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Roots and fruits: Highlighting the BIPOC Botanists legacy

Original Article

Palynology of *Stachytarpheta* species with pedicellate flowers: A group endemic to the Brazilian Cerrado

Pâmela Timóteo Patrício da Silva[1*], Pedro Henrique Cardoso[2], Andrea Pereira Luizi-Ponzo[1,3]

[1] Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas, Campus Universitário, Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza, Juiz de Fora, MG, Brazil.
[2] Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Escola Nacional de Botânica Tropical, Rio de Janeiro, RJ, Brazil.
[3] Universidade Federal de Juiz de Fora, Campus Universitário, Departamento de Botânica, Juiz de Fora, MG, Brazil.
*Corresponding author: ptimoteopatriciodasilva@gmail.com

ABSTRACT

Palynological studies provide valuable data for taxonomy, particularly in supporting species delimitation. The genus *Stachytarpheta* includes a smaller group of seven species with pedicellate flowers and salmon- or wine-colored corollas, which are restricted to the Brazilian *Cerrado*. Despite representatives of this group, known as the *S. longispicata* group, being readily distinguished from other *Stachytarpheta* taxa for bearing sessile flowers, differentiating the seven species remains challenging due to their pronounced morphological overlap. In this context, we analyze the pollen morphology of the seven species in the *S. longispicata* group, aiming to evaluate its relevance to current taxonomic circumscription. Palynological characterization was based on observations under light microscopy and scanning electron microscopy using pollen material from herbarium specimens of the seven species. Under light microscopy, we measured pollen grain parameters after chemically treating the samples. The pollen grains are monads, very large, radially symmetrical, isopolar, with an amb varying from triangular to quadrangular, and shape ranging from suboblate to prolate-spheroidal. Size, shape, sexine ornamentation, margin type, and membrane ornamentation were key to identification, supporting seven distinct species. We provide an identification key based on pollen morphology for the group.

Keywords: Palynology, Species complex, *Stachytarpheta*, Taxonomy, Verbenaceae.

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Introduction

Stachytarpheta Vahl is a monophyletic genus and the second largest within Verbenaceae, encompassing approximately 120 species of shrubs, subshrubs, and some herbs, characterized by an androecium with two fertile stamens and two staminodes (Atkins, 2005; Marx *et al.*, 2010; O'Leary& Múlgura, 2012; Cardoso *et al.*, 2021). Despite the mainly Neotropical distribution of the genus, some ruderal herb species of *Stachytarpheta* have become naturalized in tropical and subtropical regions of Africa, Asia, and Oceania. (Atkins, 2005; Cardoso *et al.*, 2021). Brazil constitutes the main center of diversity of the genus, comprising 91 species, 83 of which are endemic (Cardoso *et al.*, 2024; 2025; Cardoso & Salimena, 2025). Most of them have narrow ranges in the rupestrian grasslands (*campos rupestres*) and savannas of the *Espinhaço* Range (states of Bahia and Minas Gerais) and the *Chapada dos Veadeiros* (state of Goiás) (Atkins, 2005; Salimena & Silva, 2009; Cardoso *et al.*, 2024; Cardoso & Salimena, 2025).

Most species of *Stachytarpheta* bear sessile flowers arranged in spikes, except for a small group endemic to the Brazilian *Cerrado*, which is distinguished by pedicellate flowers organized in racemes (Atkins, 2005; Cardoso *et al.*, 2022; 2023). This group is further characterized by salmon- or wine-colored corollas, in contrast to the blue, bright red, atropurpureous, purple, black, lilac, or white corollas found in other congeners (Atkins, 2005). These distinctive traits render the group easily identifiable within the genus. There are no phylogenetic studies to determine whether the group is monophyletic so far.

