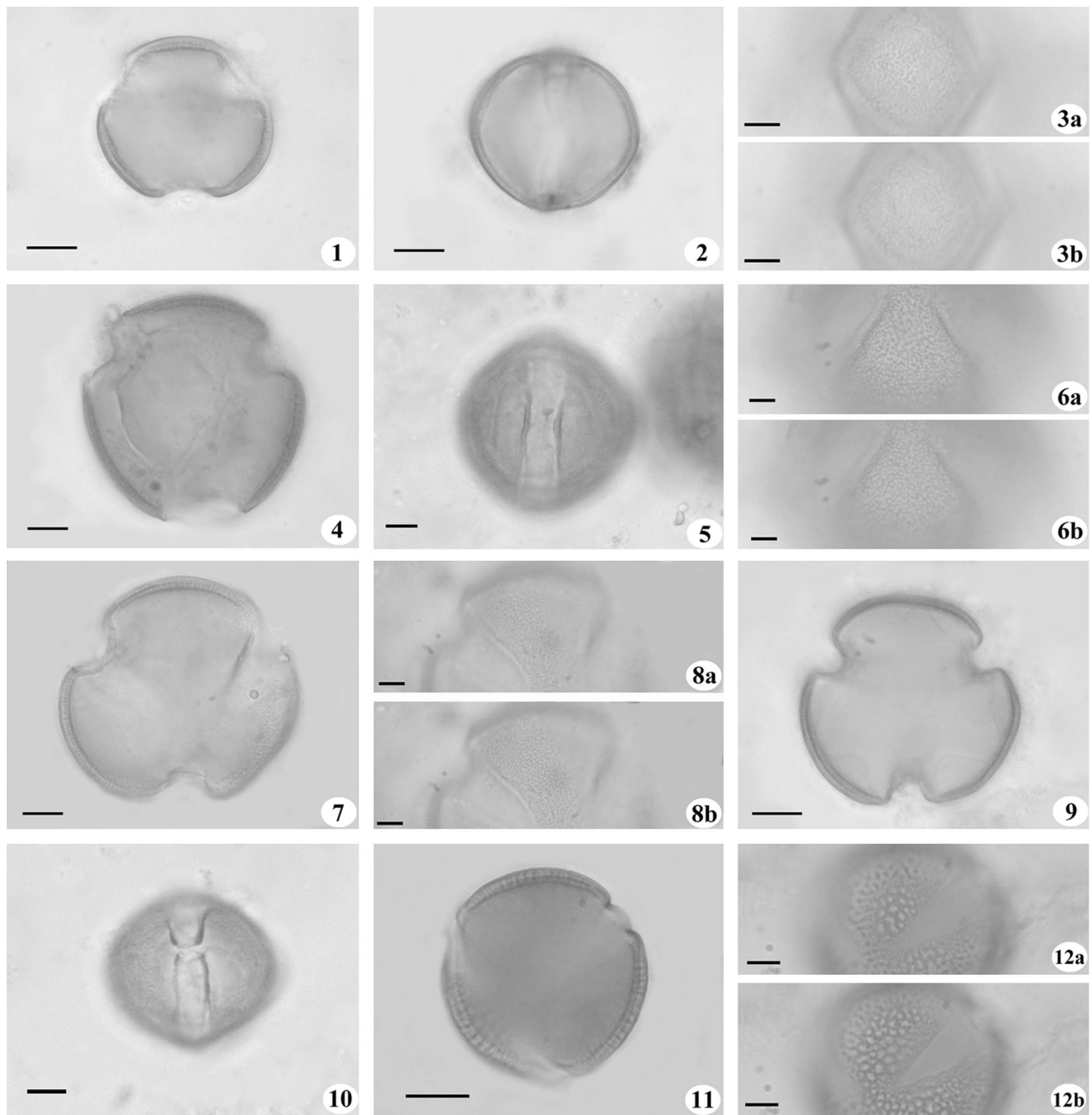
confirmed the euripalynous character to Bignoniaceae, reporting morphological variation in its pollen grains, however, she also found similar pollen morphology for the species within each studied genus.

The aim of this study was to characterize the pollen morphology of the *Fridericia* species (Bignoniaceae) from remnants forest fragments for improved knowledge and also to help the taxonomic characterization of the genus, in the search to better understand the native species from the remaining areas, for the conservation of biological resources avoiding important losses of biodiversity.

Materials and methods

We studied the pollen grains of 10 *Fridericia* native species (Appendix) from the remnant forest fragments as described by Ranga et al. (2012). The pollen materials were obtained from dried herbarium specimens supplied from SJRP and SP herbaria. Priority was given to collections of remnant forest fragments of northwest São Paulo State, however, when these were not enough we used other specimens. Pollen grains of 22 specimens were studied by light microscopy (LM) and scanning electron microscopy (SEM), and the pollen grains of five of these species had not been studied previously. For LM observations, pollen grains were acetolysed through the standard method of Erdtman (1960) with modifications by Melhem et al. (2003), and the measures were taken within seven days after their preparation (Salgado-Labouriau et al. 1965). For SEM analysis, non-acetolysed pollen grains were used according to Melhem et al. (2003). Permanent slides of light microscopy are deposited in the pollen reference collection of the Department of Biology applied to Agriculture, São Paulo State University (UNESP), Jaboticabal, Brazil.

Pollen diameters in polar and equatorial view were measured per sample under LM ($n = 25$), the other characteristics (of aperture and exine thickness) were measured in ten pollen grains. Statistical analysis was conducted to obtain the means (\bar{x}), standard deviation (s_x), standard error (s), 95 % confidence intervals (CI), coefficient of variability (V), and range (R) following Vieira (1981) and Zar (1996). The mean was calculated for exine thickness, length and width of colpus, and length and width of endoaperture. To verify whether the pollen data permitted the grouping of species, a principal component analysis (PCA) was performed using the programs FITOPAC (Shepherd 1996) and PC-ORD (McCune and Mefford 1999). For this analysis, we used the following metric variables: length of colpus (CLEN), width of colpus (CWID), equatorial diameter in equatorial view (EDEV), equatorial diameter in polar view (EDPV), polar diameter in equatorial view (PDEV), length of endoaperture (ELEN), width of endoaperture (EWID), polar



