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Talita Kely Bellonzi
Universidade de São Paulo
Gabriel Emiliano Ferreira
Universidade Estadual Paulista
Cintia Neves Souza
Universidade Estadual Paulista
Alain Chautems
Geneva Botanic Garden & University of Geneva
John L. Clark
Marie Selby Botanical Gardens
Mathieu Perret
Geneva Botanic Garden & University of Geneva
Eduardo Custódio Gasparino
eduardo.gasparino@unesp.br

Universidade Estadual Paulista

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Insight into pollen morphology in the taxonomy and biogeography of Beslerieae Bartl. (Gesneriaceae)

Talita Kely Bellonzi[1] · Gabriel Emiliano Ferreira[2,3] · Cintia Neves de Souza[2] · Alain Chautems[4] · John L. Clark[3] · Mathieu Perret[4] · Eduardo Custódio Gasparino[2*]

[1] Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras, Departamento de Biologia, Ribeirão Preto, SP, Brazil

[2] Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Biologia, Jaboticabal, SP, Brazil

[3] Marie Selby Botanical Gardens, Sarasota, FL, USA

[4] Geneva Botanic Garden & University of Geneva, Department of Plant Sciences, Chambésy, Geneva, Switzerland

* Corresponding author. E-mail address: eduardo.gasparino@unesp.br

ORCID IDs:

Talita Kely Bellonzi: https://orcid.org/0000-0003-2586-4238

Gabriel Emiliano Ferreira: https://orcid.org/0000-0003-2659-3396

Cintia Neves de Souza: https://orcid.org/0000-0002-8061-6512

Alain Chautems: https://orcid.org/0000-0002-0086-1623

John L. Clark: https://orcid.org/0000-0002-1414-6380

Mathieu Perret: https://orcid.org/0000-0003-2021-114X

Eduardo Custódio Gasparino: https://orcid.org/0000-0001-6078-7341

Abstract

The genera of the tribe Beslerieae (Gesneriaceae) exhibit remarkable diversity in growth forms, flowers, and fruits, making the identification of morphological synapomorphies challenging. Among them, *Anetanthus* Hiern ex Benth. & Hook.f., *Besleria* Plum ex L., and *Tylopsacas* Leeuwenb. remain poorly known, with unclear morphological relationships to their closest relatives. Here, we investigated whether pollen morphology can improve the characterization of Beslerieae taxa. We analyzed pollen from 20 Brazilian species of *Anetanthus*, *Besleria*, and *Tylopsacas* distributed in the Amazon and Atlantic Forest biomes. Pollen grains were acetolyzed, measured, and examined using light, scanning, and transmission electron microscopy. Qualitative characters were described, and quantitative data were analyzed using descriptive and multivariate statistics. The pollen grains are monads, isopolar, small, circular to subtriangular amb, and range from oblate to prolate. The apertures are 3-colpate, 3-(4)-colpate, or 3-colporate; long, short, or very short ectoaperture and narrow to wide; with or without margo, rounded or tapered at the polar ends; endoapertures circular or lolongate. The exine is microreticulate, microreticulate-rugulate, rugulate, or rugulate-perforate, with the sexine consistently thicker than the nexine. Variation in aperture type and exine ornamentation distinguished most species and confirmed the eurypalynous nature of Beslerieae, although these characters did not differentiate the analyzed genera. Our findings demonstrate that pollen morphology is more informative for species delimitation than for generic circumscription, reinforcing the systematic value of palynological characters for closely related and sympatric species. In contrast, no pollen morphological patterns were associated with the geographic distribution of the species.

Keywords: Amazon Rainforest, *Anetanthus*, Atlantic Rainforest, *Besleria*, pollen grains, *Tylopsacas*

Introduction

Beslerieae Bartl. belongs to Gesneriaceae Rich & Juss., a family distributed in the tropical regions of all continents (Roalson and Roberts 2016) with high species diversity in northwestern South America and southeastern Brazil (Perret et al. 2013). This tribe comprises nine genera, totaling approximately 265 species (GRC 2026), which exhibit interesting variations in growth form and habit, ranging from small rosette-shaped plants to small trees (Clark et al. 2010). Furthermore, there is high diversity in floral forms, pollination syndromes, and fruit structures, including dry capsules (septicidal, loculicidal, or both) and fleshy capsules or berries (Weber et al. 2013; Ferreira et al. 2024).

Despite the marked morphological heterogeneity of Beslerieae, the monophyly of this tribe is strongly supported by molecular data (Ogutcen et al. 2021; Ferreira et al. 2024). Based on these data and seed morphology (Burtt and Wiehler 1995; Clark et al. 2010; Weber et al. 2013; Ferreira et al. 2024), the tribe is subdivided into two subtribes: (1) Besleriinae (*Besleria* Plum. ex L., *Cremosperma* Benth., *Gasteranthus* Benth., and *Reldia* Wiehler), distributed throughout the Neotropical region, and (2) Anetanthinae (*Anetanthus* Hiern ex Benth. & Hook.f., *Cremospermopsis* L.E.Skog & L.P.Kvist, *Resia* H.E. Moore, *Shuaria* D.A.Neill & J.L.Clark, and *Tylopsacas* Leeuwenb.), distributed only in the tropical region of South America (Beaufort-Murphy 1983; Weber et al. 2013; Weber et al. 2020).

Besleria is a monophyletic genus and sister to *Cremosperma*, with about 165 described species (Weber 2004; Clark et al. 2020; Ferreira et al. 2024; GRC 2026), making it one of the largest and least explored genera of Neotropical Gesneriaceae (Wiehler 1976; Wiehler 1983; Kvist et al. 1998; Weber 2004; Weber et al. 2013; Ferreira et al. 2016; 2024). *Besleria* comprises terrestrial, perennial herbs, shrubs, subshrubs, and small trees, characterized by opposite leaves, axillary inflorescences without bracts, and corollas typically orange or yellow,

indehiscent fleshy berry fruits that frequently grow in the understory of lowland and montane forests (Wiehler 1983; Kvist et al. 1998; Weber et al. 2020). *Besleria* exhibits a hummingbird pollination syndrome, characterized by bright orange flowers that stand out in the shady forest understory (Kvist et al. 2005; Serrano-Serrano et al. 2017).

Besleria clades are challenging to define by morphological traits but are strongly correlated with geography, with infrageneric clades better explained by their regions of occurrence than by morphological synapomorphies. Thus, morphological traits have evolved independently, as indicated by recent molecular phylogenetic studies by Ferreira et al. (2024). The center of diversity of *Besleria* is in the Tropical Andes, with secondary centers in Central America, the Caribbean, and the Brazilian Atlantic Forest (Ferreira et al. 2024). In Brazil, the genus shows a disjunct distribution, occurring in both the Amazon Rainforest and the Atlantic Forest (Lopes et al. 2007; Ferreira et al. 2016). Although most species are local endemics, some are widely distributed, such as *Besleria flavovirens* Nees & Mart. and *B. laxiflora* Benth., which occur from Central America to the Brazilian Atlantic Forest, and *B. solanoides* Kunth, which range from Nicaragua to Bolivia (Kvist et al. 2005; Clavijo et al. 2015; WFO 2026; POWO 2026).

Anetanthus and *Tylopsacas* have winged seeds, an unambiguous synapomorphy shared by these two genera. The round, flat seed with developed wings is unique within Gesneriaceae (Beaufort-Murphy 1983), leading Wiehler (1976) to suggest a close relationship between the two genera, a relationship supported by Clark et al. (2010) through molecular analyses and seed morphological data. In Brazil, *Anetanthus gracilis* Hiern ex Benth. & Hook.f. represents *Anetanthus* and is found in the Cerrado and Atlantic Forest. On the other hand, the monospecific *Tylopsacas*, represented by *Tylopsacas cuneatum* Rich. & Juss. ex DC., occurs predominantly in the Amazon (Ferreira et al. 2024; Ferreira 2026; Rossini and Araujo 2026).

In addition to molecular and morphological studies in Gesneriaceae, palynology is a crucial approach for family-level studies (Gasparino et al. 2021). Smith (1996) concluded that the size and shape of pollen grains support the monophyly of the subfamily Gesnerioideae. The study of pollen-grain morphological characters in Gesneriaceae also enables differentiation among genera (Melhem and Mauro 1973) and, in some cases, even within species as demonstrated by studies by Fritze and Williams (1998) and Gasparino et al. (2011). However, palynology of Beslerieae has been underexplored, with only a few species analyzed by Nowicke (1974), Howard (1975), Williams (1978), Roubik and Moreno (1991), Fourny et al. (2010), Gasparino et al. (2011), Cortés-Ceballos et al. (2017), Cortés-Ceballos et al. (2021), and Souza et al. (2025). Thus, this study aims to characterize and describe the pollen grains of Brazilian species of Beslerieae, thereby expanding morphological knowledge of pollen within the tribe and providing data to assist in the taxonomy and systematics of the group. Furthermore, the study aims to investigate whether patterns of pollen grain morphological variation are linked to species phytogeographic distributions beyond expectations imposed by shared phylogenetic history.

Materials and Methods

Studied species

We analyzed the pollen grains of 20 Brazilian species of Beslerieae, Gesneriaceae (Table 1). The *Besleria* species analyzed, whose distribution occurs in the Brazilian Atlantic Forest, considered as “Atlantic rainforest species” are: *Besleria brevicalyx* G.E.Ferreira & Chautems, *B. diabolica* G.E.Ferreira & Chautems, *B. fluminensis* Brade, *B. grandifolia* Schott, *B. longimucronata* Hoehne, *B. macahensis* Brade, *B. melancholica* (Vell.) C.V.Morton, *B. meridionalis* C.V.Morton, and *B. selloana* Klotzsch & Hanst. Conversely, species found in the Amazon Forest, Guiana Shield, and Tropical Andes, considered "Amazon rainforest species"

are: *Besleria aggregata* (Mart.) Hanst., *B. barclayi* L.E.Skog, *B. gibbosa* (Poepp.) Hanst., *B. iara* G.E.Ferreira & M.J.G.Hopkins, *B. neblinae* Feuillet, *B. parviflora* L.E.Skog & Steyerm, and *B. solanoides* Kunth. Species in both phytogeographic domains, the Amazon and the Atlantic Rainforests, termed "Wide distribution" include *Besleria flavovirens* Nees & Mart. and *B. laxiflora* Benth. (According to the classification of Ferreira and Araujo 2026). Additionally, specimens of *Anetanthus gracilis* Hiern, found in the Cerrado and Atlantic Forest, and *Tylopsacas cuneatum* (Gleason) Leeuwenb., from the Amazon Forest, were also analyzed (Rossini and Araújo 2024; Ferreira et al. 2024).

Pollen analysis

The pollen samples were obtained from flowers with anthers in anthesis, originating from exsiccate specimens in the herbaria NY, US, MBM, SEL, and INPA (acronyms according to Thiers [continuously updated]). A total of 70 specimens were analyzed (Table 1). Permanent slides for light microscopy are deposited in the pollen reference collection of the Laboratory of Plant Morphology and Palynology (LaMPali), Department of Biology, UNESP, Jaboticabal, SP, Brazil. For pollen morphology analysis under light microscopy (LM), pollen grains were prepared using the acetolysis method by Erdtman (1960), with modifications by Melhem et al. (2003). Difficulties were encountered with the traditional acetolysis method due to rupture of the outer wall or kneading of pollen grains in some specimens. Thus, some steps were modified. First, the flowers were hydrated with water and four drops of heated glycerin to facilitate anther removal. Subsequently, the pollen was incubated in glacial acetic acid for up to 24 hours. The time in a water bath was adjusted for each species during the study, ranging from 1'20" for *Besleria flavovirens*, 1'35" for *Anetanthus gracilis*, *B. iara*, and *B. selloana*, to 1'30" for the other species.

The polar and equatorial diameters of pollen grains (n = 25) were randomly measured under a light microscope (Olympus CX22LED), in addition to the measurements of the total exine, sexine, nexine, tectum, length and width of the ectoaperture, margo of the ectoaperture (when present), length and width of the endoaperture, and length of side of the apocolpium (n = 10) (Melhem and Matos 1972). These diameter measurements were performed over seven days (Salgado-Labouriau 1973), on at least four different slides.

To analyze the exine surface in detail, we observed non-acetolyzed pollen grains under scanning electron microscopy (SEM), following the method of Melhem et al. (2003). Additionally, to describe the ultrastructure of the exine layers in detail, we performed transmission electron microscopy (TEM), following the protocols of Sabatini et al. (1963) and Haddad et al. (1998).