According to Atkins (2005), this group of pedicellate flowers with salmon or wine-colored corollas was circumscribed into seven infraspecific taxa, including subspecies and varieties, under *Stachytarpheta longispicata* (Pohl) S.Atkins. However, due to poorly defined boundaries between the taxa, Cardoso *et al.* (2022) conducted a comprehensive revision of this species complex, integrating population morphometry and morphological evidence. Based on their findings, a new delimitation was proposed, recognizing five distinct species, each found in different regions, with no geographic co-occurrence: *S. brevibracteata* (Moldenke) P.H.Cardoso, *S. longipedicellata* (Moldenke) P.H.Cardoso, *S. longispicata* (Pohl) S.Atkins, *S. minasensis* (S.Atkins) P.H.Cardoso, and *S. ratteri* (S.Atkins) P.H.Cardoso. Subsequently, based on the examination of additional herbarium collections, two new species with pedicellate flowers and salmon-colored corollas were described: *Stachytarpheta rizzoi* P.H.Cardoso and *S. longibracteata* P.H.Cardoso (Cardoso *et al.*, 2023). Thus, the group—hereafter referred to as the *S. longispicata* group—currently comprises seven accepted species (Fig. 1A-G), all with distinct, non-overlapping geographic distributions (Cardoso *et al.*, 2022; 2023).

Palynological studies have been instrumental in angiosperm taxonomy, offering valuable information for delimiting morphologically similar species (e.g., Mezzonato-Pires *et al.*, 2018; Evgeny *et al.*, 2023; Deng *et al.* 2023). A recent study by Patrício *et al.* (2024) successfully distinguished selected Brazilian species of *Stachytarpheta* sect. *Stachytarpheta* (characterized by sessile flowers embedded in the rachis excavations and corollas that are lilac, purple, white, blue, or rarely red), underscoring the significance of palynological data for the taxonomy of the genus.

Palynological data for the *Stachytarpheta longispicata* group, included in *S.* sect. *Melasanthus* Walp., remains scarce. Pohl (1827), in the original description of *S. longispicata* (under the name *Melasanthus longespicatus* Pohl), characterized its pollen grains as spherical and yellow. Later, Atkins (1991) described the pollen morphology of *S. longispicata* (sub name *S. chamissonis* Walp.), reporting an equatorial diameter ranging from 85 μm to 100 μm (n = 7 pollen grains). In this study, we conducted a comprehensive analysis of pollen morphology in the seven accepted species of the *S. longispicata* group. Our aim was to evaluate whether palynological characters support the current taxonomic delimitation proposed by Cardoso *et al.* (2022; 2023), and to provide additional evidence for species boundaries within the group, whose identification remains challenging due to their striking morphological similarities.

Material and Methods

Material examined

Sixteen specimens belonging to the *Stachytarpheta longispicata* group were analyzed, all deposited at Leopoldo Krieger Herbarium (CESJ) of the Universidade Federal de Juiz de Fora (UFJF). The seven species within the group were sampled following the circumscription and geographic distribution data provided by Cardoso *et al.* (2022; 2023). The material examined is listed below; specimens indicated with an asterisk refer to the standard material.

Stachytarpheta brevibracteata (Moldenke) P.H.Cardoso - **BRAZIL. Minas Gerais:** Delfinópolis, 11/03/2003, J.N. Nakajima 3475*, R. Romero, R.L. Volpi, R.A. Pacheco & C.A. Faria; São Roque de Minas, 06/05/2021, P.H. Cardoso 65 & W.P. Leite; *ibidem*, P.H. Cardoso 66 & W.P. Leite.

Stachytarpheta longibracteata P.H.Cardoso - **BRAZIL. Tocantins:** Mateiros, 30/01/2015, G.M. Antar 721*, H.P. Antar & U.R. Chagas.

