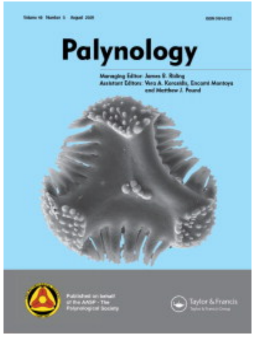

Palynology

ISSN: 0191-6122 (Print) 1558-9188 (Online) Journal homepage: www.tandfonline.com/journals/tpal20

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To cite this article: Eduardo Custódio Gasparino, Isaura de Paula Cerdan, Fernanda Vitorete Dutra, Jaqueline Alves Vieira & Daniela Sampaio (15 Sep 2025): Pollen morphology of *Ternstroemia* Mutis ex L.f. (Pentaphylacaceae): describing morphological variations in a stenopalynous taxon, Palynology, DOI: 10.1080/01916122.2025.2562506

To link to this article: https://doi.org/10.1080/01916122.2025.2562506

running title: Pollen of *Ternstroemia* (Pentaphylacaceae)

Pollen morphology of *Ternstroemia* Mutis ex L.f. (Pentaphylacaceae): describing morphological variations in a stenopalynous taxon

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Abstract

Currently classified within the Ericales, Pentaphylacaceae comprises 511 species and 14 genera with tropical and subtropical distributions. *Ternstroemia*, the genus with the most species of the family, exhibits the greatest diversity and the broadest geographic range within the family, with Brazil hosting the highest number of species. The species within this genus are challenging to delineate due to their morphological similarities, particularly in floral organs, with no detailed descriptions of their pollen morphology. This study aimed to morphologically analyze the pollen of eight *Ternstroemia* species and *Freziera atlantica* (a related Brazilian species), providing both qualitative and quantitative data on pollen grains that could help in species delimitation. Pollen grains were acetolyzed and measured, qualitative data was described, and quantitative data was analyzed using descriptive statistics. Stamen data were added to the pollen data for multivariate analyses. An artificial pollen key and illustrations in light microscopy (LM) and scanning electron microscopy (SEM) are presented. *Ternstroemia* pollen grains are monads, isopolar, small to medium, prolate spheroidal or subprolate, 3-colporate, and microreticulate. The ornamentation, presence of a costa, and a lalongate endoaperture may serve as diagnostic features for the genus, since in *F. atlantica* we observed psilate-perforate pollen, without a costa and with a circular endoaperture. Despite consistent pollen morphology, features such as amb, ectoaperture length, and the shape and position of the apertures help distinguish *Ternstroemia* species.

Key words: Ericales, palynology, pollen grains, taxonomy.

1. Introduction

Pentaphylacaceae (Ericales) is represented by 14 genera (Schönenberger et al. 2005; Tsou et al. 2016) and includes species of economic importance, especially species of *Ternstroemia*, which have medicinal value (Moreno-Quirós et al. 2017), are important for firewood production and dyes (Sakar and Devi 2017) and have ecological value as a resource for avifauna (Melo-Jr. et al. 2016) and as ornamental plants. Among the most abundant genera of Pentaphylacaceae, *Freziera* Willd. and *Ternstroemia* are represented in Brazil with a high degree of endemism (Santamaría-Aguilar et al. 2015; Vieira 2020).

Taxonomically, the genera now circumscribed in Pentaphylacaceae were previously assigned to Theaceae, Ternstroemiaceae, and Sladeniaceae. However, Tsou et al. (2016), through phylogenetic analyses based on ribosomal *ITS* and TrnL-F, proposed a new circumscription for Pentaphylacaceae, dividing the family into four tribes: Sladenieae, Pentaphylaceae, Ternstroemieae, and Frezierieae (Tsou et al. 2016; Vieira 2020). Among the genera in the family, *Ternstroemia* has a complex taxonomic history, with several changes in the circumscription of its species within families and tribes (De Candolle 1824; Ming and Bartholomew 2007; Tsou et al. 2016). Additionally, the genus has numerous synonyms (Vieira 2020). Despite the recent phylogenetic study by Tsou et al. (2016) treating *Ternstroemia* as a member of Pentaphylaceae, some authors still recognize the genus within Ternstroemiaceae (Grande 2021).

Ternstroemia Mutis ex L.f. is one of the most numerous and diverse genera of Pentaphylacaceae, occurring widely in the Subtropical and Tropical regions worldwide, with the greatest diversity of species in the Neotropical region, occurring from southern North America to southern South America (Vieira et al. 2024). Of the 511 species described for the family, 160 belong to *Ternstroemia*, with a significant number of these being endemic to the regions where they occur (Kobuski 1942a, b; Vieira et al. 2021a, b; Vieira and Sampaio 2024 [continuously updated]; Vieira et al. 2024). Brazil has the highest number of species, 27, including all the species considered by Vieira et al. (2024), in addition to *T. discoidea* Gleason and *T. klugiana* Kobuski considered by Fonseca-Cortés and Grande-Allende (2024), and Grande and Castillo (2024), but following the circumscription of *T. brasiliensis* in Vieira (2020) (*T. atlantica* J.R.Grande + *T. brasiliensis* Cambess.). In Brazil, 13 of these 27 species (47%) are endemic (Vieira 2020; Vieira and Sampaio 2024 [continuously updated]; Grande and Castillo 2024; Vieira et al. 2024).

Overall, phylogenetic studies involving Pentaphylacaceae are scarce, with Wu et al. (2007) and Tsou et al. (2016) providing limited sampling for *Ternstroemia*. A phylogenetic study for neotropical species of the genus is currently being developed (Sampaio and Vieira, personal communication). Moreover, species of the genus are difficult to delineate due to numerous morphological similarities, with diagnostic morphological characters found mainly in floral organs (Vieira 2020; Vieira and Sampaio 2024 [continuously updated]). Regarding palynology, data on the pollen morphology of *Ternstroemia* species are available only from specific studies (Keng 1962; Wei et al. 1999; Xu et al. 2011) or pollen flora, such as in Taiwan (Huang 1972), Malaysian Borneo (Jones and Pearce 2015), and China woody plants (Li et al. 2010). As previously reported by Luna and Ochoterena (2004), pollen studies for Pentaphylacaceae (including also taxa treated in Theaceae) are scattered and difficult to compare due to different techniques and terminologies. As a result, detailed palynological studies that focus on describing the morphological characteristics of pollen grains are lacking, with only Erdtman (1952) providing data on the pollen of Ternstroemieae when it was still considered a tribe within Theaceae.

Therefore, the aim of this study was to morphologically analyze the pollen of eight *Ternstroemia* species and *Freziera atlantica* (a related Brazilian species), providing both qualitative and quantitative data on pollen grains that could help in species delimitation.

2. Material and Methods

We studied the pollen grains of eight Brazilian species of *Ternstroemia* and one species of *Freziera*, in 13 specimens (Table 1); despite the reduced number of species, this number represents 35% of the Brazilian species, and of the species analyzed, only *Ternstroemia campinicola* Boom, *T. delicatula* Choisy and *T. dentata* (Aubl.) Sw. are not endemic to Brazilian territory. The pollen material used in the analysis was obtained from specimens from INPA, PACA, and SJRP herbaria (acronyms, according to Thiers 2016 – continuously updated).