Photomicrographs of pollen grains using LM were taken with an optical microscope (Leica DM1000 LED) and a video camera (Leica MC190 HD) connected to a computer. For electron micrographs, a scanning electron microscope (Zeiss EVO MA10) was used for SEM images, and a transmission electron microscope (Jeol JEM 100 CXII) was used for TEM images. Photomicrographs and electron micrographs were used to create plates illustrating the analyzed pollen grains in Adobe Photoshop CS6 Extended.

The palynological terminology was based on the glossaries of Punt et al. (2007) and Halbritter et al. (2018), and the order of pollen morphology description followed that of Bellonzi et al. (2020). The pollen size and shape were followed by Erdtman (1952) and the colpus width index by Gasparino et al. (2013). The exine thickness and polar area index (PAI) were measured following Faegri and Iversen (1966), and we followed the proposals of Erdtman (1952), Punt et al. (2007), and Soares et al. (2021) to define the type of endoaperture.

Statistical analysis

The quantitative data obtained from pollen grain measurements were used to calculate the means (x), standard deviation (sx), standard error (s), coefficient of variability (V), 95% confidence intervals (CI), and range (R), as proposed by Salgado-Labouriau et al. (1965), following Zar (2010) and Vieira (2011). Microsoft Excel® graphics were used to compare pollen grains' diameters and shapes (P/E ratio).

Using the Fitopac program (Shepherd 1996), the metric measurements of pollen grains were transformed using the natural logarithm [log(x+1)]. Subsequently, the program PC-ORD version 5.15 (McCune and Mefford 2011) was used to ordinate the covariance matrix, enabling principal component analysis (PCA) and cluster analysis (CA). First, principal component analysis was performed to evaluate the influence of the quantitative pollen grain data on species ordination. Next, a dendrogram was constructed using cluster analysis (CA; UPGMA; Euclidean distance) to assess differences and similarities among the studied species. For the PCA and CA, 11 metric variables of pollen grains were used: polar diameter in equatorial view (PDEV), equatorial diameter in equatorial view (EDEV), equatorial diameter in polar view (EDPV), shape (SHAP), width of the ectoaperture (EWID), length of the ectoaperture (ELEN), exine thickness (EXIN), sexine thickness (SEXI), nexine thickness (NEXI), tectum thickness (TECT), and polar area index (PAI).

Results

General pollen description of Beslerieae

Pollen grains studied in monads; isopolar; small size; with circular, subcircular to subtriangular amb in polar view; small, large, and very large polar area, with significant variation in shape: oblate, suboblate, oblate spheroidal, prolate spheroidal, subprolate or prolate. The aperture is 3-colpate or 3-colporate with very short, short or long, narrow, or wide ectoapertures; tapered or rounded ends, sometimes constricted, rarely syncolporate; with margo or ornamented

membrane in the colpus; endoaperture predominantly circular, rarely lolongate. The exine is tectate (rugulate, rugulate-perforate) or semitectate (fossulate, microreticulate, microreticulate-rugulate), exine ranging from very thin, thin to thick, sexine thicker than nexine (Table 2-3, Figs. 1-5).

Pollen unit, size, amb and shape

All pollen grains of the analyzed species are monads and of small size. The mean sizes of the polar diameter in the equatorial view vary from a minimum of 13.50 µm in *Besleria flavovirens* to 23.30 µm in *B. neblinae*. For the equatorial diameter in the equatorial view, the means range from 14 µm (*B. flavovirens*) to 23.50 µm (*B. macahensis*) (Table 2).

The amb of pollen grains varied from circular, subcircular, to subtriangular. There was a variation of amb within the same sample for almost all species, being exclusively circular only in *Anetanthus gracilis* (Fig. 2a), *B. diabolica* (Fig. 1h), *B. grandifolia* (Fig. 5g), *B. meridionalis* (Fig. 4h), *B. parviflora* (Fig. 4p), *B. selloana* (Fig. 3g), and *Tylopsacas cuneatum* (Fig. 4t; Fig. 5a) and exclusively subtriangular in *B. iara* (Fig. 1m; Table 3).

Regarding shape (P/E), the pollen grains are predominantly within the oblate class, ranging between suboblate (*Besleria barclayi* – Fig. 1f; *B. solanoides* – Fig. 1q), or oblate spheroidal (most species). Within the prolate class, prolate spheroidal pollen grains were observed in *Anetanthus gracilis* (Fig. 2b), *B. iara* (Fig. 1n), *B. melancholica* (Fig. 3e), *B. selloana* (Fig. 3h), subprolate in *B. diabolica* (Fig. 1j), *B. meridionalis* (Fig. 4j) or prolate in *B. neblinae* (Fig. 4n) (Table 3).

Apertures and ornamentation of exine

The pollen grains of the species analyzed are 3-colpate or 3-colporate. We identify 3-colpate pollen grains in *Besleria aggregata* (Fig. 1a-d), *B. barclayi* (Fig. 1e-g), *B. diabolica* (Fig. 1h-

l), *B. iara* (Fig. 1m-o) and *B. solanoides* (Fig. 1p-t). For these species, the ectoaperture are very short and narrow in *B. barclayi*, *B. iara*, and *B. solanoides*; short and narrow in *B. aggregata*; and ectoaperture long and narrow, with a slight constriction in *B. diabolica* (Fig. 1k). Notably, approximately 12% of the pollen grains observed in *B. diabolica* were 4-colpate (Fig. 1i - Table 4).

For species with colpate pollen grains, the observed ornamentations were as follows: rugulate (*B. aggregata* – Fig. 1d and *B. barclayi* – Fig. 1g), rugulate-perforate (*B. solanoides* – Fig. 1r-t), microreticulate (*B. iara* – Fig. o-o'), and microreticulate-rugulate (*B. diabolica* – Fig. 1l-l').

The pollen grains of the remaining studied species exhibit compound apertures (3-colporate, Figs. 2-4 – Table 4). We observed ectoaperture short and narrow in the pollen grains of *Anetanthus gracilis* (Fig. 2a-g), *Besleria brevicalyx* (Fig. 2h-m), *B. flavovirens* (Fig. 2n-p'), *B. gibbosa* (Fig. 2q-s), *B. laxiflora* (Fig. 3a-c), *B. melancholica* (Fig. 3d-f) and *B. selloana* (Fig. 3g-j'). For these species the ends of ectoaperture can be rounded in *A. gracilis* (Fig. 2a), *B. gibbosa* (Fig. 2r), *B. laxiflora* (Fig. 3a) or tapered in *B. brevicalyx* (Fig. 2h), *B. flavovirens* (Fig. 2n), *B. melancholica* (Fig. 3d), and *B. selloana* (Fig. 3g, k-l). And margo in ectoapertures were observed in only *B. melancholica* (Fig. 3e-f) and *B. selloana* (Fig. 3i). Species such as *B. gibbosa* (Fig. 2s), *B. laxiflora* (Fig. 3b), and *B. selloana* (Fig. 3h) feature a constricted ectoaperture. Meanwhile, *A. gracilis* (Fig. 2c), *B. brevicalyx* (Fig. 2l), and *B. selloana* (Fig. 3m) exhibit an ornamented membrane at the ectoaperture. Endoapertures are uniformly circular (Table 4).

In the pollen grains of *Besleria fluminensis* (Fig. 3o-q), *B. longimucronata* (Fig. 3r-s; Fig. 4a-c), *B. macahensis* (Fig. 4d-g), *B. meridionalis* (Fig. 4h-l), *B. neblinae* (Fig. 4m-o), *B. parviflora* (Fig. 4p-s), *Tylopsacas cuneatum* (Fig. 4t; Fig. 5a-f) we observed ectoaperture long and narrow. For these species rounded ends of the ectoaperture can be found in pollen grains

of *B. fluminensis* (Fig. 3o) and *B. meridionalis* (Fig. 4h-i), while tapered ends are observed in *B. longimucronata* (Fig. 3r), *B. macahensis* (Fig. 4d), *B. neblinae* (Fig. 4m), *B. parviflora* (Fig. 4p), and *T. cuneatum* (Fig. 4t; Fig. 5a). *Besleria longimucronata* pollen grains frequently exhibits long ectoapertures that converge to form syncolporates (Fig. 3s). The ectoaperture is constricted in *B. macahensis* (Fig. 4f), *B. meridionalis* (Fig. 4j), *B. neblinae* (Fig. 4o). Ornamented membrane is found in *B. meridionalis* (Fig. 4j-l), *B. parviflora* (Fig. 4r), and *B. longimucronata* (Fig. 4b-b'). The species exhibit a circular endoaperture, except for *B. parviflora* (Fig. 4r), which has lolongate endoaperture (Table 4).

The pollen grains of *Besleria grandifolia* (Plate V, 7-16) has ectoaperture long and wide, with tapered ends (Fig. 5g-h, k), slightly constricted (Fig. 5i-j), featuring an ornamented membrane on the ectoaperture (Fig. 5j, m), and a circular endoaperture (Fig. 5j) (Table 4).

Regarding ornamentation, most species with colporate pollen grains exhibit a microreticulate exine pattern (*Anetanthus gracilis* – Fig. 2d, *Besleria brevicalyx* - Fig. 2j-m, *B. flavovirens* – Fig. 2q-q', *B. fluminensis* – Fig. 3q-q', *B. grandifolia* – Fig. 5k-n, *B. macahensis* – Fig. 4g, *B. neblinae* – Fig. 4m, *B. parviflora* – Fig. 4s, *B. selloana* – Fig. 3j-k, and *Tylopsacas cuneatum* – Fig. 5c-f). Microreticulate-rugulate exine ornamentation was observed in *B. melancholica* (Fig. 3d-f), fossulate in *B. meridionalis* (Fig. 4k-l), and rugulate in *B. laxiflora* (Fig. 3c), *B. gibbosa* (Fig. 2t), and *B. longimucronata* (Fig. 4b-b').

Scanning electron microscopy (SEM) revealed perforations on the exine of *B. gibbosa* (Fig. 2t), while light microscopy (LM) showed perforations on *B. longimucronata* (Fig. 4b-b'). For *B. parviflora*, exine ornamentation was initially identified as microreticulate under light microscopy; however, scanning electron microscopy revealed an irregular surface resembling rugulae on its pollen grains (Fig. 4s).

Overall, most species have a thin exine, with exceptions in *Besleria aggregata* and *B. barclayi*, where the exine is exceptionally very thin, and in *B. neblinae*, where it is notably

thick. Across all species, the sexine layer consistently exceeds the nexine layer in thickness. Among the species studied, *Tylopsacas cuneatum* had the thinnest sexine layer (0.88 μm), while *B. gibbosa* had the thickest (1.34 μm), as shown in Table 4.

Transmission electron microscopy analysis of exine structure shows that in *Besleria grandifolia* (Fig. 5o-p), the tectum is thick and wavy, columellae are poorly defined, and the intine is thick. In *Besleria longimucronata* (Fig. 4c), the tectum is continuous, not very thick, and columellae are long. In both species, nexine 2 is thicker than nexine 1, and there is the presence of electron-dense material between columellae, likely pollenkitt (indicated by the arrow in *B. grandifolia*, Fig. 5j). In *Besleria selloana* (Fig. 3n), nexine 1 is thicker than nexine 2, and there is an absence of sexine in the aperture area (indicated by the arrow).

Quantitative data

By comparing the mean and the 95% confidence interval of the measurements of pollen grain diameters analyzed from Beslerieae (Fig. 6), a continuous range of values was observed in polar view diameter (PDEV, Fig. 6a) and particularly in equatorial view diameter (EDEV, Fig. 2b). The smallest PDEV diameters (Fig. 6a) were observed in *Besleria flavovirens* (as well as in EDEV, Fig. 6b) and *B. solanoides*, while the largest values were found in *B. neblinae* (Fig. 6a). In EDEV (Fig. 6b), *B. neblinae* exhibited the smallest diameters (the only species characterized by the prolate shape of its pollen grains), along with *B. flavovirens*. Conversely, the largest diameters were observed in *B. aggregata* and *B. macahensis* (Fig. 6b). The species *Anetanthus gracilis* and *Tylopsacas cuneatum* displayed intermediate values compared to the analyzed *Besleria* species (Fig. 6a-b).

The pollen grains shape varies among species, falling into oblate and prolate classes (Table 3). These variations are shown in Figure 7 with confidence intervals (CIs). For suboblate pollen like *B. solanoides* and *B. barclayi*, the CIs fall within the oblate class. Oblate spheroidal

pollen grains have CIs ranging from suboblate to prolate spheroidal (*B. longimucronata*, *B. aggregata*, *B. gibbosa*, *B. macahensis*, *B. flavovirens*, *B. fluminensis*, *B. grandifolia*, and *B. laxiflora*). For species like *B. parviflora*, *T. cuneatum*, and *B. brevicalyx*, oblate spheroidal grains have CIs ranging from oblate to prolate spheroidal. Prolate spheroidal pollen grains have CIs spanning from oblate spheroidal to subprolate (*B. selloana*, *A. gracilis*, *B. iara*, and *B. meridionalis*). *B. diabolica* pollen grains have only subprolate CIs. *B. melancholica* has a mean prolate spheroidal shape with CIs spanning oblate spheroidal, subprolate, and prolate. Finally, *B. neblinae* falls under the prolate class in terms of mean and CIs.