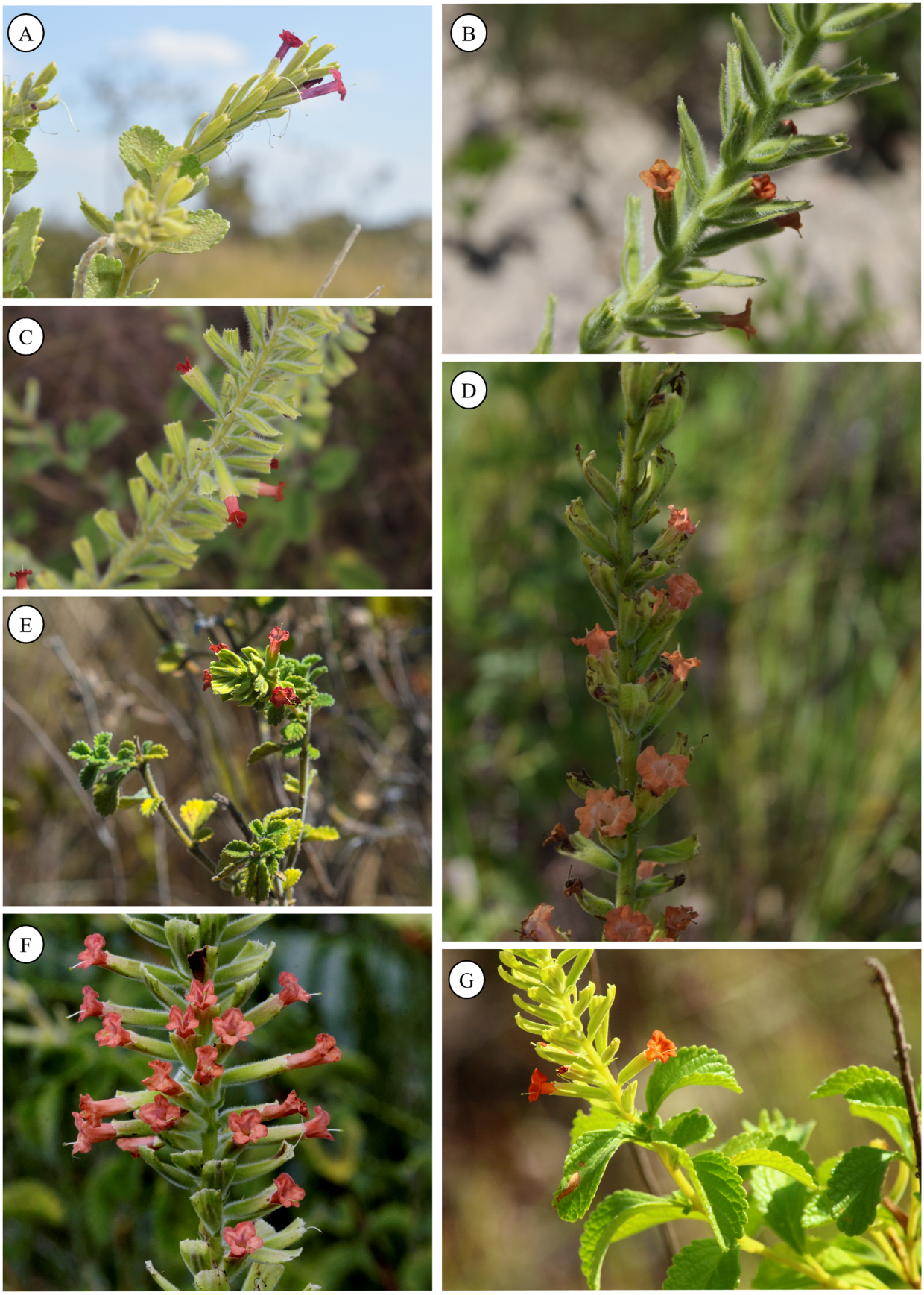

Figure 1. Photographs of individuals of the *Stachytarpheta longispicata* group. A. *Stachytarpheta brevibracteata*. B. *S. longibracteata*. C. *S. longipedicellata*. D. *S. longispicata*. E. *S. minasensis*. F. *S. ratteri*. G. *S. rizzoi*. Photo credits: A, C, D by Pedro Henrique Cardoso. B by Guilherme Antar; E by Pedro Henrique Nobre; F by Maurício Mercadante; G by Rodolph Delfino Sartin.