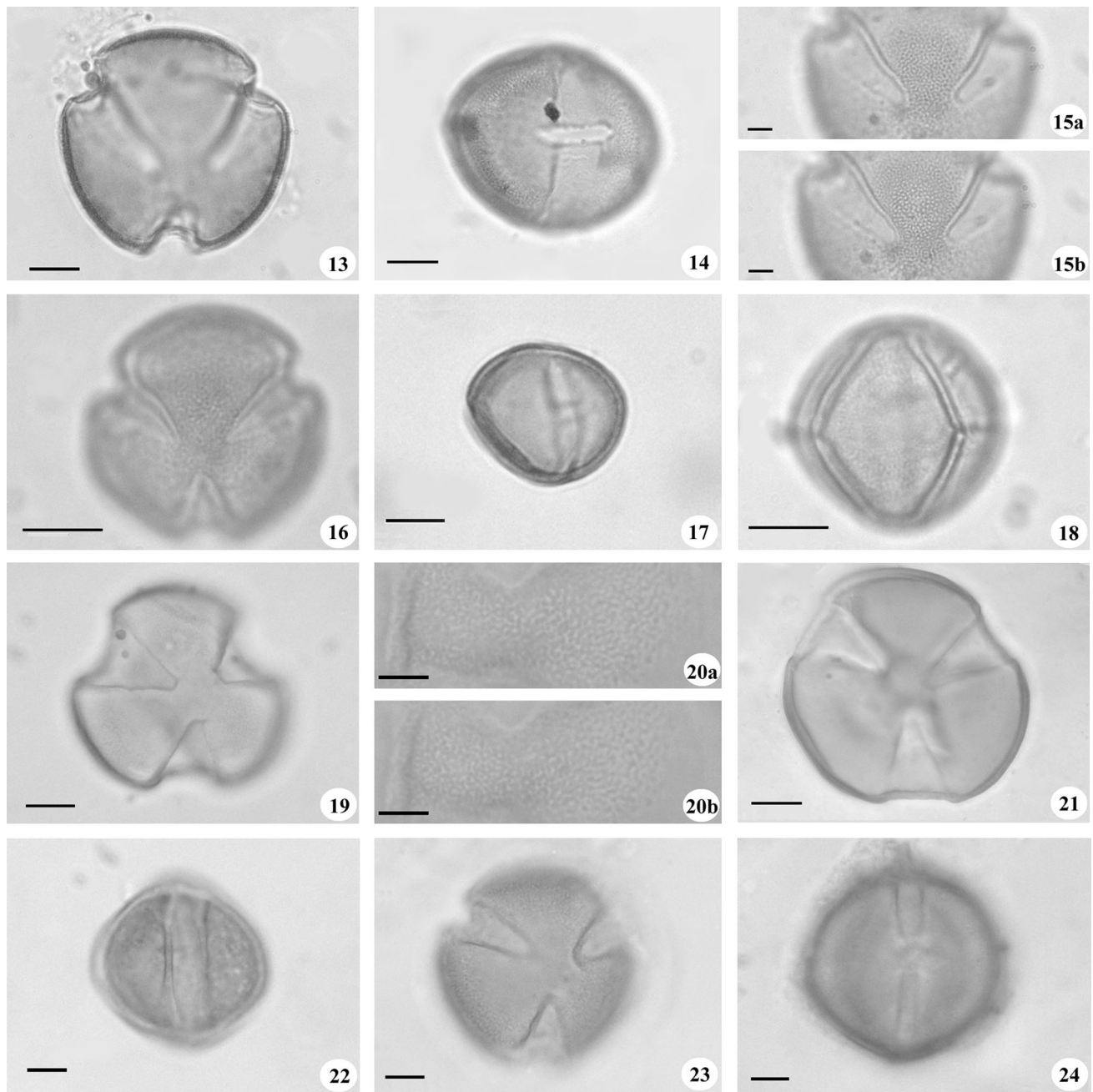
Figs. 1–12 Photomicrographs of the pollen grains of *Fridericia* Mart. (Bignoniaceae). **1–3** *F. chica* (Bonpl.) L.G.Lohmann. **1** Polar view, optical section. **2** Equatorial view, optical section. **3a–b** Ornamentation in high and low focus. **4–6** *F. craterophora* (DC.) L.G.Lohmann. **4** Polar view, optical section. **5** Equatorial view, aperture. **6a–b** Ornamentation in high and low focus. **7–8** *F. formosa*

(Bureau) L.G.Lohmann. **7** Polar view, optical section. **8a–b** Ornamentation in high and low focus. **9–10** *F. leucopogon* (Cham.) L.G.Lohmann. **9** Polar view, optical section. **10** Equatorial view, aperture. **11–12** *F. mutabilis* (Bureau and K.Schum.) L.G.Lohmann. **11** Polar view, optical section. **12a–b** Ornamentation in high and low focus. Bar = 5 μm (**3, 6, 8, 12**); 10 μm (**1–2, 4–5, 7, 9–11**)

index area (PAI), exine (EXIN), nexina (NEXI), sexine (SEXI), tectum (TECT), and shape (SHAP).

The LM photos were performed with a Leica IM50 photomicroscope, and the SEM images were scanned on JOEL, JSM5410 scanning electron microscope.

The palynological description and terminology follow Barth and Melhem (1988) and Punt et al. (2007), we used Faegri and Iversen (1966) to define the polar area index, and Gasparino et al. (2013) for width index of colpi.



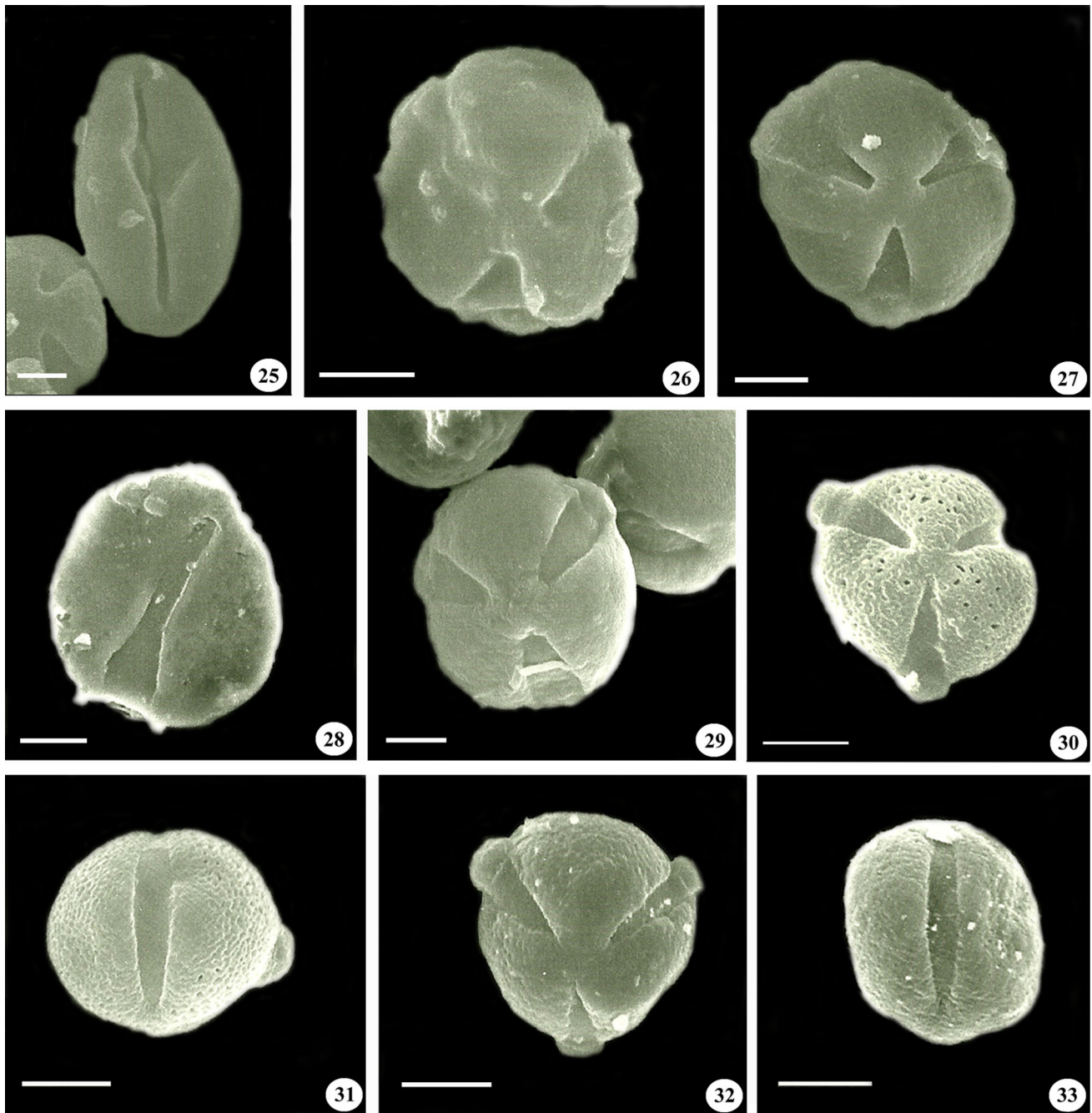
Figs 13–24 Photomicrographs of the pollen grains of *Fridericia* Mart. (Bignoniaceae). **13–15** *F. platyphylla* (Cham.) L.G. Lohmann. **13** Polar view, optical section. **14** Equatorial view, aperture. **15a–b** Ornamentation in high and low focus. **16–18** *F. pubescens* (L.) L.G. Lohmann. **16** Polar view, apocolpium. **17** Equatorial view, aperture. **18** Equatorial view, ornamentation, and lateral apertures. **19–20** *F.*