Principal component analysis (PCA) was conducted using 11 metric variables derived from the pollen grains of the analyzed Beslerieae species (Table 4). Due to the wide variation within the same species for shape, the arithmetic means of the values for this variable were used. In this exploratory analysis of quantitative data, the first two axes summarized 82.82% of the total data variability (Fig. 8). The first axis explains a significant portion, 66.76%, of the total data variation. The length of ectoaperture (ECLG) is the most influential variable on this axis, followed by the width of ectoaperture (ECWD) and the polar diameter in equatorial view (PDEV). Pollen grains from *Besleria* species with smaller values for ectoaperture length and width are positioned on the positive side of this axis, while most species of this genus, along with *Anetanthus gracilis* and *Tylopsacas cuneatum*, exhibit higher values and are situated on the negative side. In the Axis 2 accounted for 16.06% of the variability in the measured parameters and it was primarily influenced by the equatorial diameter in the equatorial view (EDEV), equatorial diameter in the polar view (EDPV), and polar diameter in the equatorial view (PDEV). Species with the smallest EDEV and EDPV measurements, such as *B. flavovirens*, *B. iara*, and *A. gracilis*, were positioned on the negative side of the axis. Conversely, species with higher values, like *B. aggregata* and *B. macahensis*, were situated on the positive side of the axis, along with *T. cuneatum* (Fig. 8).

The cluster analysis produced a similarity dendrogram, revealing two main groups (Fig. 9). The first group includes *Besleria solanoides* and *B. aggregata* due to their lower ectoaperture length values, while the second group comprises the remaining species. Among the other analyzed species, we observed the formation of two subgroups: the first with species exhibiting intermediate values of EDPV, EDEV, and ectoaperture length (ECLG) (*B. iara* and *B. flavovirens*). In the second and larger group, species with the highest values for these variables and those with closely matching values were grouped. For instance, *B. diabolica* and *B. fluminensis* showed about 90% similarity, as the difference in their ECLG and ECWD values is minimal compared to other species (*B. diabolica* ECLG = 16.37 µm, ECWD = 1.90 µm; *B. fluminensis* ECLG = 16.31 µm, ECWD = 1.91 µm). Similarly, *B. selloana* and *B. gibbosa* exhibited about 98% similarity, and *B. melancholica*, *B. meridionalis*, and *B. laxiflora* (the latter with 100% similarity) were also closely related. When analyzing the similarity relationships of pollen characteristics among *Besleria* species with the pattern of geographic distribution (Fig. 9), we found that the pollen grains of *B. flavovirens* (widely distributed) are more related to *B. iara* (Amazon Rainforest), while *B. laxiflora* (widely distributed) shows more significant similarity to *B. meridionalis* (Atlantic Rainforest, 100% similarity). It is worth highlighting the similarity among most Atlantic Rainforest species, including *B. fluminensis*, *B. diabolica*, *B. melancholica*, *B. meridionalis*, and *B. brevicalyx* (about 88% similarity), whereas for Amazon Rainforest species, *B. solanoides* and *B. aggregata* stand out (about 48% similarity, Fig. 9).

Discussion

Differences in pollen grain morphology among Beslerieae species were observed, mainly regarding the ornamentation of the exine, as well as shape and the characteristics of the type and size of the apertures, thereby confirming the eurypalynous nature of the tribe [see Howard

(1975), Roubik and Moreno (1991), Fourny et al. (2010), Gasparino et al. (2011), Cortés-Ceballos et al. (2021), and Souza et al. (2025)]. This study comprehensively describes the pollen morphology of *Anetanthus gracilis*, *Tylopsacas cuneatum*, and several *Besleria* species occurring in Brazil, significantly expanding palynological knowledge of this subtribe.

The large variation in pollen grain shapes found in Beslerieae, sometimes even within the same sample, supports previous observations, indicating that pollen shape characteristics should not be used to distinguish pollen types in Beslerieae (Gasparino et al. 2011; 2013; 2014; Souza et al. 2018; Souza 2022; Bellonzi and Gasparino 2022; Lopes et al. 2025). Conversely, qualitative data on pollen grains allow us to determine palynological similarity among genera in Beslerieae. *Anetanthus gracilis* and *Besleria selloana* (both from the Atlantic Forest) share all analyzed pollen characteristics (circular amb, 3-colporate pollen grains, short and narrow ectoaberture, and microreticulate ornamentation), and the same applies to *Tylopsacas cuneatum* and *B. neblinae* (both from the Amazon Rainforest), with 3-colporate pollen grains, long and narrow ectoaberture, and microreticulate ornamentation.

Anetanthus gracilis was characterized here as having 3-colporate pollen grains, with short ectoapertures with rounded ends, microreticulate exine, and an exine thickness of 1.40 µm. These data differ from those of Howard (1975), who described the pollen grains of *A. gracilis* as spheroidal or prolate, tricolpate, with a long, tapering ectoaperture, an ornamented membrane, foveolate to reticulate exine ornamentation, and an exine thickness of 1.0 µm.

Based on our observations, the pollen grains of *Besleria laxiflora* were classified as small, and their shapes varied within the oblate and prolate classes. However, no membrane was observed at the ectoaperture, and the exine ornamentation observed under light microscopy and SEM is clearly rugulate. These results differ from those of Roubik and Moreno (1991), who analyzed 10 species of Gesneriaceae from Barro Colorado Island in Panama, including only one species of *Besleria* (*B. laxiflora*), and characterized its pollen grains as small,

tricolpate, subprolate to spheroidal, with a persistent granular membrane in the ectoaperture and psilate exine ornamentation.

The microreticulate ornamentation and 3-colporate pollen grains with long ectoapertures observed in *Besleria macahensis* differ from the previous description, which reported this species as medium-sized, prolate, 3-colpate, with short ectoapertures, a margo, and scabrate ornamentation with perforations (Fourny et al. 2010). Differences were also observed in the other species analyzed by Fourny et al. (2010): For *B. longimucronata* and *B. melancholica,* we observed 3-colporate pollen grains, which were previously described as 3-colpate; in *B. umbrosa,* we observed pollen with long ectoapertures, whereas the authors describe the species' pollen grains as having short ectoapertures (Fourny et al. 2010).

In our study, only *B. melancholica* exhibited pollen grains with a margo in the ectoapertures, a characteristic described for all *Besleria* species by Fourny et al. (2010). Furthermore, we observed syncopate pollen grains in *B. longimucronata*, a characteristic described for this species, and in *B. melancholica*, as reported by Fourny et al. (2010). Regarding exine ornamentation, the same pattern was observed for *B. melancholica* (rugulate-perforate). For *B. longimucronata*, perforations in the exine were observed in both studies, although rugulae were also detected here. Fourny et al. (2010) studied pollen grains of Gesneriaceae species from the State of Rio de Janeiro, including *B. longimucronata*, *B. macahensis*, *B. melancholica*, and *B. umbrosa*, the latter now considered a synonym of *B. grandifolia*. The contradictions reported in the comparison between the studies can be explained by the more refined techniques used here (especially the electron microscopy data); in addition, some features, such as the details of the apertures, are difficult to visualize.

Differences were also found between our data and those reported by Gasparino et al. (2011) for *Besleria* species occurring in the State of São Paulo, particularly regarding pollen grain size, shape variation, aperture features, and exine ornamentation. For *B. selloana*, the

pollen grains analyzed here were classified only as small, whereas Gasparino et al. (2011) described them as small to medium-sized. Shaper variation was observed in our material, whereas the authors described the pollen grains as oblate spheroidal and did not report variation in shape. The pollen grains were 3-colporate in both studies. Differences were observed mainly in the aperture characteristics: the ectoapertures of *B. longimucronata* and *B. umbrosa* were characterized here as long and wide, whereas Gasparino et al. (2011) described them as long and narrow; in *B. selloana*, the ectoapertures were short and narrow here, whereas they were described as wide and narrow by those authors. The endoaperture of *B. longimucronata* was circular in our material, but lolongate in Gasparino et al. (2011). The ectoaperture ends were described here as tapering, whereas Gasparino et al. (2011) described them as tapering in *B. longimucronata* and rounded in *B. selloana* and *B. grandifolia*. Differences were also observed in exine ornamentation: *B. longimucronata* was described here as rugulate-perforate, whereas Gasparino et al. (2011) described it as psilate-perforate; *B. umbrosa* was characterized here as microreticulate, but rugulate by those authors; and *B. selloana* was described as microreticulate in both studies. Gasparino et al. (2011) analyzed pollen data from Gesneriaceae species occurring in the State of São Paulo and presented results for three species of *Besleria*: *B. longimucronata*, *B. selloana*, and *B. umbrosa*, which are currently synonymized as *B. grandifolia*.

Our results differ from those reported by Cortés-Ceballos et al. (2021), mainly because 3-porate pollen grains were not identified in the species analyzed here. The authors likely observed very short ectoapertures, which characterize colpate (brevicolpate) pollen grains. Cortés-Ceballos et al. (2021) described *Besleria brevisepala* O.L. Cortés from Colombia and analyzed pollen grains from this species and four other *Besleria* species from the Andean forests of Colombia. They reported morphological similarities between the pollen of *B. brevisepala* and other members of the genus, such as *B. solanoides*, and noted that pollen

analyses revealed diagnostic differences that allow these species to be distinguished. In their study, small pollen grains were observed, with circular amb, 3-porate apertures, and microreticulate ornamentation in *B. brevisepala*, *B. florida* Morton, *B. microphylla* Fritsch, and *B. solanoides*, or perforate ornamentation in *B. silverstoneana* Cortés.

The palynological data obtained here for Besleria are similar to those observed by Souza et al. (2025) for *Besleria affinis* C.V. Morton and *B. ardens* Decne. Our data confirm small pollen grains, with circular amb, 3-colporate apertures, long and narrow ectoapertures, lolongate or circular endoapertures, and microreticulate ornamentation, as cited by Souza et al. (2025).

Many species of *Besleria* are morphologically similar and often occur sympatrically, which makes species delimitation challenging. In this context, pollen grain morphology can offer valuable diagnostic characters to support taxonomic differentiation within the genus. A notable example of this taxonomic challenge is the case of *Besleria aggregata* and *B. iara*, which share the same geographic area and exhibit similar vegetative traits. Both species occur in the understory of humid forests, near streams, and are shrubs completely covered by indumentum. They can only be distinguished by subtle floral and foliar characteristics (Ferreira et al. 2017). Therefore, the pollen grains of these species exhibit significant differentiation in characteristics, even though both are 3-colpate: *B. aggregata* is rugulate, with short ectoaperture and rounded ends, while *B. iara* has microreticulate exine, very short ectoaperture, and tapered ends. Another distinguishing feature between the species is the exine thickness, which is very thin in *B. aggregata* and thin in *B. iara*.

When we examined the morphological differences and similarities of pollen grains and the geographic distributions of the studied taxa, we found no specific pattern characteristic of pollen from species in the Atlantic rainforest, the Amazon rainforest, or those with a wide distribution. The widely distributed species (*Besleria flavovirens* and *B. laxiflora*) share 3-

colporate pollen with short, narrow ectoapertures and rugulate or rugulate-perforate ornamentation. Despite these similarities, these species do not cluster closely in the analysis, indicating discrepancies in the quantitative pollen data (see PCA and cluster analysis). It is worth noting that, according to Ferreira (2018), these species also share the ventricose corolla shape and the pedunculate inflorescence type; in other words, palynology data confirm other morphological similarities of the species.

Note that although species distributed in the Atlantic Forest generally have 3-colporate pollen grains (except for *Besleria diabolica* with 3-colpate pollen grains), they exhibit a wider variety of ornamentation types, including rugulate, fossulate (*B. meridionalis*), microreticulate, or microreticulate-rugulate pollen grains. In contrast, species found in the Amazon rainforest typically have 3-colpate or 3-colporate pollen grains but only display rugulate or microreticulate ornamentation.