Stachytarpheta longipedicellata (Moldenke) P.H.Cardoso – **BRAZIL**. **Goiás**: Alto Paraíso de Goiás, 15/03/2020, P.H. Cardoso 59*, M. Pignal & L. Echternacht; 02/03/1972, *ibidem*, J.A. Rizzo 7762; Niquelândia, 06/01/1993, R.C. Mendonça, P.E. Noguera Silva & M. Claudio da Silva Jr. *s.n.* (CESJ 60161).*Stachytarpheta longispicata* (Pohl) S.Atkins – **BRAZIL**. **Goiás**: Cristalina, 12/03/2020, P.H. Cardoso 46*, I.M. Rollim, L.E.F. Silva & M. Trovó; *ibidem*, 12/03/2020, P.H. Cardoso 48, I.M. Rollim, L.E.F. Silva & M. Trovó.
Stachytarpheta minasensis (S.Atkins) P.H.Cardoso - **BRAZIL. Minas Gerais:** Joaquim Felício, 11/10/2022, F.R.G. Salimena 4125* & P.H. Nobre; *ibidem*, 14/09/2019, F.R.G. Salimena 4051 & P.H. Nobre; *ibidem*, 11/10/2022, F.R.G. Salimena 4124 & P.H. Nobre.
Stachytarpheta ratteri (S.Atkins) P.H.Cardoso – **BRAZIL**. **Distrito Federal**: Brasília, 07/05/2003, M.L. Fonseca 4664 & D. Alvarenga*; *ibidem*, 06/07/1999, J.G. Faria 261 *et al.*; *ibidem*, 07/03/2021, B. Schindler 56 & M. Figueira.
Stachytarpheta rizzoi P.H. Cardoso - **BRAZIL**. **Goiás**: Serra Dourada, 04/05/1969, Rizzo 4183*.

Observation by light microscopy

We described pollen grains using the method of Wodehouse (1935), with modifications proposed by Melhem *et al.* (2003). The acetolysis method used was lactic acetolysis (ACLAC) at 60 % following Raynal & Raynal (1971), to prevent morphological changes in pollen grains caused by Erdtman acetolysis (1960), as observed by another author (Atkins, 1991). The methodology used was the same performed by Patrício *et al.* (2024). We made measurements using a light microscope with an ocular micrometer. The palynological terminologies adopted were those by Erdtman (1952) and Punt *et al.* (2007). In equatorial view, the following parameters were measured: polar diameter (P), equatorial diameter (E), aperture width, and length. In polar view, we measured the following: largest diameter (DM), apocolpium side (A), exine stratification, and margin. For the standard specimens, we took measurements of P, E, DM, and A from 25 pollen grains contained in at least three slides, whenever possible. For the comparative specimens, morphometric data were based on 10 pollen grains contained on a minimum of three slides. We made all measurements within one week of slide preparation (Salgado-Labouriau, 1973).

Observation by Scanning Electron Microscopy (SEM)

Pollen grains were spread onto double-sided adhesive tape previously affixed to a numbered SEM stub. We examined samples under low vacuum in a FEI Quanta 250 scanning electron microscope (SEM).

Descriptive statistics

For parameters measured from a sample size of 25, we performed statistical analysis in Microsoft Excel 365 (version 2305) and included: size range (Xmin–Xmax), arithmetic mean (X), standard deviation (S), standard error (Sx), 95 % confidence interval (CI), and coefficient of variation (CV %). For other measurement parameters, we present only the mean and range.

Multivariate analysis

Using the PAST software (Hammer *et al.*, 2001), the morphometric data resulting from the palynological analyses were subjected to multivariate analysis. The resulting mean value of each parameter of the standard material was subjected to principal component analysis (PCA), using correlation.