For the light microscopy (LM) study, the material was processed according to Erdtman (1960) with the modifications cited by Melhem et al. (2003). Permanent light microscopy slides are archived in the Plant Morphology and Palynology Laboratory

(LaMPali) pollen reference collection at the Department of Biology, Universidade Estadual Paulista, UNESP, Jaboticabal, Brazil. The pollen grains' diameters (*n* = 25) and aperture and exine thickness (*n* = 10) were measured under light microscopy up to seven days after their preparation (Salgado-Labouriau 1973). For scanning electron microscopy (SEM) some non-acetolyzed pollen grains were used, following Melhem et al. (2003).

Statistical descriptive analysis included mean (x), standard deviation (sx), standard error (s), 95% confidence intervals (CI), coefficient of variability (V), and range (R), as per Vieira (2011) and Zar (2010). These calculations were performed for pollen grain diameter measurements. Only the arithmetic mean (x) was calculated for aperture and exine layer measurements. We used Microsoft Excel® graphs to compare pollen grain diameters and pollen shape (P/E ratio).

As a multivariate analysis and to verify if the pollen data allow the grouping of the analyzed species, a principal component analysis (PCA) was performed using the programs FITOPAC 1 (Shepherd 1996) and PC-ORD version 5.15 (McCune and Mefford 2011). Based on the pollen grains and stamens metric data, this grouping will provide information on which analyzed species are similar; the analysis also indicates the correlation of the variables with the grouping. For this analysis, we used Euclidean distance and Pearson correlation (Legendre and Gallagher 2001; Cerdan et al. 2024). The 18 metrical variables of pollen grains and stamens used for the PCA were: equatorial diameter in polar view (EDPV), polar diameter in equatorial view (PDEV), equatorial diameter in equatorial view (EDEV), shape (SHAP), length of ectoaperture (LEEC), width of ectoaperture (WIEC), length of endoaperture (LEEN), width of endoaperture (WIEN), polar area index (PAI), exine thickness (EXIN), sexine thickness (SEXI), nexine thickness (NEXI), minimum filament length (MiFL), maximum filaments length (MaFL), length minimum of the anther (LMiA), length maximum of the anther (LMaA), length minimum of the connective (LMiC), length maximum of the connective (LMaC). For this analysis, stamen data were obtained from Vieira (2020).

The qualitative pollen description was based on what was proposed by Bellonzi et al. (2020) and pollen terminology followed by Punt et al. (2007) and Halbritter et al. (2018). We used the polar area index by Faegri & Iversen (1966), the colpus width index, and the colpus length index by Gasparino et al. (2013) and Dutra et al. (2023), respectively. The definition of endoaperture shapes followed that proposed by Erdtman (1952), Punt et al. (2007), and Soares et al. (2021). The relationship between apertures and amb followed Walker & Doyle (1975). Considering that some indexes overlap in the quantitative data, we used the adjustments proposed by Soares et al. (2024).

3. Results

3.1. Pollen morphology description of species

Freziera atlantica Zorzan. & Amorim (Plate 1, Figures 1-4; Tables 2-3).

Pollen grains in monads, isopolar, small size (Table 2-3), subtriangular amb (Plate 1, Figure 1), small polar area, oblate spheroidal (Table 3); 3-colporate, angulaperturate, with fastigium (Plate 1, Figure 1), long and narrow ectoapertures, with margo; circular endoapertures not evident. Exine tectate, psilate-perforate. Thick exine and sexine thicker than nexine (Table 3). In SEM, the pollen grains are fossaperturate (Plate 1, Figure 2), and we can identify small holes in tectum less than 1 µm (Plate 1, Figures 3-4).

Ternstroemia brasiliensis Cambess. (Plate 1, Figures 5-7; Tables 2-3)

Pollen grains in monads, isopolar, small size (Table 2), subcircular amb (Plate 1, Figure 5), small polar area, prolate spheroidal (Table 3); 3-colporate, circulaperturate, with fastigium, very long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 1, Figure 7), with costa. Exine semitectate, microreticulate. Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia campinicola Boom (Plate 1, Figures 8-11a-b; Tables 2-3).

Pollen grains in monads, isopolar, medium size (Table 2-3), subtriangular amb (Plate 1, Figure 8), small polar area, subprolate (Table 3); 3-colporate, angulaperturate, with fastigium (Plate 1, Figure 8), long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 1, Figure 10), with costa. Exine semitectate, microreticulate (Plate 1, Figure 11a-b). Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia carnosa Cambess. (Plate 1, Figures 12-16; Tables 2-3).

Pollen grains in monads, isopolar, medium size (Table 2-3), subcircular amb (Plate 1, Figures 12, 15), small polar area, prolate spheroidal (Table 3); 3-colporate, circulaperturate, with fastigium (Plate 1, Figure 12), long and narrow ectoapertures, with margo (Plate 1, Figure 14) and rounded to the ends; lalongate endoapertures, with costa. Exine semitectate, microreticulate (Plate 1, Figures 15-16). Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia delicatula Choisy (Plate 1, Figures 17-20a-b; Tables 2-3).

Pollen grains in monads, isopolar, medium size (Table 2-3), circular amb (Plate 1, Figure 17), small polar area, subprolate (Table 3); 3-colporate, circulaperturate, with fastigium (Plate 1, Figure 17), long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 1, Figure 19), with costa. Exine semitectate, microreticulate (Plate 1, Figure 20a-b). Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia dentata (Aubl.) Sw. (Plate 2, Figures 1-4; Tables 2-3).

Pollen grains in monads, isopolar, small size (Table 2-3), subcircular amb (Plate 2, Figures 1, 3), small polar area, prolate spheroidal (Table 3); 3-colporate, angulaperturate, with fastigium (Plate 2, Figure 1), long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 2, Figure 2), with costa. Exine semitectate, microreticulate (Plate 2, Figures 3-4). Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia rupestris J.A.Vieira & D.Sampaio (Plate 2, Figures 5-7; Tables 2-3).

Pollen grains in monads, isopolar, small size (Table 2-3), subcircular amb (Plate 2, Figure 5), very small polar area, subprolate (Table 3); 3-colporate, circulaperturate, with fastigium, very long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 2, Figure 7), with costa. Exine semitectate, microreticulate. Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia subcaudata Kobuski (Plate 2, Figures 8-9; Tables 2-3).

Pollen grains in monads, isopolar, small size (Table 2), subtriangular amb (Plate 2, Figure 8), small polar area, prolate spheroidal (Table 3); 3-colporate, angulaperturate, with fastigium, long and narrow ectoapertures, with margo and rounded to the ends; lalongate endoapertures (Plate 2, Figure 9), with costa. Exine semitectate, microreticulate. Thin exine, and sexine thicker than nexine (Table 3).

Ternstroemia urophora Kobuski (Plate 2, Figures 10-12; Tables 2-3).

Pollen grains in monads, isopolar, medium size (Table 2), subcircular amb (Plate 2, Figure 10), small polar area, prolate spheroidal (Table 3); 3-colporate, circulaperturate, with fastigium, long and narrow ectoapertures (Plate 2, Figure 11), with margo and rounded to the ends; lalongate endoapertures (Plate 2, Figure 12), with costa. Exine semitectate, microreticulate. Thin exine, and sexine thicker than nexine (Table 3).