Quantitative data on pollen grains (analyzed metrically) may reveal similarity relationships among species; however, these patterns are not consistently structured by geographic distribution. For instance, *Besleria* species from the same region, such as *B. diabolica* and *B. fluminensis* (Atlantic rainforest), and *B. aggregata* and *B. solanoides* (Amazon rainforest), share similar pollen dimensions. Nevertheless, similar quantitative traits are also observed among species with contrasting distributions, as in *B. laxiflora* (widespread) and *B. meridionalis* (Atlantic rainforest endemic), which could not be distinguished based on pollen measurements. Qualitative data further reinforce this pattern: *Besleria laxiflora* shares key pollen features with *B. gibbosa* (Amazon rainforest), including 3-colporate pollen grains, short and narrow ectoapertures, and rugulate ornamentation. Taken together, these results indicate that pollen morphology is not directly associated with geographic distribution patterns, and may instead reflect shared evolutionary history or morphological conservatism within the group.

Conclusion

Our study identified monad, isopolar, and small pollen grains as features shared by all the Beslerieae species analyzed here. Conversely, variations in amb, polar area size, aperture type (3-colpate or 3-colporate), ectoaperture length and width, endoaperture type, and exine ornamentation (rugulate, rugulate-perforate, fossulate, microreticulate, or microreticulate-rugulate) enable the differentiation of the species studied. Analysis of both quantitative and qualitative pollen grain data did not reveal morphological patterns associated with the geographic distribution of the species. Greater variation in aperture types is found in Amazon rainforest species, while different types of pollen grain ornamentation were observed in Atlantic rainforest species.

Declaration of competing interest

The authors declare no competing interests.

Data availability

No datasets were generated or analyzed during the current study.

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Author's contribution

ECG and TKB designed and conducted the laboratory study. TKB collected the herbarium specimens. TKB, CNS, and ECG processed the data. TKB, CNS, GEF, and ECG wrote the

first draft. GEF, AC, JLC, and MP contributed taxonomic insights. All authors read and improved the final version of the manuscript.

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Table 1. List of specimens of the collected and analyzed species of the tribe Beslerieae Bartl.

Species	Voucher	Herbaria
Anetanthus gracilis Hiern	BRAZIL. Minas Gerais: Conceição do Mato Dentro, 18/I/1972, Hatschback et al. 28893	MBM
	BRAZIL. Minas Gerais: Conceição do Mato Dentro, 5/VII/2001, Souza et al. 25131	SP
	BRAZIL. Distrito Federal, 12/XI/1965, HS Irwin et al. 10247*	US
Besleria aggregata (Mart.) Hanst.	BRAZIL. Amazonas: Rio Cunhá, 29/1X/1971, Prance et al. 34647	INPA
	BRAZIL. Amazonas: Benjamin Constant, 18/II/2016, Ferreira 354-1	INPA
	BRAZIL. Amazonas: Benjamin Constant, 18/II/2016, Ferreira 354-2*	INPA
	BRAZIL. Amazonas: Benjamin Constant, 18/II/2016, Ferreira 354-3	INPA
	BRAZIL. Amazonas: Benjamin Constant, 18/II/2016, Ferreira 354-4*	INPA
Besleria barclayi L.E.Skog	COLOMBIA. Depto. del Chocó, 08/VIII/1976, Forero & Jaramillo 64525*	MBM
	COLOMBIA. Amazônia Colombiana, 08/IX/1976, Forero & Jaramillo 2654	INPA
Besleria brevicalyx G.E.Ferreira & Chautems	BRAZIL. Minas Gerais: Descoberto, 09/X/2014, Ferreira et al. 292	INPA
	BRAZIL. Minas Gerais: Descoberto, 09/X/2014, Ferreira et al. 292-1*	INPA
Besleria diabolica G.E.Ferreira & Chautems	BRAZIL. São Paulo: El Eldorado, Caverna do Diabo, 29/IX/2014, Ferreira et al. 271-2	INPA
	BRAZIL. São Paulo: El Eldorado, Caverna do Diabo, 29/IX/2014, Ferreira et al. 271-5	INPA
Besleria flavovirens Nees & Mart.	BRAZIL. Amazonas: Tabatinga, 12/X/1904, Ducre 12119	INPA
	BRAZIL. Rondônia, 19/II/1969, Prance et al. 27211	INPA
	BRAZIL. Bahia: Itacaré, 21/VII/1984, André et al. 2089	MBM
	BRAZIL. Bahia: Camacan, 15/X/2014, Ferreira et al. 313	INPA
	BRAZIL. Bahia: Camacan, 15/X/2014, Ferreira et al. 314	INPA
	BRAZIL. Bahia: Almadina, 15/I/1998, Jomar et al. 1224	MBM
Besleria fluminensis Brade	BRAZIL. Rio de Janeiro: Angra dos Reis, 02/X/2014, Ferreira et al. 276-1	INPA
	BRAZIL. Rio de Janeiro: Angra dos Reis, 02/X/2014, Ferreira et al. 276-2	INPA
Besleria gibbosa (Poepp.) Hanst.	BRAZIL. Amazonas: Rio Javari, 14/X/1976, Prance et al. 23761	INPA
	BRAZIL. Amazonas: São Gabriel da Cachoeira, 20/X/1978, Madison & Kennedy 527*	INPA
	VENEZUELA: Amazonas, Piedra de Cucuy Amazonas, 28/X/1987, Maas et al. n/n	INPA 158292
Besleria grandifolia Schott	BRAZIL. Rio de Janeiro: Petrópolis, 01/XI/1980, Krieger n/n	INPA 269189
	BRAZIL. Rio de Janeiro: Duque de Caxias, 13/IX/1986, Gonzaga n/n	INPA 262048
	BRAZIL. Rio de Janeiro: Barra do Piauí, 25/X/1988, Klein & Mamiy 568	INPA
	BRAZIL. Rio de Janeiro: Resende, 07/IX/1999, Lobão 455	INPA
	BRAZIL. Rio de Janeiro: Petrópolis, 02/XIII/2007, Tsuji 10441*	HPL
	BRAZIL. Rio de Janeiro: Petrópolis, 03/X/2014, Ferreira et al. 279-5	INPA
	BRAZIL. São Paulo: Pindamonhangaba, 02/XII/1993, Nicolau 2188*	SP
Besleria iara G.E.Ferreira & M.J.G.Hopkins	BRAZIL. Amazonas: Benjamin Constant. 08/II/2016, Ferreira 355-1	INPA
	BRAZIL. Amazonas: Benjamin Constant. 08/II/2016, Ferreira 355-2	INPA
Besleria laxiflora Benth.	BRAZIL. Roraima: Vicinity of Auaris, 06/II/1969, Prance et al. n/n	INPA 26811
	BRAZIL. Amazonas, 08/1993, Milliken 182306*	INPA

	BRAZIL. Bahia: Camacan, 14/X/2014, Ferreira et al. 303*	INPA
	BRAZIL. Bahia: Camacan, 15/X/2014, Ferreira 265834	INPA
	VENEZUELA. Santa Elena, Mata Cutia, 02/IX/1979, Rosa & Nascimento 3226	MBM
	BRAZIL. Distrito Federal, 07/IV/1982, Liesner & Medina 89753	MBM
Besleria longimucronata Hoehne	BRAZIL. Rio de Janeiro: Paraty, 20/VIII/1987, Kirizawa & Ieda 1892,	SP
	BRAZIL. Rio de Janeiro: Angra dos Reis, 05/V/2006, Tsuji 1936	HPL
	BRAZIL. Rio de Janeiro: Paraty, Estrada Paraty/Cunha, 01/X/2014, Ferreira et al. M274-3	INPA
	BRAZIL. São Paulo: Ubatuba, Praia Prumirim, 01/X/2014, Ferreira et al. M273-6*	INPA
Besleria macahensis Brade	BRAZIL. Rio de Janeiro: Santa Maria Madalena, 25/VIII/2012, Oliveira et al. 181	INPA
	BRAZIL. Rio de Janeiro: Poço Parado, 11/X/2014, Ferreira et al. 301	INPA
Besleria melancholica (Vell.) C.V.Morton	BRAZIL. Rio de Janeiro: Corcovado, II/1915, Hoehne 192	SP
	BRAZIL. Rio de Janeiro: Macaé, 22/X/1985, Leitman et al. 40	INPA
	BRAZIL. Rio de Janeiro: Petrópolis, 16/VIII/1989, Marques 121	INPA
	BRAZIL. Rio de Janeiro: Macaé, 04/III/2004, Forzza et al. 2876	INPA
	BRAZIL. Rio de Janeiro: Teresópolis, 21/XI/2006, Lucas et al. 268744	INPA
	BRAZIL. Rio de Janeiro: Macaé, 19/VII/2006, Marquete et al. 3747	INPA
	BRAZIL. Rio de Janeiro: Guapimirim, 05/X/2015, Ferreira et al. M285	INPA
Besleria meridionalis C.V.Morton	BRAZIL. Minas Gerais: Descoberto, 25/II/2000, Faria et al. no. 31061*	INPA
	BRAZIL. Minas Gerais: Descoberto, 29/IV/2000, Faria et al. no. 31139	INPA
Besleria neblinae Feuillet	BRAZIL. Amazonas: Rio Negro, XI/1987, Stevenson & Ramos n/n	INPA 167211
Besleria parviflora L.E.Skog & Steyerm.	BRAZIL. Roraima: Auaris, Amajari, 07/II/1969, Prance 9688.0	INPA
	BRAZIL. Roraima: Surucucu, 07/II/1971, Prance et al. 28686	INPA
	BRAZIL. Amazonas: Rio Javari, 02/VIII/1973, Leras et al. 40242	INPA
	BRAZIL. Amazonas: Santa Isabel do Rio Negro, 28/12/2004, Carvalho 223356	INPA
Besleria selloana Klotzsch & Hanst.	BRAZIL. Paraná: Rio Pequeno, 31/X/1973, Hatschbach 32987	MBM
	BRAZIL. São Paulo: Bertioga, 27/IX/2014, Ferreira et al. 268-2*	INPA
	BRAZIL. São Paulo: Bertioga, 27/IX/2014, Ferreira et al. 268-3	INPA
	BRAZIL. São Paulo: Bertioga, 27/IX/2014, Ferreira et al. 268-7	INPA
Besleria solanoides Kunth	COLOMBIA. Remedios: Corregimiento Santa Isabel, 03/VII/1989, Callejas et al. 215727	INPA
	PANAMA. Chiriquí, 16/VI/1987, Croat 66242*	INPA
Tylopsacas cuneatum (Gleason) Leeuwenb.	BRAZIL. Amazonas: Rio Negro, Rio Cauaburí, 13/01/1966, Silva & Bragão 272075	INPA
	VENEZUELA. Amazonas: Cerro Sipapo, 1-3/XII/1948, Maguire & Politi 27502	NY
	VENEZUELA. Amazonas: Rio Yuatua, 25/XII/1952, Maguire et al. 36893*	NY
	VENEZUELA. Amazonas, 26/XI/1984, Liesner 17322	SEL

INPA= Instituto Nacional de Pesquisas Amazônicas; Location: Brazil. Amazonas: Manaus; MBM= Herbário do Museu Botânico Municipal de Curitiba; Location: Brazil. Paraná: Curitiba; NY = New York Botanical Garden; Location: New York: New York; US= United States National Herbarium; Location: United States. Columbia: Washington; SEL = Selby Gardens Herbarium; Location: United States. Florida: Sarasota. * = Material used in photomicrographs and electron micrographs.

Table 2. Quantitative data of diameters in polar and equatorial views of pollen grains of tribe Beslerieae Bartl. (*n* = 25).