pulchella (Cham.) L.G.Lohmann. **19** Polar view, apocolpium. **20a–b** Ornamentation in high and low focus. **21–22** *F. samyoides* (Cham.) L.G. Lohmann. **21** Polar view, optical section. **22** Equatorial view, aperture. **23–24** *F. triplinervia* (Mart. ex DC.) L.G.Lohmann. **23** Polar view, apocolpium. **24** Equatorial view, aperture. Bar = 5 μ m (15, 20); 10 μ m (13–14, 16–19, 21–24)

Results

The species analyzed were: *Fridericia chica* (Bonpl.) L.G.Lohmann (Figs. 1–3, 25); *Fridericia craterophora* (DC.) L.G.Lohmann (Figs. 4–6, 26); *Fridericia formosa* (Bureau) L.G.Lohmann (Figs. 7–8); *Fridericia leucopogon* (Cham.)

L.G.Lohmann (Figs. 9–10); *Fridericia mutabilis* (Bureau and K. Schum.) L.G.Lohmann (Figs. 11–12, 27); *Fridericia platyphylla* (Cham.) L.G. Lohmann (Figs. 13–15, 28); *Fridericia pubescens* (L.) L.G. Lohmann (Figs. 16–18, 29); *Fridericia pulchella* (Cham.) L.G. Lohmann (Figs. 19–20, 30–31); *Fridericia samyoides* (Cham.) L.G. Lohmann



Figs 25–33 Electron micrographs of the pollen grains of *Fridericia* Mart. (Bignoniaceae). **25** *F. chica* (Bonpl.) L.G.Lohmann. Equatorial view. **26** *F. craterophora* (DC.) L.G.Lohmann. Polar view. **27** *F. mutabilis* (Bureau & K.Schum.) L.G.Lohmann. Polar view. **28** *F. platyphylla* (Cham.) L.G. Lohmann. Equatorial view. **29** *F. pubescens*

(L.) L.G. Lohmann. Polar view. **30–31** *F. pulchella* (Cham.) L.G.Lohmann. **30** Polar view. **31** Equatorial view. **32–33** *F. triplinervia* (Mart. ex DC.) L.G.Lohmann. **32** Polar view. **33** Equatorial view. Bar = 10 μ m

(Figs. 21–22); *Fridericia triplinervia* (Mart. ex DC.) L.G. Lohmann (Figs. 23–24, 32–33).

The *Fridericia* species (Figs. 1–33) have medium to large, isopolar pollen grains (Tables 1, 2), oblate spheroidal to subprolate, subcircular to circular amb in polar view, tricolporate, long and very large, large or narrow colpi

(Table 3), constricted or not, sometimes with margo, endoapertures lalongate. The exine is microreticulate, homobroccate or reticulate, heterobroccate (in *F. mutabilis*), the sexine is thicker as the nexine (Table 3).

Fridericia craterophora and *F. samydoides* have larger diameters in their pollen grains and size ranging from

Table 1 Morphological characterization of *Fridericia* Mart

Species	Size	P/E	Shape	PAI
<i>F. chica</i>	M	0.89	OS	0.25
<i>F. craterophora</i>	M-L	1.15	SP	0.18
<i>F. formosa</i>	M	1.00	OS	0.24
<i>F. leucopogon</i>	M	0.92	OS	0.19
<i>F. mutabilis</i>	M	0.89	OS	0.25
<i>F. platyphylla</i>	M	0.94	OS	0.16
<i>F. pubescens</i>	M	0.93	OS	0.17
<i>F. pulchella</i>	M	0.98	OS	0.18
<i>F. samydoides</i>	M-L	1.02	PS	0.17
<i>F. triplinervia</i>	M	0.94	OS	0.19

P/E ratio between polar and equatorial diameter, PAI polar area index, M medium; L large, OS oblate spheroidal, PS prolate spheroidal, SP subprolate

medium to large. For the other studied species, the pollen grains are medium, and the lowest values of diameters were observed in *F. mutabilis* and *F. chica* pollen grains. The amb was circular (*F. chica*, *F. formosa*, *F. leucopogon*, *F. mutabilis*, *F. pubescens* and *F. triplinervia*) to subcircular (*F. craterophora*, *F. platyphylla*, *F. pulchella* and *F. samydoides*), the shape observed in most species were oblate spheroidal, except for *F. craterophora* (with subprolate pollen grains) and *F. samydoides* (with prolate spheroidal pollen).

All analyzed species had three compound apertures (three colpi). The colpi are very long except for *F. chica*, *F. formosa*, and *F. mutabilis* and have high polar area index (PAI), which characterize a long aperture and not very long as in the pollen grains of the other species (Table 1). The longest colpi are observed in *F. craterophora*, *F. samydoides*, and *F. triplinervia*, and the lowest in the pollen grains of *F. formosa* and *F. mutabilis*. We observed a margo around the colpi of the pollen grains except in *F. chica*, *F. leucopogon*, *F. mutabilis*, and *F. pubescens*.

Very large colpi were found in the pollen grains of *F. chica* and *F. pulchella*, large colpi in *F. craterophora*, *F. leucopogon*, *F. platyphylla*, *F. pubescens*, *F. samydoides*, and *F. triplinervia*, and narrow in *F. formosa* and *F. mutabilis* (Table 3). The endoapertures are lalongate and sometimes difficult to visualize (as in *F. craterophora*, Fig. 5, and *F. samydoides*, Fig. 22). The smaller endoaperture were measured in *F. formosa* pollen grains, and the largest in *F. pulchella*.