3.2. Artificial pollen key

1. Psilate-perforate pollen grains ……………………..…………… *Freziera atlantica*

1'. Microreticulate pollen grains …………………………………………………... 2

2. Angulaperturate aperture position …………………...…………………...…….. 3

2'. Circulaperturate aperture position ……………………………………..…………….. 5

3. Pollen with subcircular amb ……………………………...…….. *Ternstroemia dentata*

3'. Pollen with subtriangular amb ……………………………………………...……….. 4

4. Medium pollen with subprolate shape ……………….……. *Ternstroemia campinicola*

4'. Small pollen with prolate spheroidal shape ……………..…. *Ternstroemia subcaudata*

5. Ectoapertures very long ………………………………………………………… 6

5'. Ectoapertures long ………………………………………………….…………….. 7

6. Small polar area and prolate spheroidal shape ……………. *Ternstroemia brasiliensis*

6'. Very small polar area and subprolate shape ……………...…… *Ternstroemia rupestris*

7. Circular amb and subprolate shape …………..........………….. *Ternstroemia delicatula*

7'. Subcircular amb and prolate spheroidal shape 8

8. Ectoaperture length > 18.00 μm ……………………………… *Ternstroemia carnosa*

8'. Ectoaperture length ≤ 18.00 μm ……........................………… *Ternstroemia urophora*

3.3. Pollen size and shape

The Pentaphylacaceae species analyzed here have pollen grains varying from small to medium (Figure 1). The pollen grains of *Freziera atlantica*, *Ternstroemia brasiliensis*, *T. dentata*, *T. rupestris*, and *T. subcaudata* are small, while those of the other species are medium (Tables 2-3). The smallest diameter values were observed in *Freziera atlantica* (Figure 1A-B), while the largest diameters were recorded in the pollen of *Ternstroemia carnosa* and *T. urophora*. The polar diameter data in equatorial view (PDEV, Figure 1A) indicate values greater than 25 μm for the pollen of *T. delicatula*, *T. campinicola*, *T. carnosa* and *T. urophora;* when we observe the equatorial diameter values in equatorial view (EDEV, Figure 1B), only *T. carnosa* and *T. urophora* have data greater than 25 μm for the diameters. The species analyzed form three distinct groups based on their polar diameters in an equatorial view (Figure 1A). *Freziera atlantica* shows the lower values, *T. subcaudata*, *T. rupestris*, *T. dentata*, and *T. brasiliensis* have intermediate values, and the other species have larger pollen grain diameters. Regarding the equatorial diameter and view, all analyzed species display similar pollen grain diameters, forming a large continuous group (Figure 1B).

The shape of the pollen grains analyzed here varied from subprolate in *Ternstroemia campinicola*, *T. delicatula*, and *T. rupestris*, or prolate spheroidal in the other species (Table 3). Although this characteristic is generally variable in pollen grains, the species analyzed here in the genus *Ternstroemia* maintain a subprolate to prolate spheroidal shape in their pollen grains, unlike *Freziera atlantica*, which presents more oblate pollen grains (in this case, oblate spheroidal – Figure 2). Among the *Ternstroemia* species, *T. subcaudata* and *T. dentata* have prolate spheroidal pollen grains that range to oblate spheroidal (Figure 2). The other species in the genus have prolate spheroidal pollen grains that vary to subprolate, except for *T. delicatula*, which has subprolate to prolate pollen grains (Figure 2).

The data obtained here regarding pollen shape for *Ternstroemia* species and *Freziera atlantica* indicate the considerable variability of this character in these taxa (Figure 2). Despite this, we were able to observe that for *Ternstroemia* species, this variation can be: prolate to subprolate (*T. delicatula*), subprolate to prolate spheroidal (*T. carnosa*, *T. urophora*, *T. brasiliensis*, *T. campinicola* and *T. rupestris*), prolate spheroidal to oblate spheroidal (*T. subcaudata* and *T. dentata*); for *Freziera atlantica*, this variation in shape can record suboblate, oblate spheroidal and prolate spheroidal pollen (Figure 2).

3.4. Principal component analysis (PCA) with quantitative data

When analyzing the quantitative data of pollen and stamens explored through multivariate analysis, we found that the results express 65.57 % of the variability of the metric data measured, which were obtained from the 18 metric variables (Table 4). The first axis of the PCA summarized 34.72% of the analyzed data (Figure 3).

On this axis, the values of width of endoaperture (Table 4) stand out, which have a moderate correlation with the axis, while the other variables have a low or insignificant correlation; thus, the positioning of the species along axis 1, according to the metric data of the pollen and stamens was ordered based on the contribution of all the variables included in the analysis. Thus, it can be observed that *Freziera atlantica* stands out, isolated from the *Ternstroemia* species, on the negative side of the axis, most likely due to the exine values of the pollen grains (NEXI and EXIN). In contrast, the other species are distributed in the central and positive part of axis 1 (Figure 3). We can highlight this group consisting of the species *Ternstroemia*, *T. urophora*, and *T. subcaudata* on the right side of axis 1, separated from the other species by high values of most variables (except NEXI and EXIN).

Axis 2 of the PCA explains 30.85% of the total variability of the data used in this analysis, highlighting the moderate correlation of maximum filaments length (MaFL – Table 4) with this axis. We can observe the influence of this second analysis component, forming three clusters for the *Ternstroemia* species. On the positive side of axis 2, *T. campinicola* (Figure 3) stands out, with the highest values for the variables most related to axis 2; on the negative side of axis 2, *T. brasiliensis* and *T. subcaudata* are found, separated from the other species by the MiFL and MaFL; and finally, a third cluster in the central region of axis 2 with the other *Ternstroemia* species, indicating intermediate values for all the variables analyzed. It is worth noting that, regarding axis 2 of the PCA, *Freziera atlantica* pollen grains are associated with *T. brasiliensis* and *T. subcaudata* based on the data analyzed.

4. Discussion

For palynology, morphological characters related to the type and number of apertures and the exine ornamentation are the primary taxonomic diagnostics (Melhem et al. 2003; Gasparino 2022). *Ternstroemia* and *Freziera atlantica* species analyzed here present 3-colporate pollen grains, generally with a long or very long and narrow ectoaperture (colpus) and psilate-perforate (*F. atlantica*) or microreticulate ornamentation. Additionally, diagnostic characters distinguishing the genera analyzed here include circular endoapertures in *Freziera atlantica* pollen grains and lalongate endoapertures in the *Ternstroemia* species. Furthermore, a thin exine and costa were observed in the pollen grains of the *Ternstroemia* species. In contrast, in the *Freziera atlantica* pollen grains we observed a thick exine and the absence of a costa.

Defined by Iversen & Troels-Smith (1950) and Punt et al. (2007), and present in the pollen grains of *Ternstroemia* species, costa is an internal thickening of the nexine around the endoaperture. This thickening appears in light microscopy as a margo at the endoaperture. Note that this morphological characteristic was not observed in the pollen of *Freziera atlantica*. The fastigium, observed for *F. atlantica* e *Ternstroemia* pollen grains, is defined as a separation of the exine layers in the region of the pollen apertures, forming a cavity (Reitsma 1966; Punt et al. 2007).