	Equatorial view								Polar View
	Polar diameter				Equatorial diameter				Equatorial diameter
Species	**(R) x ± s_x**	**s**	**CI**	**V**	**(R) x ± s_x**	**s**	**CI**	**V**	**(R) x**
A. gracilis	(15.00 – 22.50) 17.40 ± 0.42	2.10	16.53 – 18.27	12.08	(12.50 – 20.00) 16.00 ± 0.38	1.91	15.21 – 16.79	11.93	(15.00 – 22.50) 16.80
B. aggregata	(17.50 – 25.00) 20.90 ± 0.45	2.27	19.96 – 21.84	10.85	(20.00 – 25.00) 22.60 ± 0.31	1.53	21.97 – 23.23	6.76	20.00 – 25.00) 22.00
B barclayi	(15.00 – 20.00) 16.70 ± 0.28	1.39	16.13 – 17.27	8.33	(17.50 – 22.50) 19.90 ± 0.23	1.14	19.43 – 20.37	5.71	(15.00 – 22.50) 19.10
B. brevicalyx	(17.50 – 25.00) 19.80 ± 0.29	1.43	19.21 – 20.39	7.22	(15.00 – 20.00) 17.80 ± 0.36	1.81	17.05 – 18.55	10.19	(17.50 – 22.50) 20.10
B. diabolica	(17.50 – 25.00) 21.90 ± 0.36	1.81	21.15 – 22.65	8.26	(15.00 – 22.50) 19.00 ± 0.35	1.77	18.27 – 19.73	9.30	(17.50 – 22.50) 19.70
B. flavovirens	(12.50 – 15.00) 13.50 ± 0.25	1.25	12.98 – 14.02	9.26	(12.50 – 17.50) 14.00 ± 0.29	1.44	13.40 – 14.60	10.31	(12.50 – 15.00) 13.40
B. fluminensis	(15.00 – 22.50) 18.30 ± 0.40	2.01	17.47 – 19.13	10.96	(15.00 – 22.50) 19.10 ± 0.35	1.75	18.38 – 19.82	9.16	(17.50 – 20.00) 18.90
B. gibbosa	(17.50 – 22.50) 19.90 ± 0.27	1.35	19.34 – 20.46	6.77	(17.50 – 25.00) 21.50 ± 0.32	1.61	20.83 – 22.17	7.51	(17.50 – 22.50) 20.60
B. grandifolia	(15.00 – 20.00) 16.90 ± 0.30	1.49	16.28 – 17.52	8.83	(15.00 – 20.00) 17.50 ± 0.29	1.44	16.90 – 18.10	8.25	(15.00 – 20.00) 17.90
B. iara	(15.00 – 20.00) 17.40 ± 0.27	1.35	16.84 – 17.96	7.74	(12.50 – 17.50) 15.70 ± 0.37	1.84	14.94 – 16.46	11.74	(15.00 – 20.00) 16.90
B. laxiflora	(15.00 – 22.50) 18.60 ± 0.36	1.78	17.87 – 19.33	9.57	(15.00 – 22.50) 19.00 ± 0.38	1.91	18.21 – 19.79	10.05	(15.00 – 20.00) 18.00
B. longimucronata	(15.00 – 20.00) 17.30 ± 0.25	1.23	16.79 – 17.81	7.13	(17.50 – 22.50) 19.30 ± 0.31	1.53	18.67 – 19.93	7.95	(17.50 – 20.00) 19.30
B. macahensis	(20.00 – 25.00) 22.30 ± 0.29	1.43	21.71 – 22.89	6.41	(20.00 – 27.50) 23.50 ± 0.53	2.28	22.56 – 24.44	9.71	(20.00 – 25.00) 23.20
B. melancholica	(15.00 – 22.50) 19.90 ± 0.37	1.84	19.14 – 20.66	9.23	(15.00 – 22.50) 17.80 ± 0.46	2.32	16.84 – 18.76	13.02	(15.00 – 22.50) 19.70
B. meridionalis	(17.50 – 22.50) 19.80 ± 0.29	1.43	19.21 – 20.39	7.22	(15.00 – 20.00) 17.30 ± 0.32	1.60	16.64 – 17.96	9.25	(17.50 – 20.00) 18.50
B. neblinae	(22.50 – 25.00) 23.30 ± 0.24	1.19	22.81 – 23.79	5.11	(12.50 – 17.50) 14.70 ± 0.30	1.50	14.08 – 15.32	10.20	(15.00 – 20.00) 18.40
B. parviflora	(12.50 – 20.00) 15.50 ± 0.29	1.44	14.90 – 16.10	9.31	(12.50 – 20.00) 15.90 ± 0.32	1.59	15.24 – 16.56	10.03	(15.00 – 17.50) 16.50
B. selloana	(20.00 – 25.00) 22.00 ± 0.29	1.44	21.40 – 22.60	6.56	(17.50 – 22.50) 20.60 ± 0.33	1.66	19.92 – 21.28	8.05	(17.50 – 22.50) 21.20
B. solanoides	(12.50 – 15.00) 13.60 ± 0.25	1.27	13.08 – 14.12	9.31	(15.00 – 17.50) 17.10 ± 0.19	0.94	16.71 – 17.49	5.47	(15.00 – 17.50) 16.20
T. cuneatum	(17.50 – 22.50) 19.10 ± 0.28	1.42	18.51 – 19.69	7.44	(15.20 – 20.00) 19.30 ± 0.27	1.35	18.74 – 19.86	7.02	(17.50 – 22.50) 19.70

A = *Anetanthus*, B. = *Besleria, T* = *Tylopsacas*, R = range, *x* = mean (µm), *sx* = standard deviation (µm), *s* = standard error (µm), CI = confidential interval in 95% (µm), V = coefficient of variability (%).

Table 3. Morphological characterization and metric data of tribe Beslerieae Bartl. pollen grains.

Species	Amb	P/E	Shape	Aperture	END	PAI	Ectoaperture		WCI	Endoaperture		EO	Exine thickness			
							Length	Width		Length	Width		Ex	Tec	Sex	Nex
Anetanthus gracilis	C	1.09	PS	3-colporate	CIR	0.54	14.30	1.31	12.21	4.47	2.90	MIC	1.40°	0.35°	1.01°	0.38°
Besleria aggregata	C, SC, ST	0.92	OS	3-colpate	-	0.69	4.58	1.22	18.58	-	-	RUG	1.35	0.32	1.02	0.33
B barclayi	SC, ST	0.80	SO	3-colpate	-	0.82	12.37	1.76	11.87	-	-	RUG	1.25	0.28	0.95	0.30
B. brevicalyx	C, SC	0.99	OS	3-colporate	CIR	0.66	16.91	1.88	10.57	4.20	3.42	MIC	1.33	0.34	1.00	0.33
B. diabolica	C	1.15	SP	3(-4)-colpate	-	0.45	16.37	1.90	9.99	-	-	MIC-RUG	1.47	0.39	1.11	0.35
B. flavovirens	C, ST	0.96	OS	3-colporate	CIR	0.71	9.47	1.49	9.40	3.74	3.24	MIC	1.15	0.29	0.90	0.25
B. fluminensis	SC	0.96	OS	3-colporate	CIR	0.44	16.31	1.91	10.04	4.44	2.74	MIC	1.54	0.37	1.19	0.35
B. gibbosa	C, SC	0.93	OS	3-colporate	CIR	0.58	17.62	1.90	8.85	3.74	3.15	RUG-PER	1.73	0.36	1.34	0.39
B. grandifolia	C	0.97	OS	3-colporate	CIR	0.37	14.58	2.85	6.13	5.34	4.20	MIC	1.62	0.35	1.27	0.35
B. iara	ST	1.11	PS	3-colpate	-	1.00	12.90	0.91	17.25	-	-	MIC	1.28	0.31	0.91	0.37
B. laxiflora	C, SC, ST	0.98	OS	3-colporate	CIR	0.53	16.25	1.49	12.72	5.07	3.35	RUG	1.41	0.40	1.04	0.37
B. longimucronata	SC, ST	0.90	OS	3-colporate	CIR	0.46	11.96	1.79	10.76	3.41	3.00	RUG-PER	1.52	0.37	1.19	0.33
B. macahensis	C, SC, ST	0.95	OS	3-colporate	CIR	0.35	17.39	2.41	9.76	4.26	3.56	MIC	1.70	0.42	1.33	0.37
B. melancholica	C, ST	1.11	PS	3-colporate	CIR	0.66	14.48	1.56	11.38	4.70	3.40	MIC-RUG	1.30	0.34	0.95	0.35
B. meridionalis	C	1.14	SP	3-colporate	CIR	0.50	15.06	1.58	10.97	3.78	2.83	FOS	1.46	0.39	1.09	0.37
B. neblinae	SC	1.59	P	3-colporate	CIR	0.45	16.59	1.82	8.09	3.60	2.70	MIC	1.55	0.35	1.18	0.37
B. parviflora	C	0.97	OS	3-colporate	LO	0.40	12.36	1.65	9.62	4.21	2.70	MIC	1.46	0.36	1.10	0.36
B. selloana	C	1.07	PS	3-colporate	CIR	0.67	18.31	1.99	10.34	3.34	2.34	MIC	1.55	0.35	1.18	0.37
B. solanoides	SC, ST	0.80	SO	3-colpate	-	0.84	3.93	1.09	15.70	-	-	RUG-PER	1.28	0.31	0.99	0.29
Tylopsacas cuneatum	C	0.99	OS	3-colporate	CIR	0.39	3.57	1.48	13.00	2.74	2.82	MIC	1.49	0.29	0.88	0.61

C = Circular, SC = Subcircular; ST = Subtriangular; P/E = Ratio between polar and equatorial diameter in equatorial view; O = Oblate, SO = Suboblate, OS = Oblate spheroidal, PS = Prolate spheroidal, P = Prolate, SP = Subprolate, END = Endoaperture; CIR = Circular; LO = Lolongate; EO = Exine ornamentation; MIC = Microreticulate, MIC – RUG = Microreticulate- rugulate, RUG = Rugulate, RUG-PER = Rugulate-perforate; PAI = Polar area index; WCI = Width colpus index; Ex = Exine total; Tec = Tectum thickness; Sex = Sexine thickness; Nex = Nexine thickness. Measurements in µm; $n = 10$, ° $n < 10$.

Table 4. Pearson & Kendall correlation coefficients for pollen grain metric variables of the first and the second axis of PCA ordination in tribe Beslerieae Bartl. species.

Variables		Principal components	
		Axis 1	Axis 2
EDVP	(Equatorial diameter in polar view)	-0.1196	0.4987
PDEV	(Polar diameter in equatorial view)	-0.2228	0.3797
EDEV	(Equatorial diameter in equatorial view)	-0.0579	0.5903
ECLG	(Ectoaperture length)	-0.9008	-0.3245
ECWD	(Ectoaperture width)	-0.2675	0.2671
EXIN	(Exine thickness)	-0.0988	0.1540
SEXI	(Sexine thickness)	-0.0857	0.1579
NEXI	(Nexine thickness)	-0.0439	0.0334
TECT	(Tectum thickness)	-0.0403	0.0397
SHAP	(Shape)	-0.0842	-0.1112
P.A.I	(Polar area index)	0.1481	-0.1351