The exine is semitectate, microreticulate, and homobroccate in pollen grains of all studied species, except for *F. mutabilis*, where it could be observed reticulate and heterobroccate pollen grains (Fig. 12). For the microreticulate pollen grains, the muri are psilate and straight, while the reticula of *F. mutabilis* showing sinuous muri. There are no differences between

pollen grain ornamentation between apocolpium and mesocolpium for all the studied species. It was not possible to distinguish the layers of nexine (nexine 1 and nexine 2) because it is very thin. We observed for all studied pollen grains similar values of thicknesses of the sexine and nexine.

Under SEM, could be observed traces of another tissue in the pollen grains (Figs. 25–33), which can sometimes cover the microreticulum's lumina (Figs. 25–28, 32–33). The colpi membranes are psilate with some small granules (Figs. 30–33). The colpi margo is also psilate (Fig. 28).

Based on measurements made and comparing the means and the interval of the diameters of pollen grains studied (Fig. 34a, b) we observed that, on all of the diameters measured the pollen grains of *F. mutabilis* and *F. chica*, these species separate from other species for presenting the lowest values.

In equatorial view, *F. craterophora* presents the highest values of polar diameter of its pollen grains, while in equatorial diameter, although *F. craterophora* has high values, the larger pollen grains were observed in *F. triplinervia*. The pollen grains of *F. leucopogon*, *F. pubescens*, *F. platyphylla*, *F. pulchella*, *F. formosa*, *F. triplinervia*, and *F. samydoides* in equatorial view constitute a group by polar diameter. In this group the lowest values were observed in *F. leucopogon*, and the larger in *F. samydoides*.

As for the measures of the equatorial diameter in equatorial view, *F. leucopogon*, *F. pubescens*, *F. pulchella*, *F. formosa*, *F. platyphylla*, *F. samydoides*, and *F. craterophora* represent a continuous group, with the largest diameters observed in *F. craterophora*, and lowest in *F. leucopogon* (Fig. 34b).

The relationships among the studied species through the quantitative data were explored using the PCA analysis (Fig. 35). It is an exploratory analysis of data and the first two axes contained 65.7 % of the total variability of the analyzed data. The first component of the analysis explained 49.5 % of the variance based on the values of the diameters, width of colpi and tectum (negative side of the axis), and also the values of PAI (positive side, Fig. 35). Thus, the species placed on the negative side of the axis 1 (on left) present the highest values for most metric variables analyzed in their pollen grains, except *F. chica*, *F. formosa*, *F. mutabilis*, and *F. leucopogon* which were placed on the positive side (on right) because the measures of polar area index (PAI). *F. mutabilis* appears distant from the other species mainly due to the low values to the diameters of its pollen, the same happens to *F. chica*.

The second component explained 16.1 % of the variability of the data, where the length and width of endoaperture (ELEN and EWID) and values of sexine (SEXI) were the most important variables for ordination of species on the negative side of axis 2 (Fig. 35). The same way the

Table 2 Quantitative data of pollen grains of *Fridericia* Mart. species in polar and equatorial views, $n = 25$

Species	(R) $x \pm s_x$	s	CI	V
Equatorial diameter in polar view				
<i>F. chica</i>	(25.71–33.42) 30.65 ± 0.29	1.47	(30.04–31.25)	4.79
<i>F. craterophora</i>	(46.28–51.42) 49.36 ± 0.47	2.35	(48.40–50.33)	4.75
<i>F. formosa</i>	(43.71–48.85) 45.66 ± 0.31	1.54	(45.03–46.29)	3.36
<i>F. leucopogon</i>	(35.99–41.14) 38.57 ± 0.15	0.74	(38.26–38.87)	1.92
<i>F. mutabilis</i>	(25.61–33.33) 30.17 ± 0.47	1.81	(29.17–31.17)	6.00
<i>F. platyphylla</i>	(38.57–43.71) 39.97 ± 0.53	1.77	(38.78–41.16)	4.42
<i>F. pubescens</i>	(38.57–43.71) 41.24 ± 0.35	1.74	(40.52–41.95)	4.21
<i>F. pulchella</i>	(41.14–48.85) 43.36 ± 0.49	2.29	(42.34–44.37)	5.27
<i>F. samydoides</i>	(35.99–46.28) 39.90 ± 0.56	2.79	(38.75–41.05)	6.99
<i>F. triplinervia</i>	(38.57–43.71) 41.55 ± 0.24	1.22	(41.05–42.05)	2.92
Polar diameter in equatorial view				
<i>F. chica</i>	(25.71–33.42) 29.10 ± 0.32	1.61	(28.44–29.77)	5.54
<i>F. craterophora</i>	(46.28–59.13) 51.11 ± 0.58	2.90	(49.91–52.31)	5.68
<i>F. formosa</i>	(41.14–43.71) 42.34 ± 0.34	1.33	(41.60–43.07)	3.14
<i>F. leucopogon</i>	(35.99–43.71) 38.15 ± 0.38	1.92	(37.36–38.94)	5.03
<i>F. mutabilis</i>	(25.71–30.85) 27.42 ± 0.41	1.59	(26.55–28.30)	5.79
<i>F. platyphylla</i>	(38.57–43.71) 40.31 ± 0.38	1.92	(39.52–41.11)	4.77
<i>F. pubescens</i>	(35.99–43.71) 38.67 ± 0.43	2.16	(37.78–39.56)	5.59
<i>F. pulchella</i>	(38.57–43.70) 40.90 ± 0.73	2.43	(39.22–42.59)	5.93
<i>F. samydoides</i>	(35.99–51.42) 43.60 ± 0.70	3.52	(42.15–45.05)	8.07
<i>F. triplinervia</i>	(38.57–48.85) 43.09 ± 0.52	2.60	(42.02–44.16)	6.04
Equatorial diameter in equatorial view				
<i>F. chica</i>	(30.85–33.42) 32.60 ± 0.24	1.22	(32.10–33.10)	3.75
<i>F. craterophora</i>	(38.57–51.42) 44.43 ± 0.57	2.83	(43.26–45.59)	6.37
<i>F. formosa</i>	(41.14–43.71) 42.34 ± 0.34	1.34	(41.59–43.09)	3.14
<i>F. leucopogon</i>	(38.57–43.71) 41.55 ± 0.24	1.22	(41.05–42.05)	2.92
<i>F. mutabilis</i>	(25.71–33.42) 30.68 ± 0.47	1.81	(29.68–31.68)	5.90
<i>F. platyphylla</i>	(41.14–43.71) 42.68 ± 0.26	1.29	(42.15–43.21)	3.01
<i>F. pubescens</i>	(38.57–43.71) 41.75 ± 0.37	1.86	(40.99–42.52)	4.45
<i>F. pulchella</i>	(38.60–46.30) 41.84 ± 0.78	2.59	(40.04–43.64)	6.20
<i>F. samydoides</i>	(38.57–48.85) 42.88 ± 0.68	3.38	(41.49–44.28)	7.88
<i>F. triplinervia</i>	(43.71–51.42) 47.41 ± 0.37	1.83	(46.66–48.16)	3.86