When we analyzed only the pollen grains of *Ternstroemia* species, we found similarities in terms of the type and number of apertures and the pattern of exine ornamentation. However, discrete morphological variations can be identified as to the size, the amb, the size of the polar area, the shape, the position of the apertures, and the length of the ectoapertures; these characters are used to distinguish taxa (pollen key).

In this study, we observed a variation in pollen size from small to medium, with values similar to those observed by Jones and Pearce (2015). Keng (1962), analyzing the pollen of Theaceae, describes small pollen for Ternstroemioideae; however other genera close to *Friziera* and *Ternstroemia* analyzed here have also been reported with size variation from small to medium (Maguire et al. 1972; Seetharam 1985; Luna and

Ochoterena 2004). For Jones and Pearce (2015), *Ternstroemia* pollen EDEV values are higher than PDEV values, indicating that the P/E ratio differs, which directly affects the shape classes of the pollen grains (Erdtman 1952).

The amb in *Ternstroemia* pollen grains varied from subtriangular, circular to subcircular, and the position of the apertures from angulaperturate to circulaperturate (in LM). Therefore, despite being discrete characteristics, these qualitative data allow the distinction of species groups.

Previous studies on the palynology of Pentaphylacaceae, including related taxonomic entities (such as Theaceae), describe the difficulty in identifying the endoapertures for colporate pollen grains, which are defined as colporoidate (Luna and Ochoterena 2004). In this context, Lee (1987) cites 3-colporate pollen grains for Ternstroemioideae and 3-colporoidate for Theoideae. This fact was also observed by Erdtman (1952) and Keng (1962).

Erdtman (1952), when describing the pollen grains of Ternstroemieae, mentions difficulty in identifying the endoapertures for species of the tribe (3-colporoidate pollen grains), which in our study was only observed in *Freziera atlantica*, since the endoapertures of the pollen grains of *Ternstroemia* species are easily visible. So, the endoapertures of *F. atlantica* pollen grains are difficult to visualize and can be considered colporoidate, whereas for *Ternstroemia* species the colpori are well defined and demarcated. The terminology (colporoidate) that indicates poorly defined endoapertures (Erdtman 1952) is not used here because it is considered obsolete (Punt et al. 2007).

Ternstroemia brasiliensis and *T. rupestris* presented pollen grains with very long ectoapertures (colpi); however, only *T. rupestris* presents a very small polar area. The relationship between the measurement of the apocolpium side and the equatorial diameter in the polar view defines the polar area index (Faegri and Iversen 1966). This calculation was initially proposed by Iversen and Troels-Smith (1950), who identified classes for the polar area and suggested a relationship with the length of the ectoapertures. However, these calculations do not consider the polar diameter in the equatorial view of the pollen grains, which can significantly influence the definition of the length of the ectoapertures (see Dutra et al. 2023). This explains the variation in the polar area index for *T. brasiliensis* and *T. rupestris* even in species presenting pollen with very long ectoapertures.

When defining characters and character states in a study of the phylogenetic relationships of Theaceae and related groups, Luna and Ochoterena (2004) reviewed the variations in the ornamentation of pollen grains of these taxa, considering two distinct types: (0) very evident, and (1) smooth or with little ornamentation. The authors define as very evident the ornamentation that presents visible supratectal elements, while for psilate, granulate or rugulate pollen grains these were considered smooth or with little ornamentation (Luna and Ochoterena 2004). Keng (1962) described the pollen grains of Ternstroemioideae as smooth, data corroborated by Weitzman (1987), who described psilate, granulate or rugulate pollen grains for *Freziera* species and less evident ornamentation for Ternstroemioideae. Our results confirm a less evident pollen ornamentation (smooth or psilate) for the pollen grains of *Freziera atlantica* as observed by Keng (1962) and Weitzman (1987); however, the microreticulum identified in the pollen grains of *Ternstroemia* species evidence more elaborate ornamentation. Similar results were described by Wei et al. (1999) when observing pollen grains with rugulate or rugulate-reticulate ornamentation for Pentaphylacaceae and Sladeniaceae. Reticulate pollen grains were described by Jones and Pearce (2015) for the genus *Ternstroemia*, diverging from the data obtained here since we identified microreticulate pollen grains.

Multivariate analysis (PCA) allowed us to identify which metric data of pollen grains and stamens can help distinguish Pentaphylacaceae genera (separating *Freziera atlantica* from *Ternstroemia* species) and form species groups within *Ternstroemia*, which can be used in future taxonomic study for the group. It is essential to emphasize that the analysis of quantitative data and the morphological description (qualitative data) can enrich future analyses by clarifying the relationships between species or groups of species that are currently controversial. Note that of the variables used in this analysis, only the width values of endoapertures showed a moderate correlation with the axes, while the other variables have a low or insignificant correlation, according to the parameters of Hinkle et al. (2003).

It is worth noting that, despite being discreet, qualitative differences in pollen, together with stamen data, contribute to the distinction of species or groups of species (as can be presented in Cluster analysis). These qualitative data, combined with morphometric data (identified in the PCA analysis), contribute to elucidating attributes with taxonomic potential for *Ternstroemia*. Thus, the present study identifies morphological patterns in pollen that can be explored in the future since palynological studies in Pentaphylacaceae are scarce.

5. Conclusions

Although we only analyzed a few species, our study identified some morphological features of pollen grains in Pentaphylacaceae that can help distinguish the genera, such as the presence of costae and lalongate endoapertures that characterize *Ternstroemia* species. These features were not identified in the pollen grains of *Freziera atlantica*, and the endoapertures are difficult to visualize. This differentiation of the genera was also confirmed in the quantitative data of the pollen grains.

With 3-colporate pollen grains, lalongate endoapertures, costa, and microreticulate ornamentation, *Ternstroemia* species present a similar pollen morphology, characterizing a stenopalynous group. However, discrete details of the pollen grains such as shape, amb, ectoaperture length, and polar area index can aid in distinguishing species groups.

Conflicts of Interest

The authors reported no potential conflicts of interest.

Acknowledgments: The authors are grateful to Fapesp (2022/05573-8) and CNPq (#309555/2021-3) for E.C. Gasparino, the curators of the herbaria for pollen sampling, and the Laboratório de Microscopia Eletrônica, FCAV, for providing technical assistance with scanning electron microscopy.