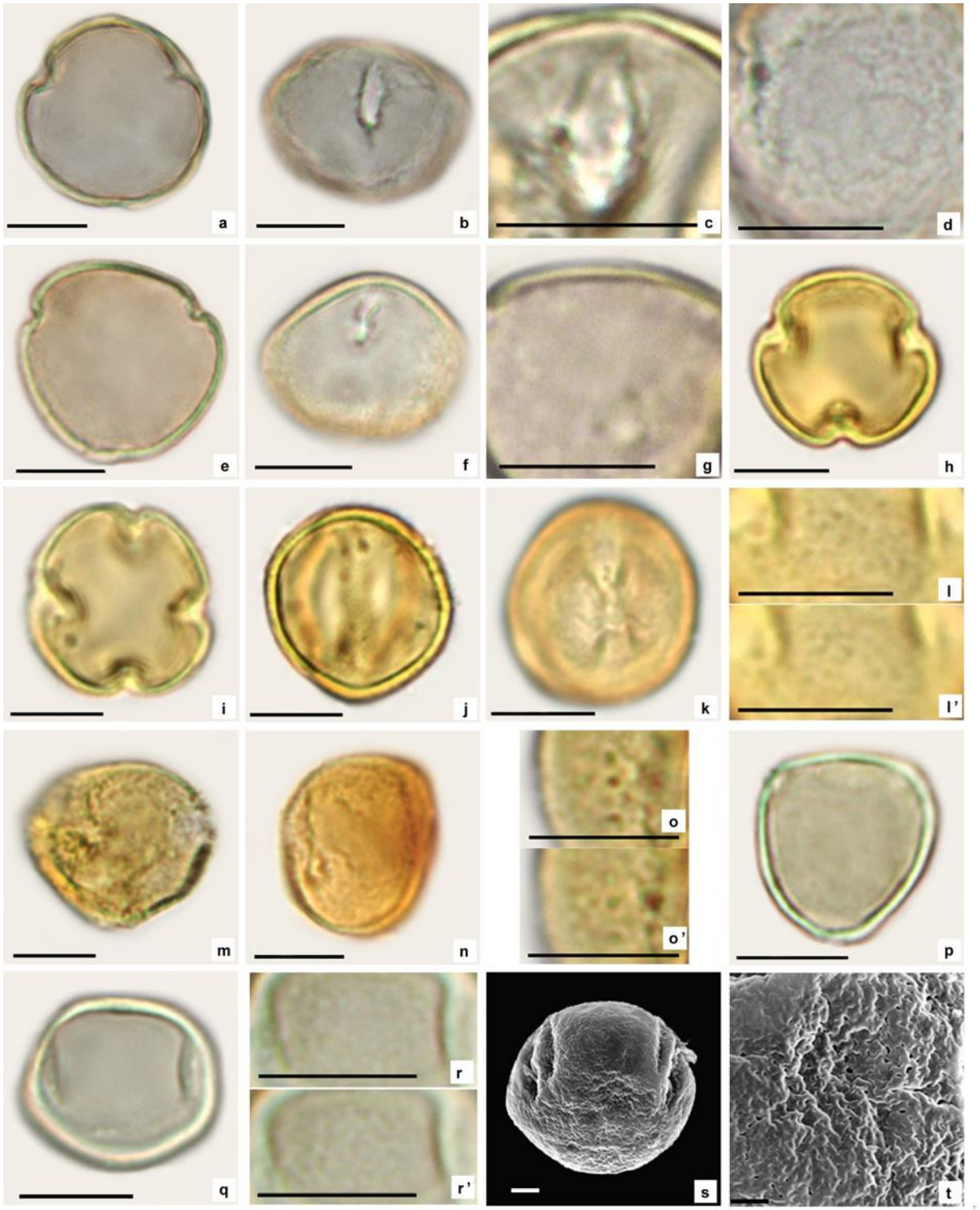


Fig. 1. Light microscopy and scanning electron micrographs of the Tribe Beslerieae Bartl. pollen grains. (a-d) *Besleria aggregata* (Mart.) Hanst.; (a) Polar view. (b) Equatorial view. (c) Detail of brevicolpus. (d) Ornamentation details. (e-g) *Besleria barclayi* L.E.Skog; (e) Polar view. (f) Equatorial view. (g) Ornamentation details. (h-l') *Besleria diabolica* G.E.Ferreira & Chautems; (h) Polar view. (i) Polar view, 4-colporate pollen grain. (j) Polar view. (k) Equatorial view with emphasis to the ectoaperture with constricted. (l-l') Ornamentation in high and low focus. (m-o') *Besleria iara* G.E.Ferreira & M.J.G.Hopkins; (m) Polar view. (n) Equatorial view. (o-o') Ornamentation in high and low focus. (p-t) *Besleria solanoides* Kunth.; (p) Polar view. (q) Equatorial view. (r-r') Ornamentation in high and low focus. (s) Equatorial view (SEM). (t) Ornamentation details (SEM). Scale bars, 5 µm (s–t), 10 µm (a–r).

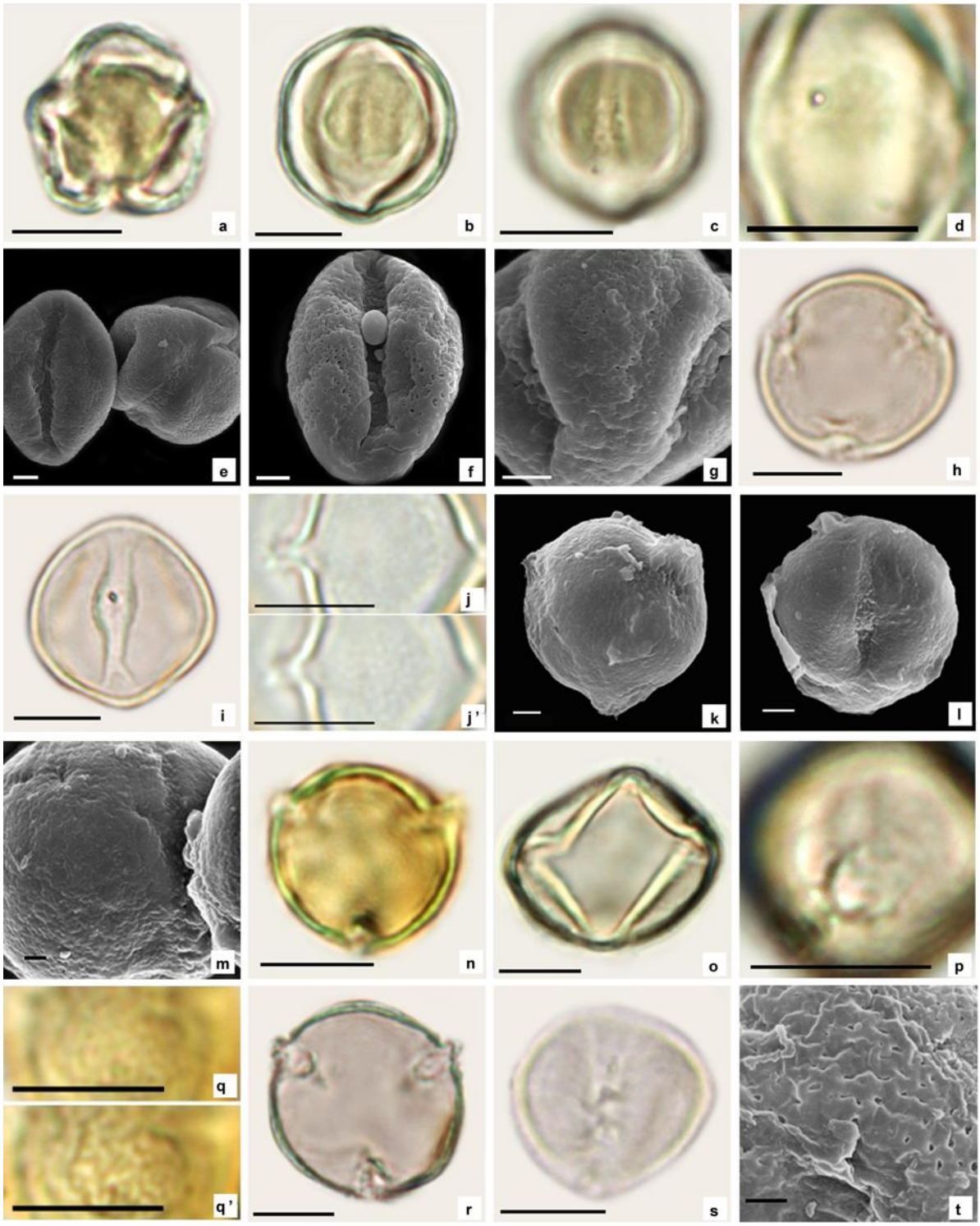


Fig. 2. Light microscopy and scanning electron micrographs of the Tribe Beslerieae Bartl. pollen grains. (a-g) *Anetanthus gracilis* Hiern; (a) Polar view. (b) Equatorial view. (c) Equatorial view with emphasis to the membrane ornamented. (d) Ornamentation details. (e) Equatorial and polar view (SEM). (f) Equatorial view. (g) Ornamentation details (SEM). (h-m) *Besleria brevicalyx* G.E.Ferreira & Chautems; (h) Polar view. (i) Equatorial view. (j-j') Ornamentation in high and low focus. (k) Polar view (SEM). (l) Equatorial view (SEM). (m) Ornamentation details (SEM). (n-q') *Besleria flavovirens* Nees & Mart.; (n) Polar view. (o) Equatorial view. (p) Equatorial view with emphasis to the circular endoaperture. (q-q') Ornamentation in high and low focus. (r-t) *Besleria gibbosa* (Poepp.) Hanst.; (r) Polar view. (s) Equatorial view. (t) Ornamentation details (SEM). Scale bars, 5 µm (e-g, k-m, t), 10 µm (a-d, h-j, n-s)

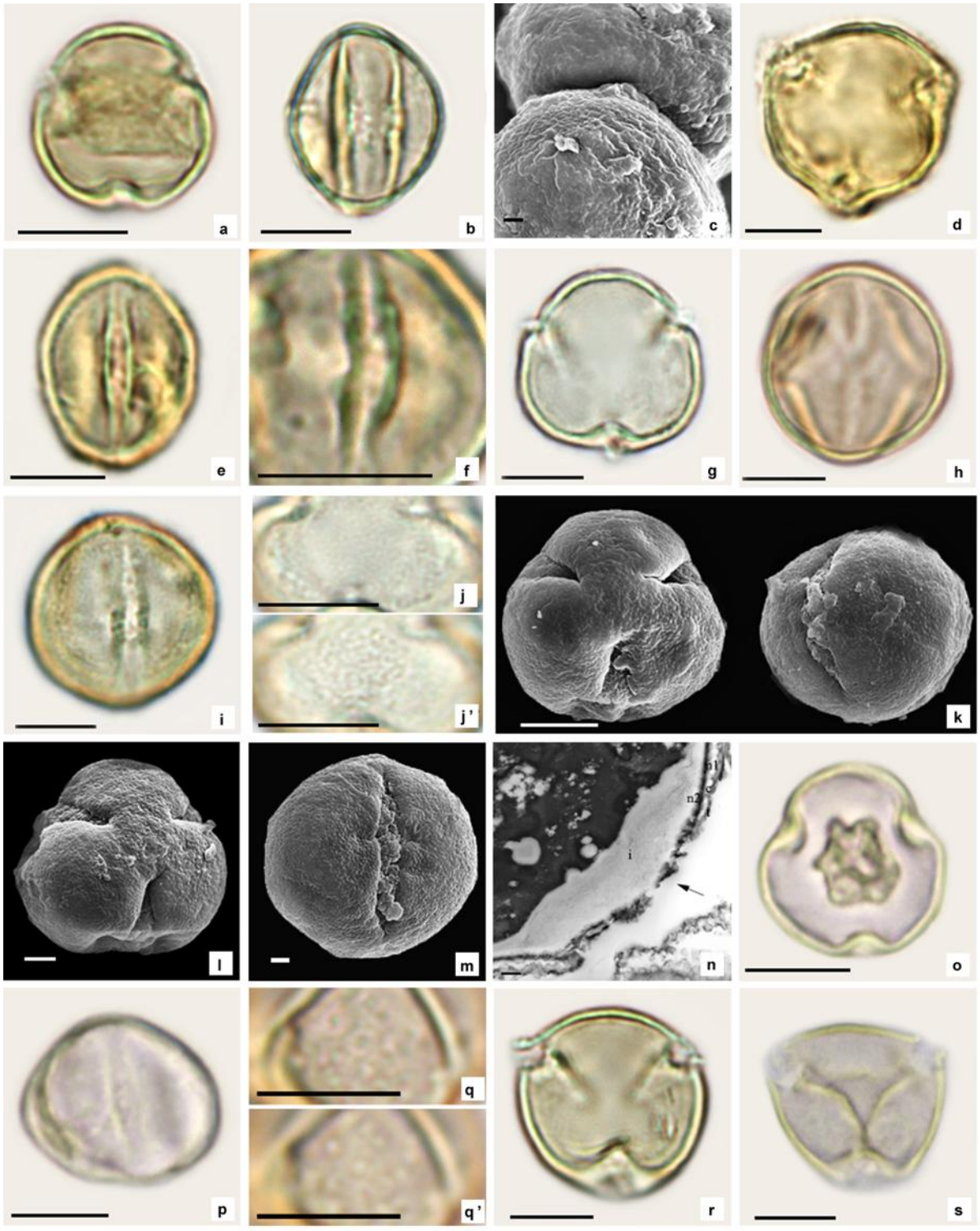


Fig. 3. Light microscopy, scanning and transmission electron microscopy of the Tribe Beslerieae Bartl. pollen grains. (a-c) *Besleria laxiflora* Benth. (a) Polar view. (b) Equatorial view. (c) Ornamentation details (SEM). (d-f) *Besleria melancholica* (Vell.) C.V.Morton; (d) Polar view. (e) Equatorial view. (f) Equatorial view with emphasis to the margo. (g-n) *Besleria selloana* Klotzsch & Hanst.; (g) Polar view. (h) Equatorial view. (i) Equatorial view with emphasis to the margo. (j-j') Ornamentation in high and low focus. (k) Polar and equatorial view (SEM). (l) Polar view (SEM). (m) Equatorial view (SEM). (n) Exine structure (TEM) (t= tectum, c= columella, n1=nexine 1, n2= nexine 2, i = intine). (o-q') *Besleria fluminensis* Brade; (o) Polar view. (p) Equatorial view. (q-q') Ornamentation in high and low focus. (r-s) *Besleria longimucronata* Hoehne.; (r) Polar view. (s) Polar view, syncolporate. Scale bars, 2 µm (n), 5 µm (c, l-m), 10 µm (a-b, d-k, n-s).