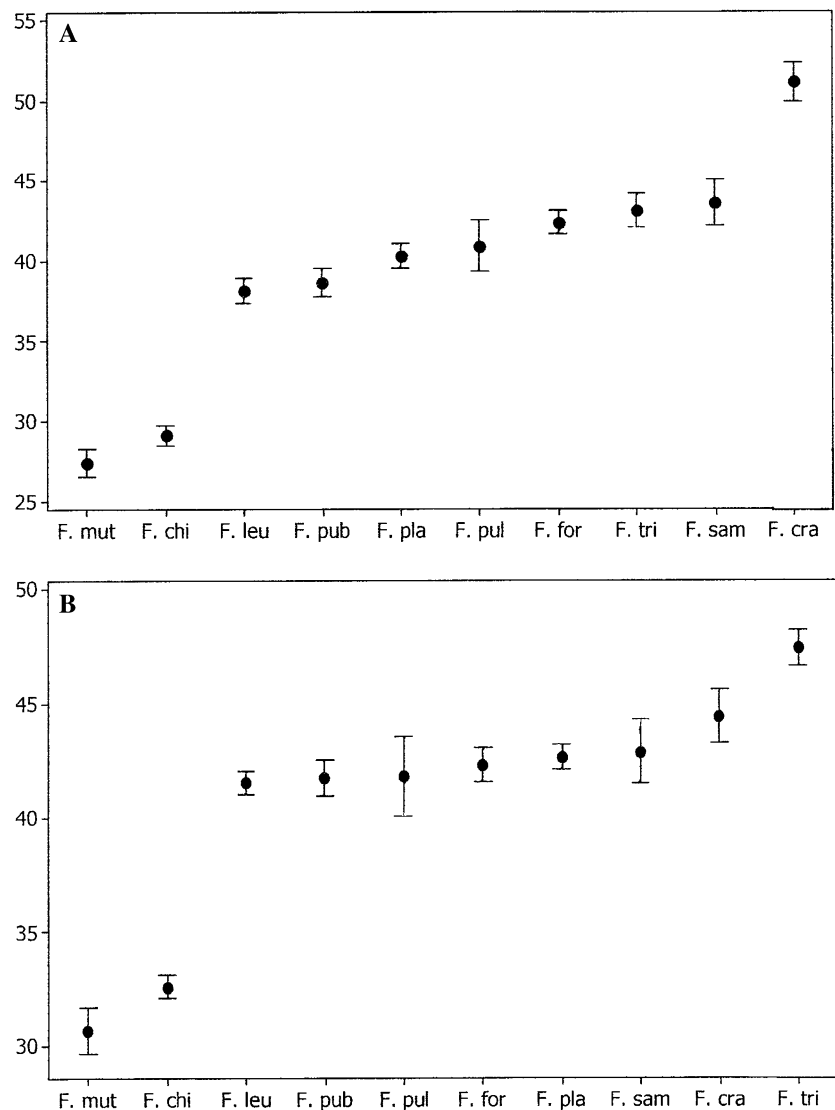
R range, x mean (μm), s_x standard deviation (μm), s standard error (μm), CI confidential interval in 95 % (μm), V coefficient of variability (%)

Table 3 Measures (in μm) of *Fridericia* Mart. pollen grains in equatorial view, apertures and exine. $n = 10$

Species	Colpus		Endoaperture		Ex	Sex	Nex	WCI
	Length	Width	Length	Width				
<i>F. chica</i>	28.02	6.68	2.57	6.17	2.35	1.85	0.50	4.88
<i>F. craterophora</i>	34.71	8.25	2.85	8.74	2.50	2.00	0.50	5.38
<i>F. formosa</i>	18.51	5.40	3.60	5.40	2.45	1.95	0.50	7.86
<i>F. leucopogon</i>	29.57	6.43	2.57	6.68	2.35	1.85	0.50	6.46
<i>F. mutabilis</i>	16.20	4.37	3.86	6.17	2.30	1.80	0.50	7.02
<i>F. platyphylla</i>	29.05	7.71	5.14	8.74	2.20	1.70	0.50	5.53
<i>F. pubescens</i>	31.62	7.74	5.17	9.00	2.48	1.98	0.50	5.39
<i>F. pulchella</i>	24.42	9.00	5.14	11.57	2.43	1.93	0.50	4.65
<i>F. samydoides</i>	34.71	7.68	2.89	8.03	2.22	1.72	0.50	5.58
<i>F. triplinervia</i>	33.94	7.97	3.09	8.74	2.35	1.85	0.50	5.94

Ex exine thickness, Sex sexine thickness, Nex nexina thickness, WCI width colpus index

Fig. 34 Representation of confidence interval of mean in 95 % of the pollen grains of *Fridericia* Mart. (Bignoniaceae). **a** Polar diameter in equatorial view. **b** Equatorial diameter in equatorial view. The *higher* and *lower boundaries* showing the confidence interval; the *average circle* showing the arithmetic mean. F. chi = *Fridericia chica*, F. cra = *Fridericia craterophora*, F. for = *Fridericia formosa*, F. leu = *Fridericia leucopogon*, F. mut = *Fridericia mutabilis*, F. plat = *Fridericia platyphylla*, F. pub = *Fridericia pubescens*, F. pul = *Fridericia pulchella*, F. samy = *Fridericia samydoides*, and F. tri = *Fridericia triplinervia*. The values are in μm



shape of pollen grains (SHAP), added to the other metric variables, helped the placement of other species on the positive side of axis 2. The high values of length endoaperture (ELEN) contribute to the placement of *F. platyphylla*, *F. pulchella*, and *F. pubescens* on the negative side of axis 2 (Table 4). And the ratio between the polar and equatorial diameters (P/E), which gives the shape of the pollen grains, contributed to the species ordination on the positive side of axis 2 (*F. craterophora*, *F. samydoides* and *F. formosa*).

Discussion

Bignoniaceae has been traditionally described as a euripalynous family (Ferguson and Santisuk 1973; Silvestre 1984; Silvestre and Melhem 1989; Bove 1993, 1994; Gonçalves-Esteves and Crespo 1994). However, as previously reported,

some authors point the homogeneity in the pollen morphology within its genera (e.g. Bove 1994). When reviewing the palynological literature of the family, it was possible to identify some differences in *Fridericia* pollen grains described by performing studies (Table 5).

The species examined here were previously treated on papers of Corrêa-Gomes (1951), Buurman (1977), Gentry and Tomb (1979), Silvestre (1984), Silvestre and Melhem (1989), and Bove (1993, 1994).