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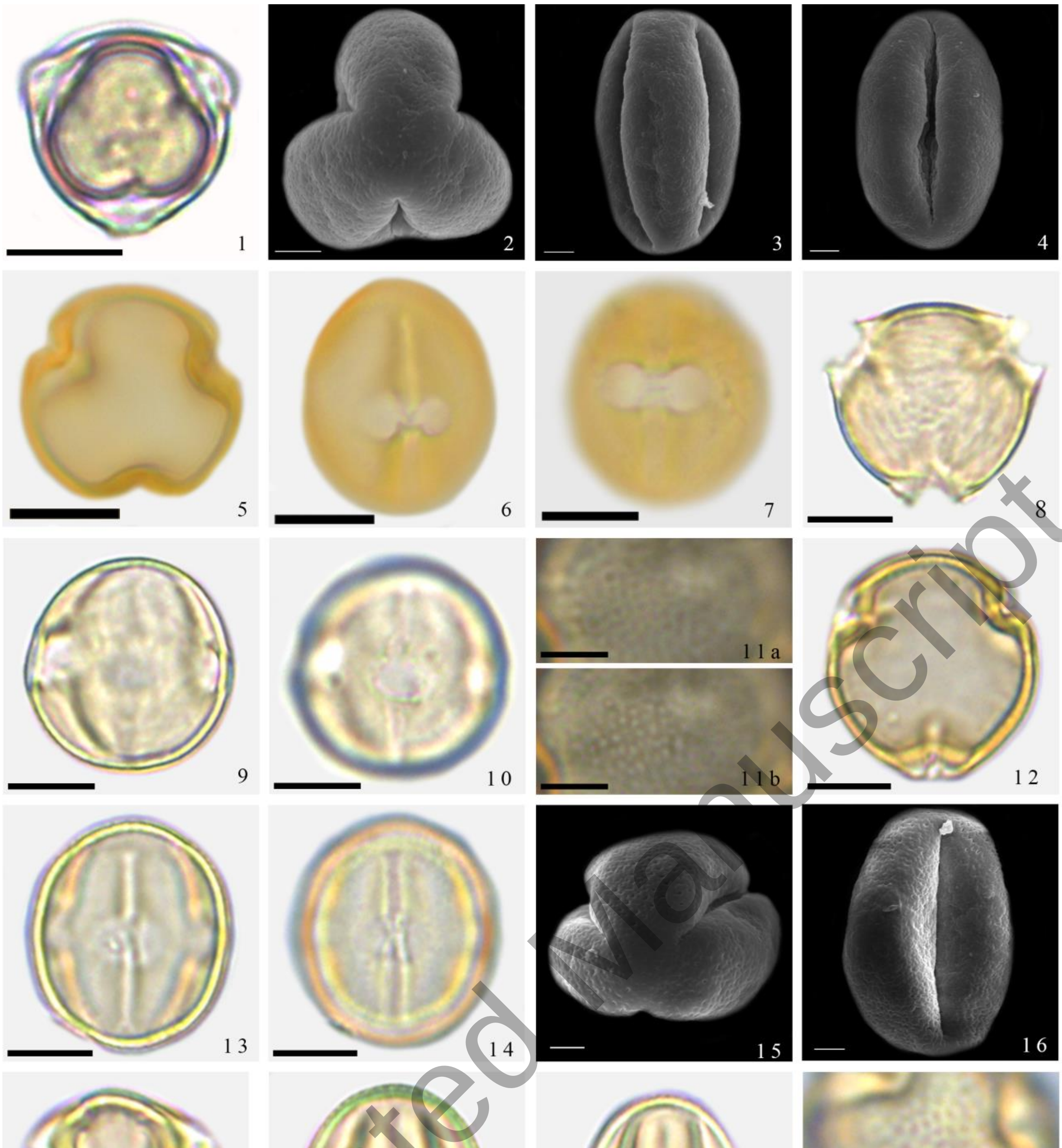

Plate 1. Photomicrographs and electron micrographs of the pollen grains of *Freziera* and *Ternstroemia* species (Pentaphylacaceae). 1-4. *Freziera atlantica*; 5-7. *Ternstroemia brasiliensis*; 8-11. *Ternstroemia campinicola*; 12-16. *Ternstroemia carnosa*; 17-20. *Ternstroemia delicatula*. Polar view (1, 2, 5, 8, 12, 15, 17), equatorial view, aperture (3, 4, 6, 9, 10, 13, 14, 16, 18, 19), details of ornamentation (7, 11a, 11b, 20a, 20b). Bars: 10 µm =1, 5, 6, 7, 8, 9, 10, 12, 13, 14, 17, 18, 19. 5 µm = 11a, 11b, 20a, 20b. 2 µm. = 2, 3, 4, 15, 16.

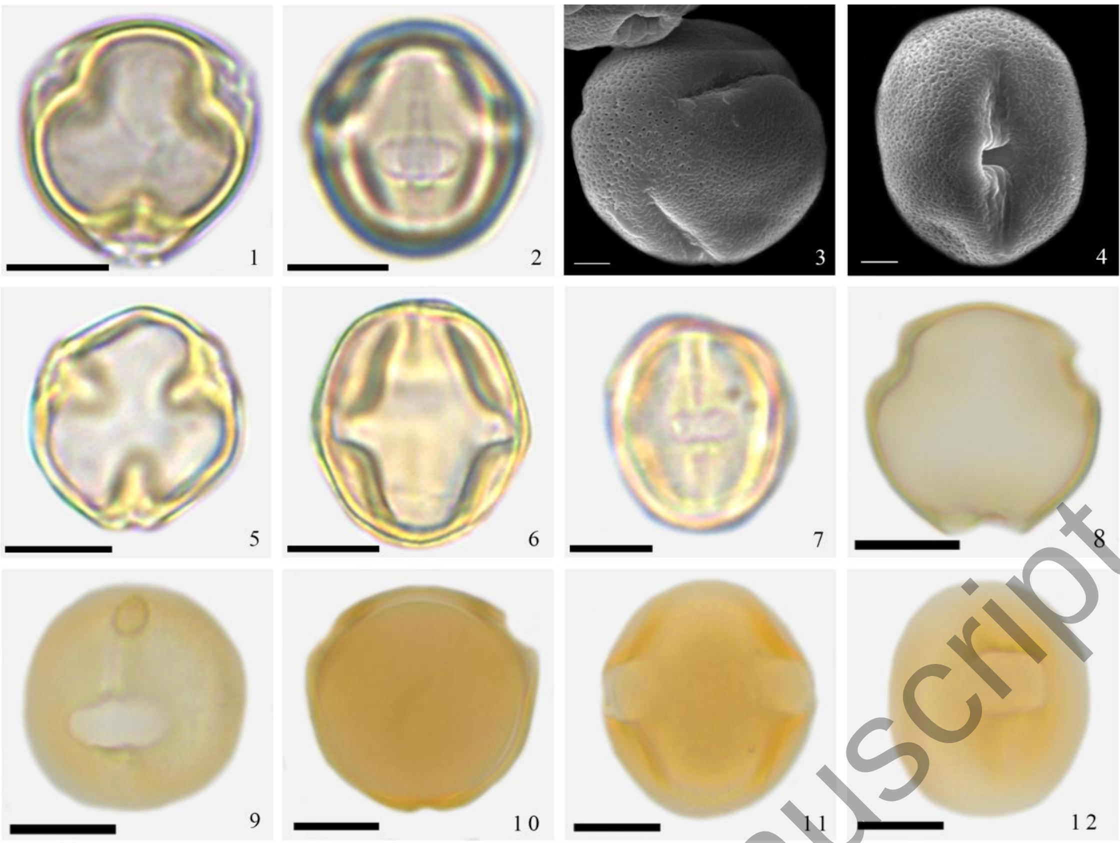

Plate 2. Photomicrographs and electron micrographs of the pollen grains of *Ternstroemia* species (Pentaphylacaceae). 1-4. *Ternstroemia dentata*. 5-7. *Ternstroemia rupestris*. 8-9. *Ternstroemia subcaulata*. 10-12. *Ternstroemia urophora*. Polar view (1, 3. 5, 8, 10), equatorial view, aperture (2, 4, 6, 7, 9, 11, 12), details of ornamentation (3,4). Bars: 10 µm =1, 2, 5-12. 2 µm = 3, 4.