Results

Description of pollen grains

The studied species (Fig. 1, Fig. 2, Fig. 3) present pollen grains that are monads, with a very large average size (Tab. 1; Tab. 2; Tab. 3; Tab. 4), radial symmetry, and isopolarity; amb varying from triangular (Fig. 2A), subtriangular, subcircular to quadrangular (Fig. 2K), and the shape varying from suboblate, oblate-spheroidal to prolate-spheroidal (Tab. 1). The pollen grains are tricolpate, sometimes tetracolpate, with narrow, anguloaperturate colpi (Fig. 2D). The pollen grains surface is verrucate, with verrucae either regularly distributed (Fig. 2A) or more distantly spaced (Fig. 2K). The pollen grains exhibit margins (Fig. 2, 3) and membranes (Figs. 2A, 2C, 2D, 2G–I, 2J, 2L, 3C-D).

Stachytarpheta brevibracteata – Pollen grains very large (Tab. 2; Tab. 3), with triangular (Fig. 2A), subtriangular, and subcircular amb, oblate-spheroidal shape (Tab. 1), and 3-colpate (Fig. 2A). Ornamentation with verrucae regularly distributed (Fig. 2A–C). Simple margin, with aligned and fused verrucae (Fig. 2C). The apertural membrane is psilate with occasional perforations (Fig. 2C).

Stachytarpheta longibracteata – Pollen grains very large (Tab. 2; Tab. 3), with triangular (Fig. 2E), subtriangular, and quadrangular amb (Fig. 2K), oblate-spheroidal shape (Tab. 1), and 3-colpate (Fig. 2E) or 4-colpate (Fig. 2K). Ornamentation with verrucae regularly distributed (Fig. 2D-F). Simple margin, with aligned and fused verrucae (Fig. 2E-F). The apertural membrane is psilate with occasional granules.

Stachytarpheta longipedicellata – Pollen grains very large (Tab. 2; Tab. 3), with triangular (Fig. 2G), subtriangular, and subcircular amb, oblate-spheroidal shape (Tab. 1), and 3-colpate (Fig. 2G). Ornamentation with verrucae regularly distributed (Fig. 2G–I). Simple margin, with fused verrucae (Fig. 2I). The apertural membrane is perforate (Fig. 2I).

Stachytarpheta longispicata – Pollen grains very large (Tab. 2; Tab. 3), with triangular (Fig. 2J), subtriangular, and subcircular amb, suboblate shape (Tab. 2; Tab. 3), and 3-colpate (Fig. 2J). Ornamentation with verrucae regularly distributed (Fig. 2J). Simple margin, with fused verrucae (Fig. 2J). The apertural membrane is psilate with occasional perforations.