Buurman (1977) studied *Fridericia* under the name *Arrabidea* and he describes the genus with tricolpate pollen grains, long colpi, and finely reticulate or pisilate tectum. To *F. samydoides*, the author presents diameters ratio similar found here (about 30–45 μm), but we observed tricolporate pollen grains not tricolpate. Corrêa-Gomes (1951) and Gentry and Tomb (1979) described the pollen of *F. chica* (still under the name *Arrabidaea chica* (Bonpl.) Verl.) as 3-colpate and scabrous-perforated, the specimens

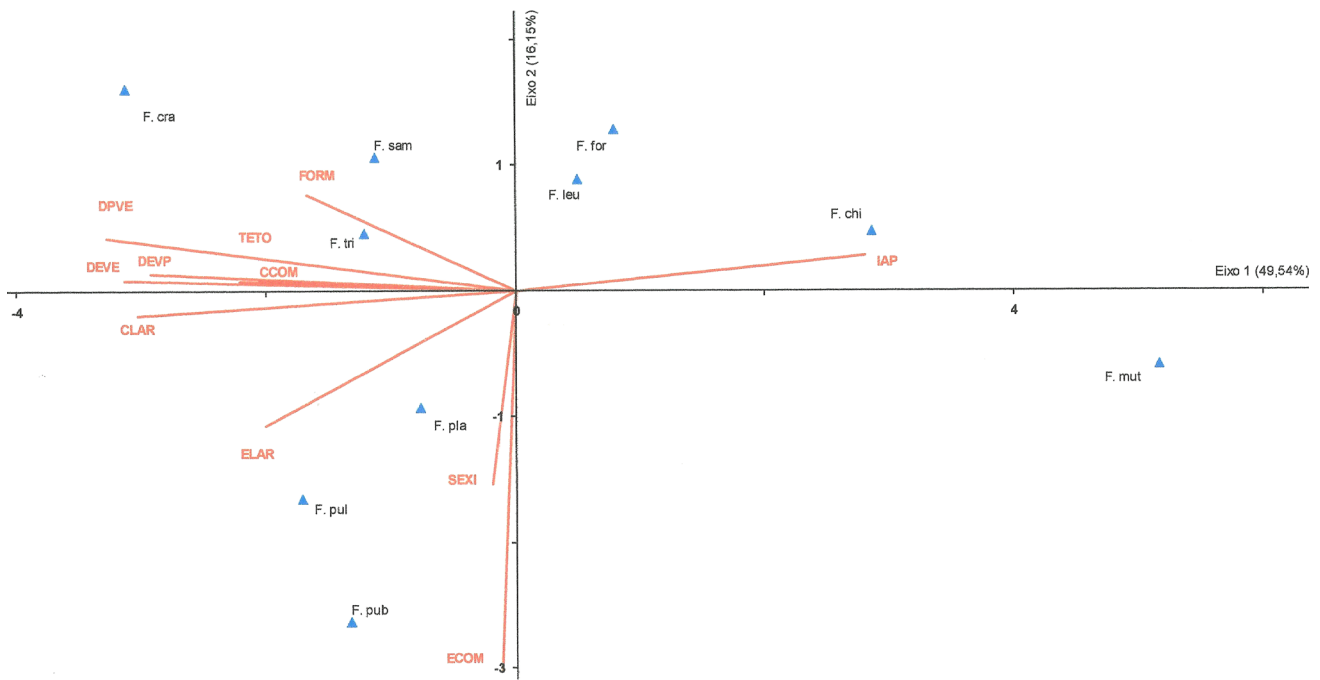


Fig. 35 Principal component analysis performed with the pollen metrical variables from *Fridericia* Mart. species. F. chi = *Fridericia chica*, F. cra = *Fridericia craterophora*, F. for = *Fridericia formosa*, F. leu = *Fridericia leucopogon*, F. mut = *Fridericia mutabilis*,

F. pla = *Fridericia platyphylla*, F. pub = *Fridericia pubescens*, F. pul = *Fridericia pulchella*, F. samy = *Fridericia samydoides*, and F. tri = *Fridericia triplinervia*

Table 4 Pearson and Kendall correlation coefficients for pollen grains metric variables of the first and the second axis of PCA ordination in *Fridericia* Mart. species

Variables	Principal components	
	Axis 1	Axis 2
EDPV	-0.3509	0.1371
PDEV	-0.3716	0.2357
EDEV	-0.3634	0.1093
CLEN	-0.2942	0.1075
CWID	-0.3573	-0.1589
ELEN	-0.0729	-0.6245
EWID	-0.2907	-0.3721
EXIN	-0.1132	-0.0518
SEXI	-0.0914	-0.4457
TECT	-0.3051	0.1059
SHAP	-0.2658	0.3147
PAI	0.3432	0.1921

here analyzed we observed pollen grains with 3-colporate microrreticulate ornamental, differing from the results previously reported for the species.

By the features described by Corrêa-Gomes (1951), Buurman (1977), and Gentry and Tomb (1979), it is clear that there is a difference to set the composed aperture in the

Fridericia pollen grains, which can be due the difficulty in visualizing the endoaperture.

The pollen grains of *F. chica*, *F. pulchella*, and *F. samydoides* were studied by Silvestre (1984), the author describes 3-colporate apertures, oblate spheroidal shape, scabrate-perforate exine, and diameters ranging between 28–29 µm (for *F. chica*) and 43–58 µm (in *F. samydoides*). Our results agree with Silvestre (1984) regarding the aperture type and shape (for *F. chica* and *F. pulchella*). However, we observed prolate spheroidal shape (in *F. samydoides*), microreticulate sexine (in the three species) and diameter values slightly smaller in *F. chica*, and higher than those observed by the author to *F. pulchella* and *F. samydoides*. In her study, Silvestre (1984) does not show measures values to endoaperture, stating that this feature is difficult to see.

Silvestre and Melhem (1989) studied the pollen grains of four *Fridericia* species, they observed 3-colporate pollen grains, *F. chica* and *F. samydoides* with subtriangular amb and oblate spheroidal pollen, and *F. pulchella* with sub-circular amb and suboblate shape. The endoapertures were circular (in *F. chica*) and lalongate (*F. pulchella* and *F. samydoides*). The three species here studied showed similar characteristics, but differs to present lalongate endoaperture and circular amb (in *F. chica*), prolate spheroidal shape and subcircular amb (in *F. samydoides*), and oblate spheroidal pollen grains in *F. pulchella*.

Table 5 Important pollen characters described about the pollen morphology of *Fridericia* Mart. species

	Ferguson and Santisuk (1973)	Silvestre (1984)	Silvestre and Melhem (1989)	Bove (1994)	Gonçalves-Esteves and Crespo (1994)
Small to medium pollen grains	Medium to large pollen grains	Medium to large pollen grains	Medium to large pollen grains	Medium to large pollen grains	Large pollen grains
Spheroidal to prolate	Oblate to oblate spheroidal	Suboblate to oblate spheroidal	Suboblate to oblate spheroidal	Subspheroidal to prolate	Prolate
Subcircular	Circular to subtriangular	Subcircular to subtriangular	Subcircular to subtriangular	Circular or subcircular	Subcircular
Tricolporate	Tricolporate with circular or lalongate endoaperture	Tricolporate with circular or lalongate endoaperture	Tricolporate with unclear endoaperture	Tricolporate, tricolporoidate or tricolporate with circular or lalongate endoaperture	Tricolporate with lalongate endoaperture
Finely reticulate	Finely reticulate or scabrate-perforate	Finely reticulate or microreticulate	Finely reticulate or microreticulate	Reticulate to microreticulate	Reticulate

The pollen morphology of 33 Bignoniaceae species of the Atlantic Forest of southern Brazil was analyzed by Bove (1993). To *F. chica*, *F. leucopogon*, and *F. samydoides* the pollen grains were described as tricolporate and microreticulate. The diameters of pollen grains studied by the author ranged from 24–35 μm (in *F. chica*), 30–57 μm (in *F. leucopogon*) to 40–64 μm (in *F. samydoides*). Our study confirm the Bove (1993) data regarding the ornamentation and aperture type. However, the diameter values of *F. samydoides* pollen grains are smaller. To *F. chica* and *F. leucopogon* the diameter values of the pollen grains are similar to those observed by the author.