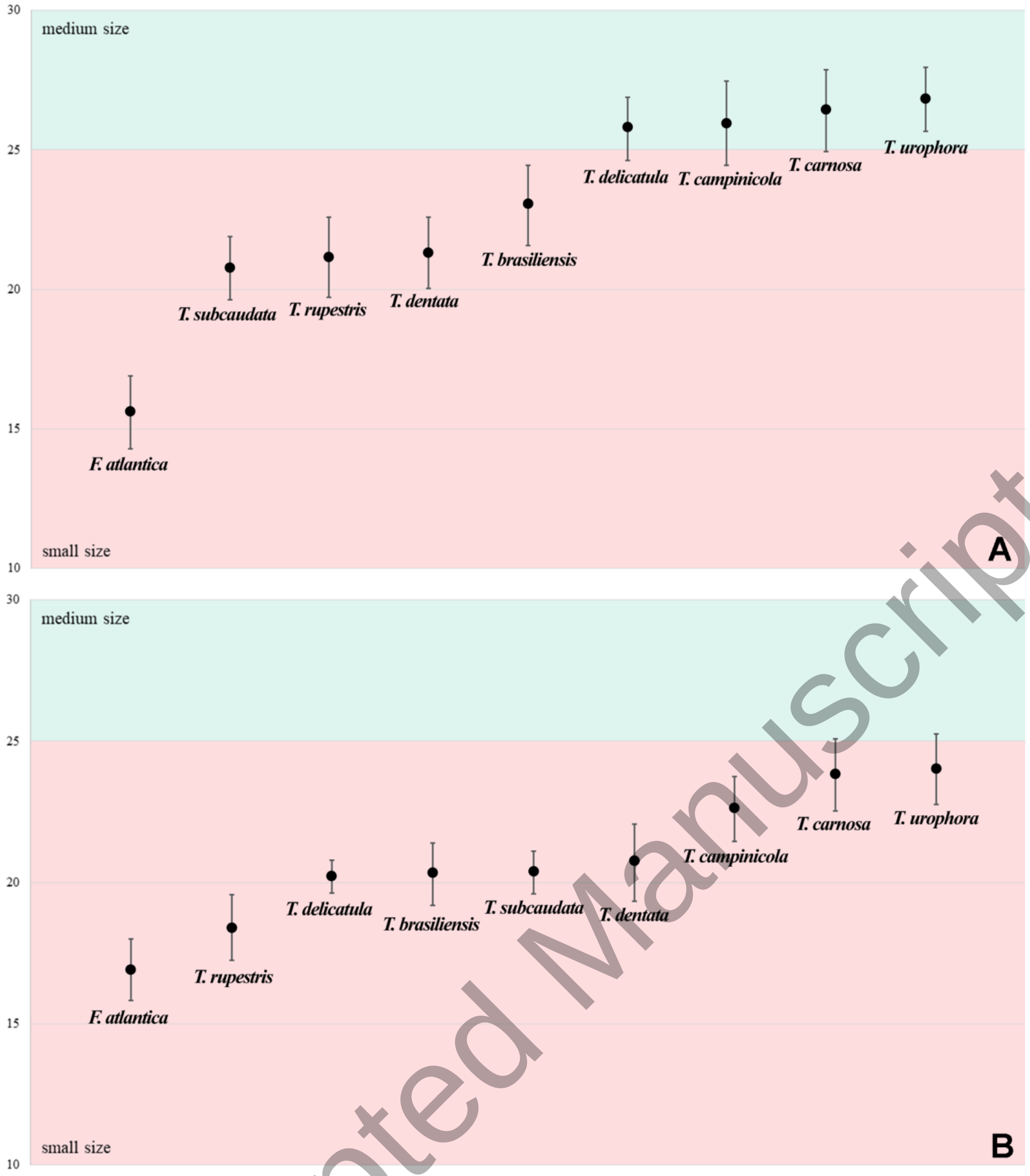

Figure 1. Representation of confidence interval of mean in 95 % of the pollen grains of *Freziera* and *Ternstroemia* (Pentaphylacaceae). A. Polar diameter in equatorial view. B. Equatorial diameter in equatorial view. The higher and lower boundaries show the confidence interval; The average circle shows the arithmetic mean. The values are in µm. (F. atla = *Freziera atlantica*; T. bras = *Ternstroemia brasiliensis*; T. camp = *Ternstroemia campinicola*; T. carn = *Ternstroemia carnosa*; T. deli = *Ternstroemia delicatula*; T. dent = *Ternstroemia dentata*; T. rupe = *Ternstroemia rupestris*; T. subc = *Ternstroemia subcaudata*; T. urop = *Ternstroemia urophora*).

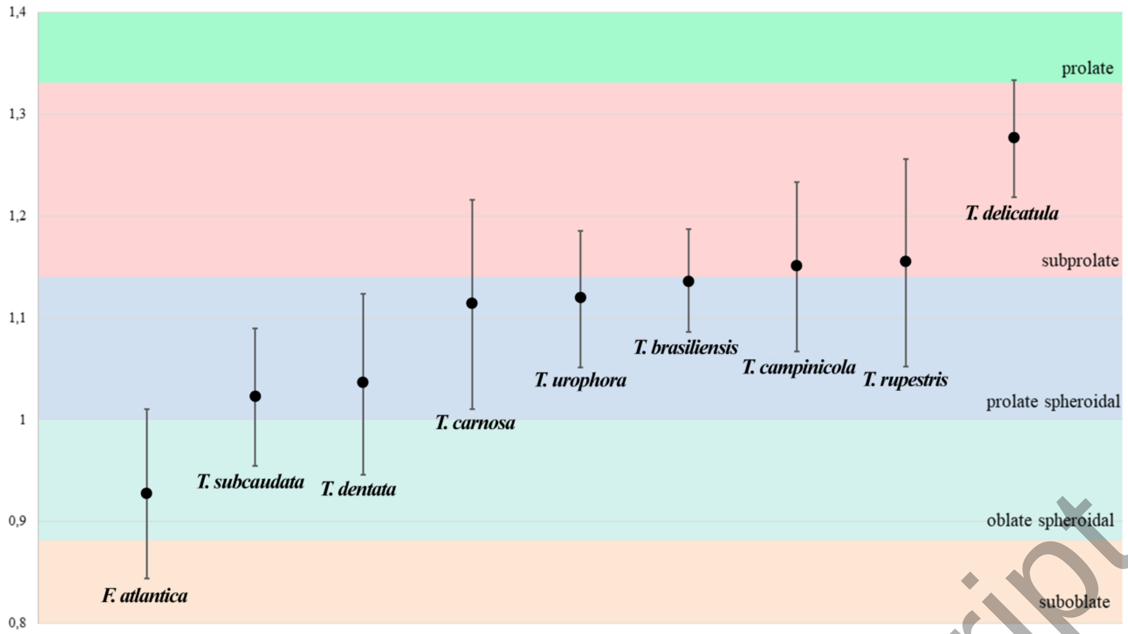

Figure 2. Graphical representation of the arithmetic means and sample standard deviation of the P/E ratio (shape) of *Freziera* and *Ternstroemia* (Pentaphylacaceae) pollen grains. The bars represent the limits of the sample standard deviation, and the circles represent the arithmetic mean. (F. atla = *Freziera atlantica*; T. bras = *Ternstroemia brasiliensis*; T. camp = *Ternstroemia campinicola*; T. carn = *Ternstroemia carnosa*; T. deli = *Ternstroemia delicatula*; T. dent = *Ternstroemia dentata*; T. rupe = *Ternstroemia rupestris*; T. subc = *Ternstroemia subcaudata*; T. urop = *Ternstroemia urophora*).