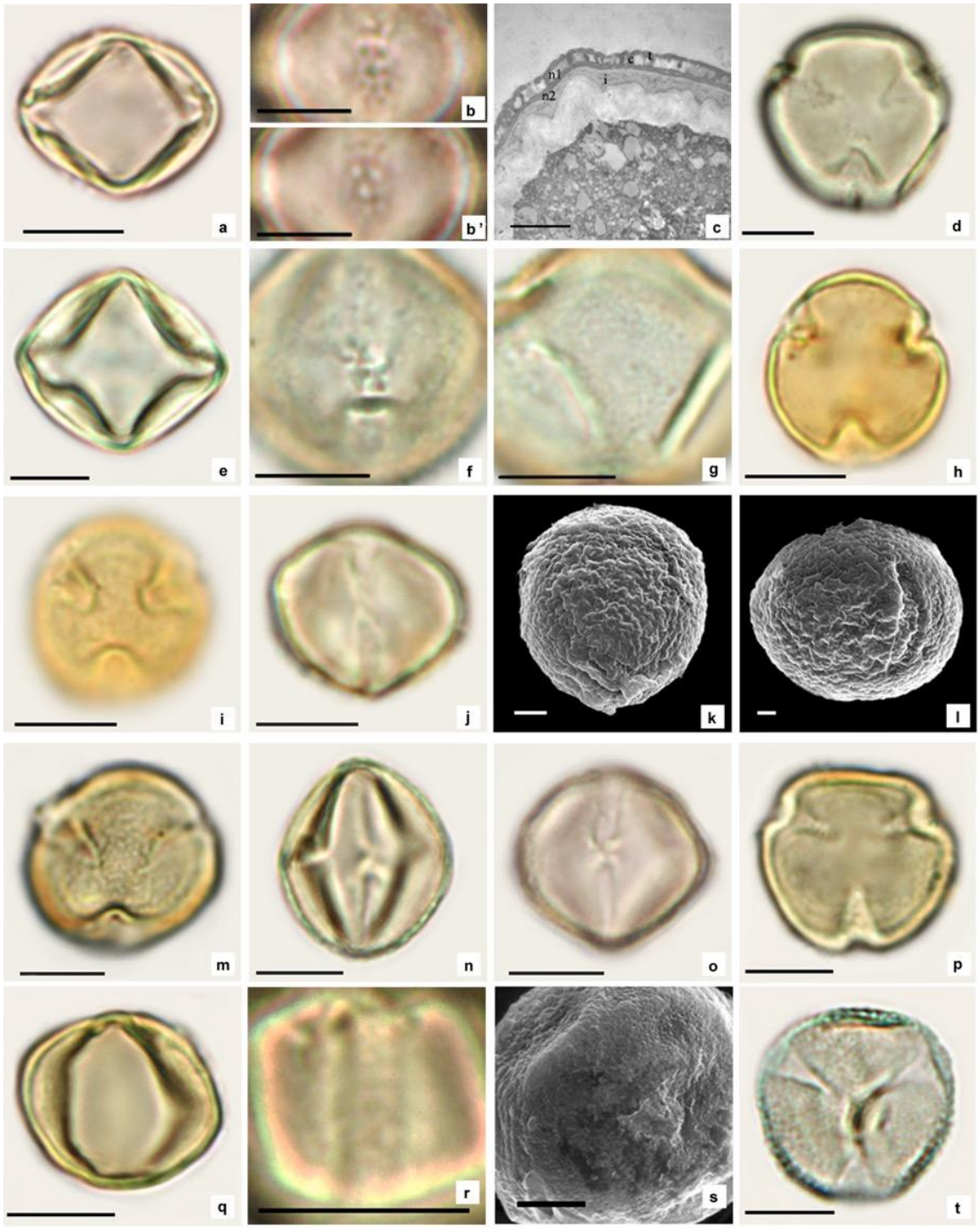


Fig. 4. Light microscopy and scanning electron micrographs of the Tribe Beslerieae Bartl. pollen grains. (a-c) *Besleria longimucronata* Hoehne.; (a) Equatorial view. (b-b') Ornamentation in high and low focus. (c) Exine structure (TEM) (t= tectum, c= columella, n1=nexine 1, n2= nexine 2, i = intine). (d-g) *Besleria macahensis* Brade; (d) Polar view. (e) Equatorial view. (f) Emphasis with constricted ectoaperture. (g) Ornamentation details. (h-l) *Besleria meridionalis* C.V.Morton; (h) Polar view. (i) Polar view with emphasis on the ends. (j) Equatorial view with emphasis to the ectoaperture with constricted. (k) Polar view (SEM). (l) Equatorial view (SEM). (m-o) *Besleria neblinae* Feuillet; (m) Polar view with emphasis to the ends. (n) Equatorial view. (o) Equatorial view with emphasis to the ectoaperture with constricted. (p-s) *Besleria parviflora* L.E.Skog & Steyerm.; (p) Polar view with emphasis to the ends. (q) Equatorial view. (r) Equatorial view with emphasis to the membrane ornamented. (s) Ornamentation detail (SEM). (t) *Tylopsacas cuneatum* (Gleason) Leeuwenb.; (t) Polar view Scale bars, 0.5 µm (c), 5 µm (k-l, s), 10 µm (a-b, d-j, m-r, t).

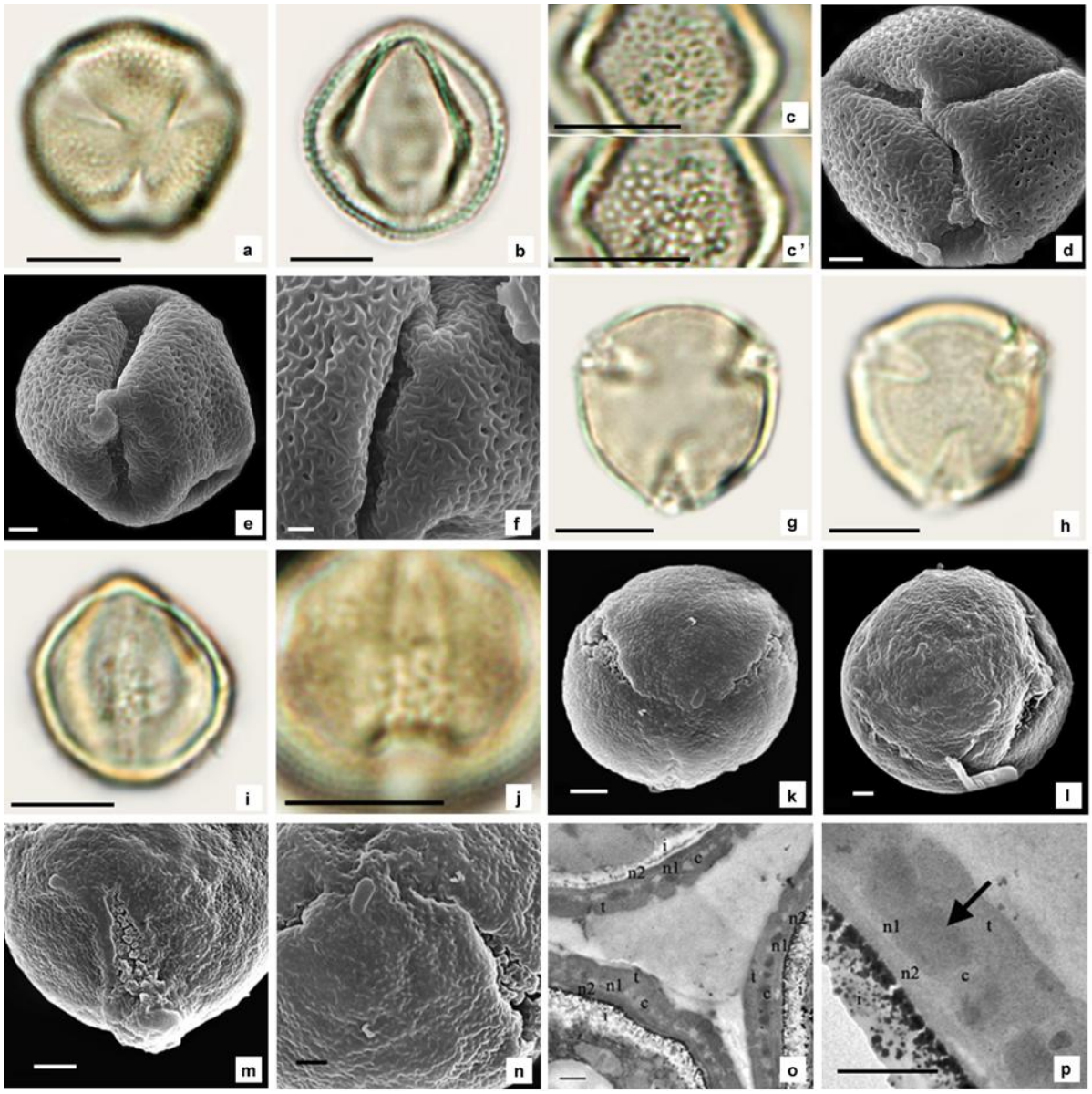


Fig. 5. Light microscopy, scanning and transmission electron microscopy of the Tribe Beslerieae Bartl. pollen grains. (a-f) *Tylopsacas cuneatum* (Gleason) Leeuwenb.; (a) Polar view with emphasis on the ends. (b) Equatorial view. (c-c') Ornamentation details. (d) Polar view (SEM). (e) Equatorial view (SEM). (f) Ornamentation detail (SEM). (g-p) *Besleria grandifolia* Schott.; (g) Polar view. (h) Polar view with emphasis to the ends. (i) Equatorial view. (j) Equatorial view with emphasis to the membrane ornamented. (k) Polar view (SEM). (l) Equatorial view (SEM). (m) Emphasis to the membrane ornamented (SEM). (n) Ornamentation detail (SEM). (o-p) Exine structure (TEM) (t= tectum, c= columella, n1=nexine 1, n2= nexine 2, i = intine). Scale bars, 0.4 µm (o), 0.5 µm (p), 5 µm (d-f, k-n), 10 µm (a-c, g-j).

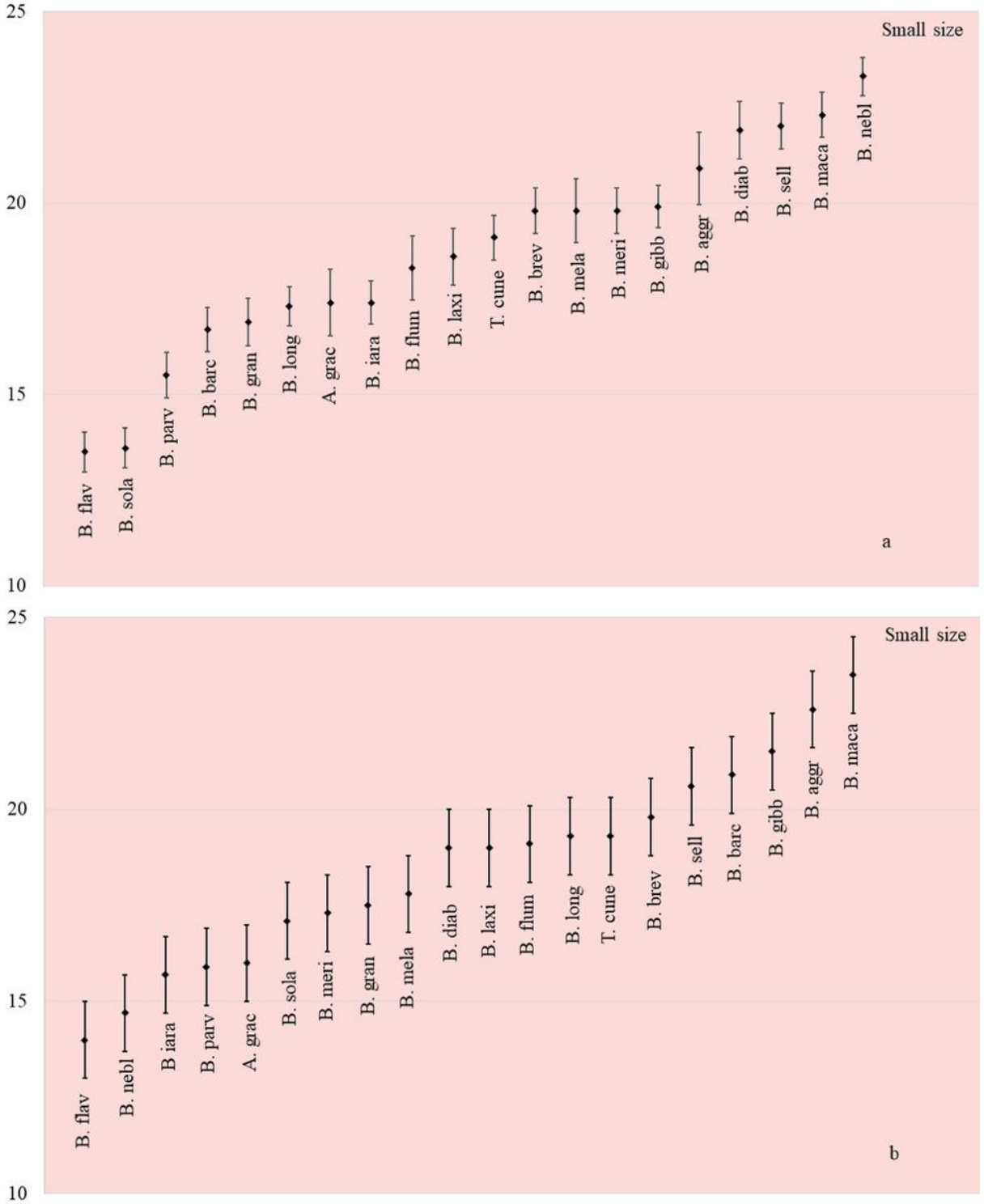


Fig. 6. Representation of confidence interval of mean in 95 % of the pollen grains of Beslerieae species. a) Polar diameter in equatorial view (PDEV). b) Equatorial diameter in equatorial view (EDEV). The higher and lower boundaries showing the confidence interval; The average circle showing the arithmetic mean A. grac = *Anetanthus gracilis*, B. aggr = *Besleria aggregata*, B. barc = *Besleria barclayi*, B. brev = *Besleria brevicalyx*, B. diab = *Besleria diabolica*, B. flav = *Besleria flavovirens*, B. flum = *Besleria fluminensis*, B. gibb = *Besleria gibbosa*, B. gran = *Besleria grandifolia*, B. laxi = *Besleria laxiflora*, B. long = *Besleria longimucronata*, B. maca = *Besleria macahensis*, B. mela = *Besleria melancholica*, B. meri = *Besleria meridionalis*, B. nebl = *Besleria neblinae*, B. parv = *Besleria parviflora*, B. sell = *Besleria selloana*, B. sola = *Besleria solanoides* and T. cune = *Tylopsacas cuneatum*. The values are in µm.