Bove (1994) in her paper about the pollen morphology of climber Bignoniaceae from Southern Brazil, classified the pollen grains of *F. chica*, *F. leucopogon*, and *F. samydoides* in the tricolporate-microreticulate type, whereas *F. mutabilis* was placed in the tricolporate-reticulate type. The author observed also medium pollen grains, suboblate (in *F. chica*), oblate spheroidal (*F. mutabilis*), prolate spheroidal (*F. samydoides*), and prolate (*F. leucopogon*). In the present study, the pollen grains showed the same characteristics as the aperture type, amb and ornamentation, however, were observed pollen grains tricolporate pollen grains in *F. mutabilis* and a variation on the pollen grains shape for the studied species.

For *Fridericia craterophora*, *F. formosa*, *F. platyphylla*, *F. pubescens*, and *F. triplinervia* no data was found about the pollen morphology, and therefore this study is the first report of pollen for them.

The PCA analysis showed the closeness between *F. samydoides* and *F. triplinervia* (negative side of axis 1), which was due to the similarity found in their pollen grains. Though, according to Rodrigues (2012), these species have some morphological differences in the flower and petiole size (larger in *F. triplinervia*) and in the calyx shape (tubular in *F. triplinervia* and campanulate in *F. samydoides*). The same occurs with *F. formosa* and *F. leucopogon* (placed on the positive side of axis 1, PCA), although quantitative data are similar, the margo of colpi in *F. leucopogon* pollen grains allow the separation of these species.

The results demonstrate that *Fridericia* is a stenopalous genus, especially when we observe the quantitative data of the pollen grains. However, there are characters like variations in the ornamentation (microreticulum and reticulum), shape, amb, and colpi margo allowing identification of the species. The quantitative data, although similar, also help in the differentiation as evidenced by the principal component analysis performed.

Acknowledgments The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP–2012/09942-6) for the scholarship of the first author.

Appendix: Specimens examined

Fridericia chica (Bonpl.) L.G.Lohmann: BRASIL. São Paulo: Magda, 15 Dec. 2007, *N.T. Ranga et al. 03* (SJRP); BRASIL. São Paulo: Magda, 19 Nov. 2009, *N.T. Ranga et al. 01* (SJRP); BRASIL. São Paulo: Matão, 24 Jan. 2009, *N.T. Ranga et al. 02* (SJRP).

Fridericia craterophora (DC.) L.G.Lohmann: BRASIL. São Paulo: Barretos, 14 Feb. 2009, *N.T. Ranga et al. 07* (SJRP); BRASIL. São Paulo: Palestina, 20 Oct. 2010, *N.T. Ranga et al. 05* (SJRP); BRASIL. São Paulo: Onda Verde, 1 Feb. 2007, *N.T. Ranga et al. 30* (SJRP).

Fridericia formosa (Bureau) L.G.Lohmann: BRASIL. Minas Gerais: Belo Horizonte, 6 Feb. 1919, *A. Gehrt s/n* (SP3173); BRASIL. Minas Gerais: Paraisópolis, 15 Apr. 1927, *F.C. Hoehne s/n* (SP20195).

Fridericia leucopogon (Cham.) L.G.Lohmann: BRASIL. São Paulo: Sales, 28 Mar. 2007, *N.T. Ranga et al. 43* (SJRP); BRASIL. São Paulo: Sales, 28 Mar. 2007, *N.T. Ranga et al. 44* (SJRP); BRASIL. São Paulo: Barretos, 5 Mar. 2009, *N.T. Ranga et al. 08* (SJRP).

Fridericia mutabilis (Bureau & K. Schum.) L.G.Lohmann: BRASIL. Minas Gerais: Coronel Pacheco, Fazenda da Liberdade, 13 Oct. 1945, *E.P. Heringer s/n* (SP53198).

Fridericia platyphylla (Cham.) L.G. Lohmann: BRASIL. São Paulo: Nova Granada, Fazenda São João, 21 May 2010, *M.C. Rodrigues 241* (SJRP).

Fridericia pubescens (L.) L.G. Lohmann: BRASIL. Minas Gerais: Fortaleza de Minas, mata do Paiol, 3 Apr. 2009, *A. Robotino s/n* (SJRP30343). BRASIL. São Paulo: Vinhedo, Condomínio Estância Marambaia, 27 Jan. 2002, *J.R. Guillaumon s/n* (SJRP28794).

Fridericia pulchella (Cham.) L.G. Lohmann: BRASIL. São Paulo: Mogi-Guaçu, Fazenda Campininha, 17 Sep. 1984, *M. Kirizawa & W. Mantovani 1313* (SP); BRASIL. São Paulo: Sarapui, 30 Oct. 1977, *O. Yano 17* (SP).

Fridericia samyoides (Cham.) L.G. Lohmann: BRASIL. São Paulo: Onda Verde, 31 Jul. 2008, *N.T. Ranga et al. 16* (SJRP); BRASIL. São Paulo: Onda Verde, 31 Jul. 2008, *N.T. Ranga et al. 17* (SJRP).

Fridericia triplinervia (Mart. ex DC.) L.G. Lohmann: BRASIL. São Paulo: Onda Verde, 01 Jan. 2007, *N.T. Ranga et al. 48* (SJRP); BRASIL. São Paulo: Sales, 28 Mar. 2007, *N.T. Ranga et al. 46* (SJRP); BRASIL. São Paulo: União Paulista, 12 Jul. 2010, *N.T. Ranga et al. 02* (SJRP).