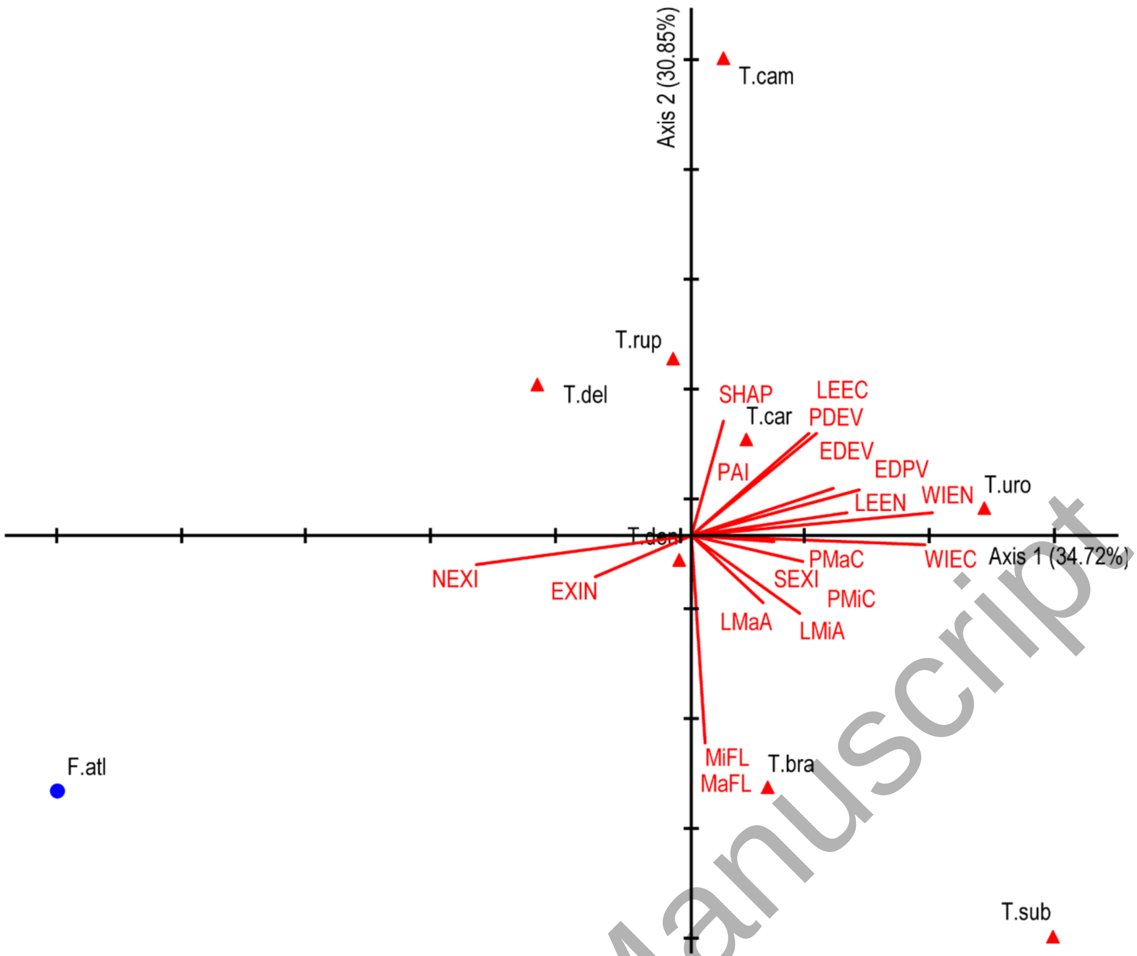

Figure 3. Principal component analysis performed with the pollen and stamens metrical variables from *Freziera* and *Ternstroemia* species (Pentaphylacaceae). (F. atl = *Freziera atlantica*; T. bra = *Ternstroemia brasiliensis*; T. cam = *Ternstroemia campinicola*; T. car = *Ternstroemia carnosa*; T. del = *Ternstroemia delicatula*; T. den = *Ternstroemia dentata*; T. rup = *Ternstroemia rupestris*; T. sub = *Ternstroemia subcaudata*; T. uro = *Ternstroemia urophora*; EDPV = equatorial diameter in polar view; PDEV = polar diameter in equatorial view; EDEV = equatorial diameter in equatorial view; SHAP = shape; LEEC = length of ectoaperture; WIEC = width of ectoaperture; LEEN = length of endoaperture; WIEN = width of endoaperture; PAI = polar area index; EXIN = exine thickness; SEXI = sexine thickness; NEXI = nexine thickness; MiFL = minimum filament length; MaFL = maximum filaments length; LMiA = length minimum of the anther; LMiA = length maximum of the anther; PMiC = length minimum of the connective; PMiC = length maximum of the connective).

Table 1. Voucher specimens of *Freziera* and *Ternstroemia* species (Pentaphylacaceae).

Taxon	Locality	Herbarium
Freziera atlantica Zorzan. & Amorim	Brazil: Rio de Janeiro, Petrópolis, Pico do Bonet (J.A.Vieira et al. 529)	SJRP
Ternstroemia brasiliensis Cambess.	Brazil: Bahia, Igatu, Estrada Mucugê-Igatu (J.A.Vieira et al. 825)	SJRP
	Brazil: Santa Catarina, Barra do Sul, Araquari (P. R. Reitz 5142)	PACA
	Brazil: Bahia, Mucugê, Poço do Fogão (J.A.Vieira et al. 519)	SJRP
Ternstroemia campinicola Boom	Brazil: Amazonas, Barcelos (W.A. Rodrigues 10485)	INPA
Ternstroemia carnosa Cambess.	Brazil: Bahia, Diamantina (F.A. Almeda 9609)	SJRP
	Brazil: Minas Gerais, Joaquim Felicio (V.C.Souza et al 25633)	SJRP
	Brazil: Bahia, Rio de Contas, Morro Bittencourt (J.A.Vieira et al. 492)	SJRP
Ternstroemia delicatula Choisy	Brazil: Pará, Oriximiná (C.A.C.Ferreira et al 1458)	SJRP
Ternstroemia dentata (Aubl.) Sw.	Brazil: Pará, Alter do Chão, Trilha para Pico da Piroca (J.A.Vieira et al. 698)	SJRP
Ternstroemia rupestris J.A.Vieira & D.Sampaio	Brazil: Minas Gerais, Santana do Riacho, Serra da Serpentina (J.A.Vieira et al. 762)	SJRP
Ternstroemia subcaudata Kobuski	Brazil: Amazonas, Manaus, Igarapé Cachoeira Alta Tarumã (W.A. Rodrigues 4637)	INPA
Ternstroemia urophora Kobuski	Brazil: Amazonas, Manaus, Igarapé Tarumã-Mirim (P. Campos n/n)	INPA

INPA = Institution: Instituto Nacional de Pesquisas Amazônicas Location: Brazil, Amazonas, Manaus. PACA = Institution: Instituto Anchietano de Pesquisa/UNISINOS; Location: Brazil, Rio Grande do Sul, São Leopoldo. SJRP = Institution: Universidade Estadual Paulista; Location: Brazil, São Paulo, São José do Rio Preto.