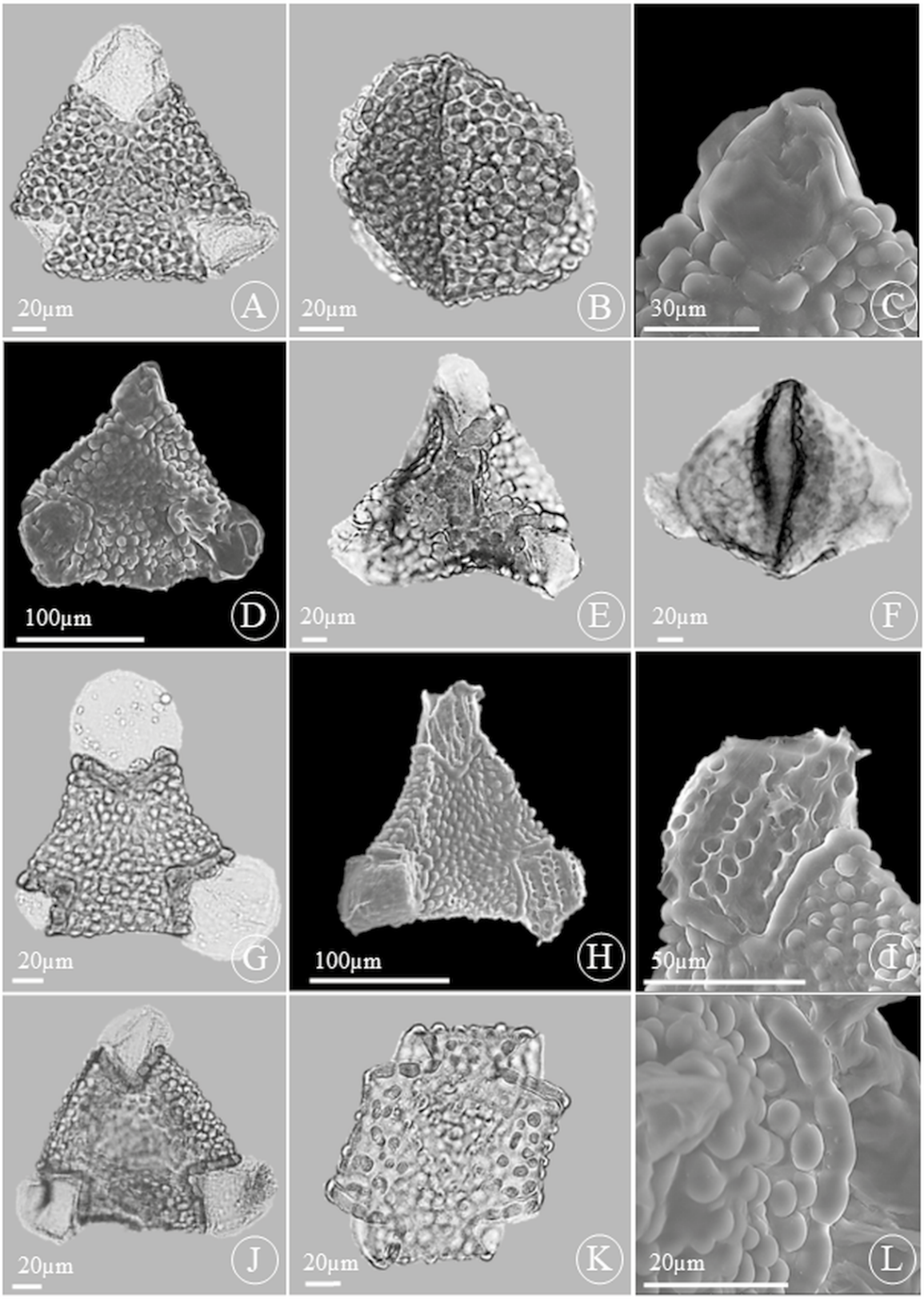

Figure 2. Photomicrographs (ML) and electromicrographs (SEM) of pollen grains of the studied species. A–D. *Stachytarpheta brevibracteata*. A. Polar view (ML - non-acetolized). B. Equatorial view (ML - acetolized). C. Details of membrane and margin (SEM - non-acetolized). D. Polar view (SEM - non-acetolized). E–F. *S. longibracteata*. E. Polar view (ML - non-acetolized). F. Equatorial view (ML - non-acetolized). G-I. *S. longipedicellata*. G. Polar view (ML - non-acetolized). H. Polar view (SEM - non-acetolized). I. Details of membrane and margin (SEM - non-acetolized). J. *S. longispicata*. J. Polar view (ML - non-acetolized). K–L *S. minasensis*. K. Polar view (ML - acetolized). L. Details of membrane and margin (SEM - non-acetolized).

Table 1. Morphometric data of pollen grains from the standard material of the *Stachytarpheta longispicata* group. P = polar diameter (µm); E = equatorial diameter (µm); DM = largest diameter (µm); A = side of the apocolpus (µm); C = length (µm); L = width (µm).

Species	P	E	P/E	DM	A	Nexine	Sexine	Margin	Aperture	
									C	L
S. brevibracteata	117.30	120.90	0.97	133.29	57.80	2.07	4.80	7.10	30.00	83.75
S. longibracteata	128.38	130.30	0.98	107.13	64.50	1.47	5.52	10.25	15.71	100.00
S. longipedicellata	107.50	113.50	0.94	128.14	54.10	5.95	2.25	10.25	9.37	103.12
S. longispicata	113.54	107.59	1.05	125.91	62.10	2.17	5.20	8.25	18.75	83.43
S. minasensis	108.63	106.50	1.02	124.02	67.40	2.05	6.02	7.80	27.00	90.17
S. ratteri	115.12	104.41	1.10	129.07	65.54	2.70	5.75	7.52	24.28	99.28
S. rizzoi	103.61	106.90	0.96	86.52	59.70	1.80	4.42	7.27	17.91	62.91