References

- Apg III (2009) An update of the Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161:105–121
- Barth OM, Melhem TS (1988) Glossário ilustrado de Palinologia. Editora da Universidade Estadual de Campinas, Campinas
- Bove CP (1993) Pollen morphology of the Bignoniaceae from a south Brazilian Atlantic forest. *Grana* 32:330–337
- Bove CP (1994) Morfologia polínica de Bignoniaceae (Lianas) do Brasil Meridional. *Rev Bras Biol* 54:273–291
- Bove CP, Barth OM (1992) Catálogo sistemático do pólen das plantas arbóreas do Brasil Meridional. XXV-Bignoniaceae. *Rev Bras Biol* 52:283–292
- Burelo-Ramos CM, Lorea-Hernández FG, Vovides AP (2009) Palynological survey of subtribe Pithecocteniinae (Bignoniaceae, Bignoniaceae). *Bot J Linn Soc* 159:155–162
- Buurman J (1977) Pollen morphology of the Bignoniaceae. *Pollen Spores* 19:447–519
- Chen S, Guan K, Zhou Z, Olmstead RG, Cronk Q (2005) Molecular phylogeny of *Incarvillea* (Bignoniaceae) based on ITS and *trnL-F* sequences. *Am J Bot* 92:625–633
- Corrêa-Gomes JC Jr (1951) Contribuição ao conhecimento das Bignoniaceae brasileiras. IV. *Rev Bras Biol* 11:49–52
- Corrêa-Gomes JC Jr (1955) Contribuição à sistemática das Bignoniaceae brasileiras. *Arquivos do Serviço Florestal* 9:261–296
- Corrêa-Gomes JC Jr (1957) Bignoniaceae. In *Flora de Itatiaia*. *Rodriguésia* 20:111–129
- De Sampaio AJ (1934) Caracteres de menor frequência nas Bignoniaceae. *Arq Mus Nac* 36:75–85
- Erdtman G (1952) Pollen morphology and plant taxonomy—Angiosperms. *Alquist and Wiksell, Stockholm*
- Erdtman G (1960) The acetolysis method. A revised description. *Sven Bot Tidskr* 54:561–564
- Fægri G, Iversen J (1966) Textbook of modern pollen analysis, 2nd edn. *Scandinavian University Books, Copenhagen*
- Ferguson IK, Santisuk T (1973) Notes on the pollen morphology of some Asiatic Bignoniaceae. *Kew Bull* 28:187–197
- Fischer E, Theisen I, Lohmann LG (2004) Bignoniaceae. In: Kadereit JW (ed) *The families and genera of vascular plants, vol. 7 Lamiales*. Springer, Berlin, pp 9–38
- Fonseca GAB (1985) The vanishing Brazilian Atlantic forest. *Biol Conserv* 34:17–34
- Gasparino EC, Cruz-Barros MAV, Chautems A (2013) Pollen morphology in Brazilian species of *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrader (Gesneriaceae). *Grana* 52:258–274
- Gentry AH (1973) Bignoniaceae. In: Crosby MR (ed) *Flora of Panama*. *Ann. Missouri Bot. Gard.* 60:781–977
- Gentry AH (1980) Bignoniaceae. Part I. Tribes Crescentieae and Tourretieae. *Flora Neotrop Monogr* 25:1–131
- Gentry AH, Tomb AS (1979) Taxonomic implications of Bignoniaceae Palynology. *Ann Mo Bot Gard* 66:756–777
- Gonçalves-Esteves V, Crespo SRM (1994) Estudos polínicos em plantas de restinga do estado do Rio de Janeiro—Bignoniaceae. *Juss. Bol Mus Nac* 91:1–13
- Grose SO, Olmstead RG (2007a) Evolution of a charismatic Neotropical clad: Molecular phylogeny of *Tabebuia* s.l., Crescentieae, and allied genera (Bignoniaceae). *Syst Bot* 32:650–659
- Grose SO, Olmstead RG (2007b) Taxonomic revisions in the polyphyletic genus *Tabebuia* s.l. (Bignoniaceae). *Syst Bot* 32:660–670
- Kronka FJN, Matsukuma CK, Nalon MA, Delcali IH, Rossi M, Mattos IFA, Shinike MS, Pontinhas AAS (1993) Inventário Florestal do Estado de São Paulo. Instituto Florestal, São Paulo
- Li J (2008) Phylogeny of *Catalpa* (Bignoniaceae) inferred from sequences of chloroplast *ndhF* and nuclear ribosomal DNA. *J Syst Evol* 46:341–348
- Lohmann LG (2003) Phylogeny, classification, morphological, diversification, and biogeography of Bignoniaceae (Bignoniaceae, Lamiales). University of Missouri, St. Louis, Tese de Doutorado

- Lohmann LG (2006) Untangling the phylogeny of neotropical lianas (Bignoniae, Bignoniaceae). *Am J Bot* 93:304–318
- Lohmann LG (2013) Bignoniaceae. In: R. C. Forzza (coord.), *Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. <http://floradobrasil.jbrj.gov.br/2012/FB112305>. Accessed Aug 2013
- Lohmann LG, Taylor CM (2013) A new generic classification of Bignoniae (Bignoniaceae) based on molecular phylogenetic data and morphological synapomorphies. *Ann Missouri Bot Gard* (in press)
- Lohmann LG, Ulloa Ulloa C (2012) Bignoniaceae. In: iPlants prototype checklist [online]. <http://www.iplants.org>. Accessed Aug 2013
- Lohmann LG, Bell CD, Calió MF, Winkworth RC (2013) Pattern and timing of biogeographical history in the Neotropical tribe Bignoniae (Bignoniaceae). *Bot J Linn Soc* 171:154–170
- Lopes MMM (2005) Bignoniaceae durante de um fragmento florestal, em Viçosa, Zona da Mata Mineira: florística e aspectos ecológicos. Universidade Federal de Viçosa, Viçosa, Dissertação de Mestrado
- McCune B, Mefford MJ (1999) PC-ORD Multivariate analysis of ecological data. MjM Software Desing, Oregon
- Melhem TS, Cruz-Barros MAV, Corrêa AMS, Makino-Watanabe H, Silvestre-Capelato MSF, Gonçalves-Estevês VL (2003) Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). *Bol Inst Bot* 16:1–104
- Myers N, Mittermeier RA, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853–858
- Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. *Rev Paleobot Palynol* 143:1–81
- Ranga NT, Rezende AA, Cavasan O, Toniato MTZ, Cielo Filho R, Stranghetti V (2012) Caracterização florística de remanescentes de vegetação nativa da região noroeste do Estado de São Paulo. In: Necchi O Jr (ed) *Fauna e Flora de fragmentos florestais remanescentes da região Noroeste do Estado de São Paulo*. Holos Editora, Ribeirão Preto, pp 105–135
- Rodrigues MC (2012) Bignoniáceas de dezoito fragmentos florestais remanescentes no Noroeste Paulista. Dissertação de Mestrado, Universidade Estadual Paulista, Botucatu
- Salgado-Labouriau ML (1973) Contribuição à Palinologia dos Cerrados. Academia Brasileira de Ciências, Rio de Janeiro, p 219
- Salgado-Labouriau ML, Barth OM (1962) Pollen grains of the “Cerrado”-II. *An Acad Bras Ciênc* 34:87–100
- Salgado-Labouriau ML, Vanzolini PE, Melhem TS (1965) Variation of polar axes and equatorial diameters in pollen grains of two species of *Cassia*. *Grana Palynol* 6:98–105
- Shepherd GJ (1996) Fitopac 1: manual do usuário. Departamento de Botânica, Universidade Estadual de Campinas, Campinas
- Silvestre MS (1984) Contribuição ao Estudo Palinológico das Bignoniaceae. Dissertação de Mestrado, Universidade Estadual de Campinas, São Paulo
- Silvestre MS, Melhem TS (1989) Flora polínica da Reserva do Parque Estadual das Fontes do Ipiranga. *Hoehnea* 16:211–228
- Spangler RE, Olmstead RG (1999) Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences *rbcl* and *ndhF*. *Ann Mo Bot Gard* 86:33–46
- Suryakanta S (1973) Pollen morphological studies in the Bignoniaceae. *J Palynol* 9:45–82
- Turner IM (1996) Species loss in fragments of tropical rain forest: a review of the evidence. *J Appl Ecol* 33:200–209
- Vieira S (1981) Introdução à Bioestatística. Campus, Rio de Janeiro
- Zar JH (1996) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs
- Zyhra ML, Sytsma KJ, Olmstead RG (2004) Delimitation of Malagasy tribe Coleeae and implications for fruit evolution in Bignoniaceae inferred from a chloroplast DNA phylogeny. *Plant Syst Evol* 245:55–67