Table 2. Quantitative data of *Freziera* and *Ternstroemia* pollen grain diameters.

Species	Polar diameter in equatorial view				Equatorial diameter in equatorial view			
	$(X^{min} – X^{max})$ $\bar{x}$ $\pm s_x$	s	CI	CV	$(X^{min} – X^{max})$ $\bar{x}$ $\pm s_x$	s	CI	CV
Freziera atlantica	(12.50 – 17.50) 15.60±0.26	1.31	15.06 – 16.14	8.38	(15.00 – 17.50) 16.90±0.22	1.09	16.45 – 17.35	6.45
Ternstroemia brasiliensis	(20.00 – 25.00) 23.00±0.29	1.44	22.40 – 23.60	6.28	(17.50 – 22.50) 20.30±0.22	1.10	19.85 – 20.75	5.41
Ternstroemia campinicola	(23.75 – 30.00) 25.95±0.30	1.50	25.33 – 26.57	5.78	(20.00 – 25.00) 22.60±0.23	1.14	22.13 – 23.07	5.03
Ternstroemia carnosa	(25.00 – 30.00) 26.40±0.29	1.46	25.80 – 27.00	5.52	(22.50 – 25.00) 23.80±0.25	1.63	23.27 – 24.33	5.36
Ternstroemia delicatula	(25.00 – 27.50) 25.75±0.23	1.14	25.28 – 26.22	4.43	(20.00 – 22.50) 20.20±0.12	0.59	19.96 – 20.44	2.92
Ternstroemia dentata	(20.00 – 22.50) 21.30±0.25	1.27	20.77 – 21.83	5.98	(17.50 – 22.50) 20.70±0.27	1.35	20.14 – 21.26	6.54
Ternstroemia rupestris	(17.50 – 22.50) 21.15±0.29	1.44	20.56 – 21.74	6.81	(17.50 – 20.00) 18.40±0.23	1.17	17.92 – 18.88	6.36
Ternstroemia subcaudata	(20.00 – 22.50) 20.75±0.23	1.14	20.28 – 21.22	5.50	(20.00 – 22.50) 20.35±0.15	0.77	20.03 – 20.67	3.77
Ternstroemia urophora	(25.00 – 27.50) 26.80±0.23	1.15	26.33 – 27.27	4.27	(22.50 – 25.00) 24.00±0.25	1.25	23.48 – 24.52	5.21

X^{min}=lowest value observed in the sample (µm); X^{max}= highest value observed in the sample (µm); $\bar{x}$= arithmetic mean (µm); s_x= standard deviation of the mean (µm); s= sample standard deviation (µm); CI= 95% confidence interval (µm); CV= coefficient of variability (%).

Table 3. Morphological characterization and measures (μm) of *Freziera* and *Ternstroemia* pollen grains, apertures and exine. *n* = 10.

Species	Size	EDPV	AS	P/E	Shape	Ectoaperture			Endoaperture		CWI	CLI	PAI	Exine	Sexine	Nexine
						length	width		length	width						
Freziera atlantica	S	16.40	6.18	0.92	OS	9.51	0.77		2.31	2.63	21.90	0.60	0.38	1.98	1.23	0.75
Ternstroemia brasiliensis	S	20.10	6.07	1.13	PS	17.25	1.41		2.74	9.14	14.40	0.75	0.30	1.65	1.30	0.35
Ternstroemia campinicola	M	22.85	9.14	1.15	SP	18.87	1.26		4.04	9.69	17.90	0.72	0.40	1.57	1.24	0.33
Ternstroemia carnosa	M	23.55	9.31	1.11	PS	19.67	1.70		5.34	9.62	14.00	0.74	0.40	1.73	1.34	0.39
Ternstroemia delicatula	M	19.17	5.70	1.27	SP	17.57	0.96		3.00	5.47	21.00	0.68	0.26	1.59	1.22	0.37
Ternstroemia dentata	S	20.10	5.77	1.03	PS	14.40	1.65		2.92	7.36	12.60	0.67	0.29	1.54	1.19	0.35
Ternstroemia rupestris	S	18.50	4.53	1.15	SP	16.31	1.22		3.52	6.83	15.10	0.76	0.24	1.53	1.22	0.31
Ternstroemia subcaudata	S	20.70	7.18	1.02	PS	14.35	1.93		4.32	8.41	10.50	0.69	0.35	1.59	1.28	0.31
Ternstroemia urophora	M	23.60	10.70	1.12	PS	17.73	1.67		5.32	10.09	14.40	0.66	0.45	1.74	1.38	0.36

S= small; M= medium; EDPV = equatorial diameter in polar view; AS = apocolpium side; OS= oblate spheroidal; SP= subprolate; PS= prolate spheroidal; CWI= colpus width index; CLI= colpus length index; PAI= polar area index.

Table 4. Pearson and Kendall correlation coefficients for pollen grain and stamens metric variables of the first and second axes of PCA ordination in *Freziera* and *Ternstroemia* species (Pentaphylacaceae).

Variables		**Principal components**	
		Axis I	Axis II
EDPV	(equatorial diameter in polar view)	0.1533	0.0852
PDEV	(polar diameter in equatorial view)	0.1863	0.1845
EDEV	(equatorial diameter in equatorial view)	0.1346	0.0825
SHAP	(shape)	0.0279	0.0559
LEEC	(length of ectoaperture)	0.2389	0.2293
WIEC	(width of ectoaperture)	0.2607	-0.0570
LEEN	(length of endoaperture)	0.2998	0.1238
WIEN	(width of endoaperture)	0.5728	0.1890
PAI	(polar area index)	0.0156	0.0021
EXIN	(exine thickness)	-0.0528	-0.0372
SEXI	(sexine thickness)	0.0260	-0.0080
NEXI	(nexine thickness)	-0.1362	-0.0539
MiFL	(minimum filament length)	0.1081	-0.4281
MaFL	(maximum filaments length)	0.0053	-0.6855
LMiA	(length minimum of the anther)	0.3478	-0.3107
LMiA	(length maximum of the anther	0.2526	-0.2584
PMiC	(length minimum of the connective)	0.2741	-0.1431
PMiC	(length maximum of the connective)	0.2932	0.0268