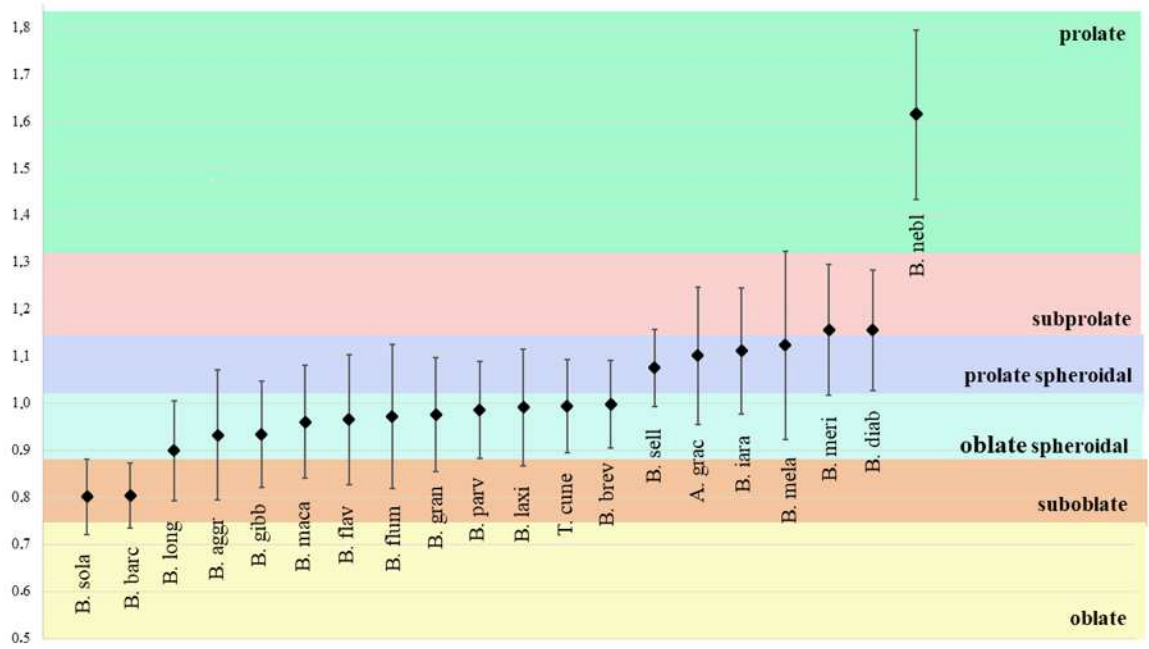


Fig. 7. Graphical representation of the arithmetic means and sample standard deviation of the P/E ratio (shape) of pollen grains of Beslerieae species. The bars represent the limits of the sample standard deviation, and the rhombus represent the arithmetic mean. A. grac = *Anetanthus gracilis*, B. aggr = *Besleria aggregata*, B. barc = *Besleria barclayi*, B. brev = *Besleria brevicalyx*, B. diab = *Besleria diabolica*, B. flav = *Besleria flavovirens*, B. flum = *Besleria fluminensis*, B. gibb = *Besleria gibbosa*, B. gran = *Besleria grandifolia*, B. laxi = *Besleria laxiflora*, B. long = *Besleria longimucronata*, B. maca = *Besleria macahensis*, B. mela = *Besleria melancholica*, B. meri = *Besleria meridionalis*, B. nebl = *Besleria neblinae*, B. parv = *Besleria parviflora*, B. sell = *Besleria selloana*, B. sola = *Besleria solanoides* and T. cune = *Tylopsacas cuneatum*.

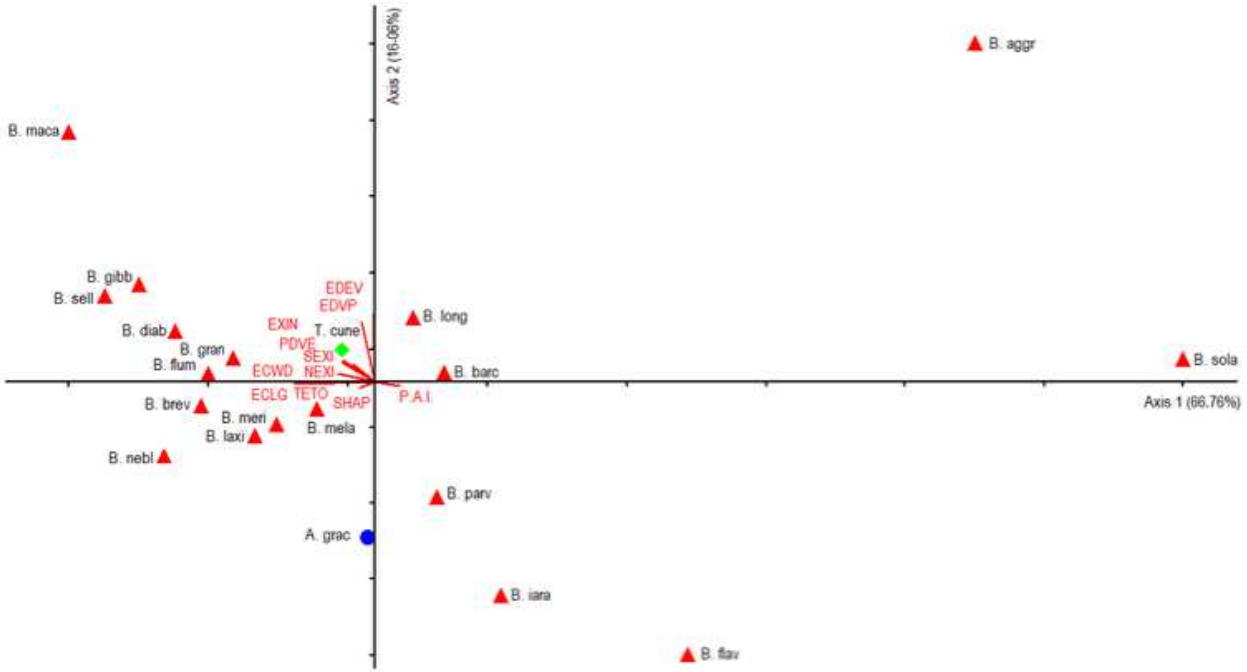


Fig. 8. Principal component analysis performed with the pollen metrical variables from Tribe Beslerieae Bartl. species. A. grac = *Anetanthus gracilis*, B. aggr = *Besleria aggregata*, B. barc = *Besleria barclayi*, B. brev = *Besleria brevicalyx*, B. diab = *Besleria diabolica*, B. flav = *Besleria flavovirens*, B. flum = *Besleria fluminensis*, B. gibb = *Besleria gibbosa*, B. gran = *Besleria grandifolia*, B. laxi = *Besleria laxiflora*, B. long = *Besleria longimucronata*, B. maca = *Besleria macahensis*, B. mela = *Besleria melancholica*, B. meri = *Besleria meridionalis*, B. nebl = *Besleria neblinae*, B. parv = *Besleria parviflora*, B. sell = *Besleria selloana*, B. sola = *Besleria solanoides* and T. cune = *Tylopsacas cuneatum*.

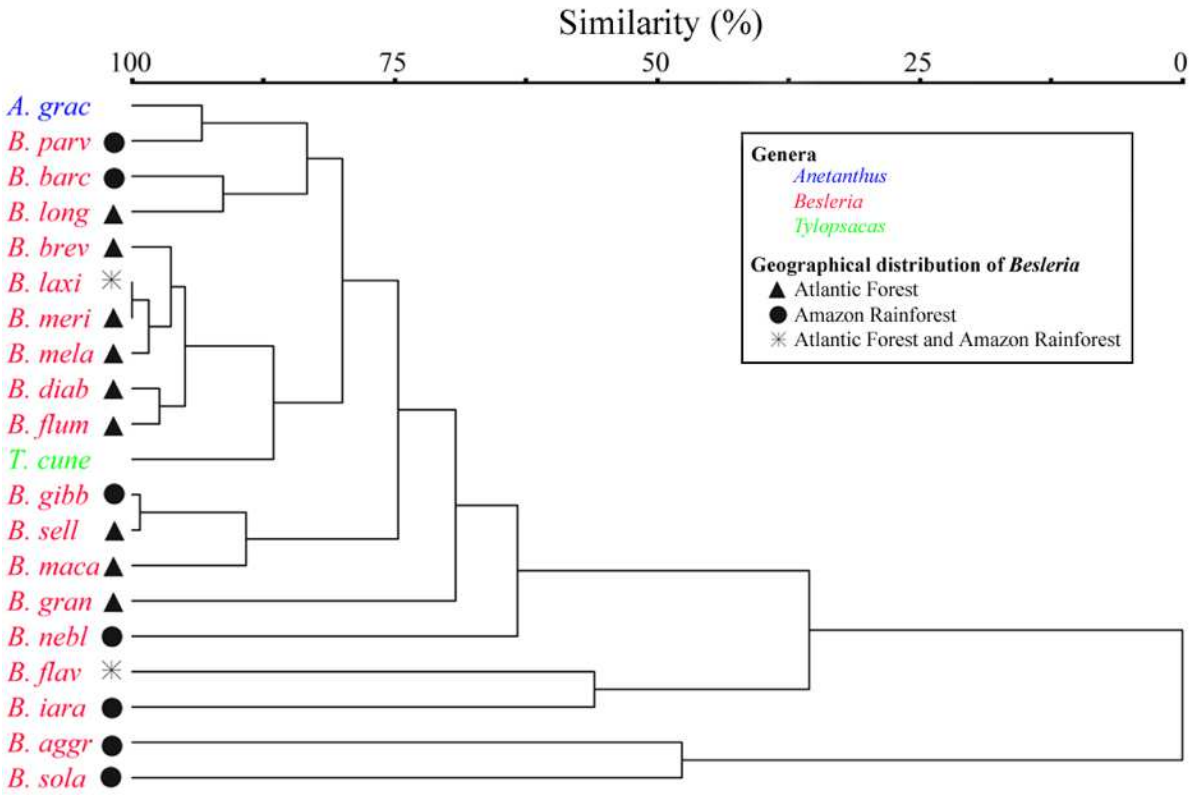


Fig. 9. Cluster analysis (UPGMA and Euclidean distance) performed with the pollen grains metrical variables from Tribe Beslerieae Bartl. species. *A.* grac= *Anetanthus gracilis,* B. *aggr = Besleria aggregata*, *B. barc = Besleria barclayi*, *B. brev = Besleria brevicalyx*, *B. diab = Besleria diabolica*, *B. flav = Besleria flavovirens*, B. *flum = Besleria fluminensis*, *B. gibb = Besleria gibbosa*, *B. gran = Besleria grandifolia*, *B. laxi = Besleria laxiflora*, *B. long = Besleria longimucronata*, *B. maca = Besleria macahensis*, *B. mela = Besleria melancholica*, *B. meri = Besleria meridionalis*, *B. nebl = Besleria neblinae*, *B. parv = Besleria parviflora*, *B. sell = Besleria selloana*, *B. sola = Besleria solanoides* and *T. cune = Tylopsacas cuneatum.*

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