Table 2. Morphometric data for the polar diameter (P) of pollen grains of the standard material of the *Stachytarpheta longispicata* group. P = polar diameter (µm); Xmin = minimum (µm); Xmax = maximum (µm); X = mean (µm); sX = standard error (µm); S = standard deviation (µm); CI = 95 % confidence interval (µm); CV = coefficient of variability (%).

Species (P)	Xmin - Xmax	X ± sX	S	IC (95 %)	CV (%)
S. brevibracteata	100.00 - 135.00	117.30 ± 1.92	9.62	113.33 - 121.27	8.20
S. longibracteata	105.00 - 162.50	128.38 ± 2.71	13.59	122.77 - 133.99	10.58
S. longipedicellata	80.00 - 125.00	107.50 ± 2.08	10.40	103.21 - 111.79	9.67
S. longispicata	77.50 - 142.50	113.54 ± 2.68	13.44	107.99 - 119.09	11.83
S. minasensis	85.00 - 125.00	108.63 ± 1.80	9.04	104.90 - 112.36	8.32
S. ratteri	92.50 - 130.00	115.12 ± 1.74	8.87	111.52 - 118.72	7.57
S. rizzoi	87.50 - 122.50	103.61 ± 1.84	9.20	99.82 - 107.40	8.88

Table 3. Morphometric data for the equatorial diameter (E) of pollen grains of the standard material of the *Stachytarpheta longispicata* group. E = equatorial diameter (µm); Xmin = minimum (µm); Xmax = maximum (µm); X = mean (µm); sX = standard error (µm); S = standard deviation (µm); CI = 95 % confidence interval (µm); CV = coefficient of variability (%).

Species (E)	Xmin - Xmax	X ± sX	S	CI (95 %)	CV (%)
S. brevibracteata	110.00 - 137.50	120.90 ± 1.43	7.17	117.94 - 123.86	5.93
S. longibracteata	110.00 - 180.00	130.30 ± 3.44	17.20	123.20 - 137.40	13.20
S. longipedicellata	82.50 - 147.50	113.50 ± 3.67	18.38	105.92 - 121.08	16.19
S. longispicata	77.50 - 132.50	107.59 ± 2.46	12.30	102.52 - 112.66	11.43
S. minasensis	80.00 - 135.00	106.50 ± 2.71	13.55	100.91 - 112.09	12.72
S. ratteri	87.50 - 122.50	104.41 ± 2.15	10.78	99.96 - 108.86	10.32
S. rizzoi	87.50 - 132.50	106.90 ± 2.16	10.80	102.44 - 111.36	10.11

Table 4. Morphometric data of pollen grains from the comparison material of the *Stachytarpheta longispicata* group. P = polar diameter (µm); E = equatorial diameter (µm); DM = largest diameter (µm).

Species	Exsiccates	P	E	P/E	DM
S. brevibracteata	P.H. Cardoso 66 & W.P. Leite	99.54	123.74	0.80	98.86
	P.H. Cardoso 65 & W.P. Leite	99.04	119.54	0.82	111.89
S. longipedicellata	R.C. Mendonça *et al.* (CESJ 60161)	85.95	103.74	0.82	98.55
	J. A. Rizzo 7762	92.37	110.90	0.83	85.46
S. longispicata	P.H. Cardoso 48 *et al.*	115.75	103.75	1.11	94.52
S. minasensis	F.R.G. Salimena 4051 & P.H. Nobre	100.77	109.66	0.91	107.19
	F.R.G. Salimena 4124 & P.H. Nobre	92.37	107.44	0.85	112.13
S. ratteri	B. Schindler 56 & M. Figueira	105.90	107.50	0.98	107.75
	J.G. Faria *et al.* 261	100.27	92.75	1.08	110.17

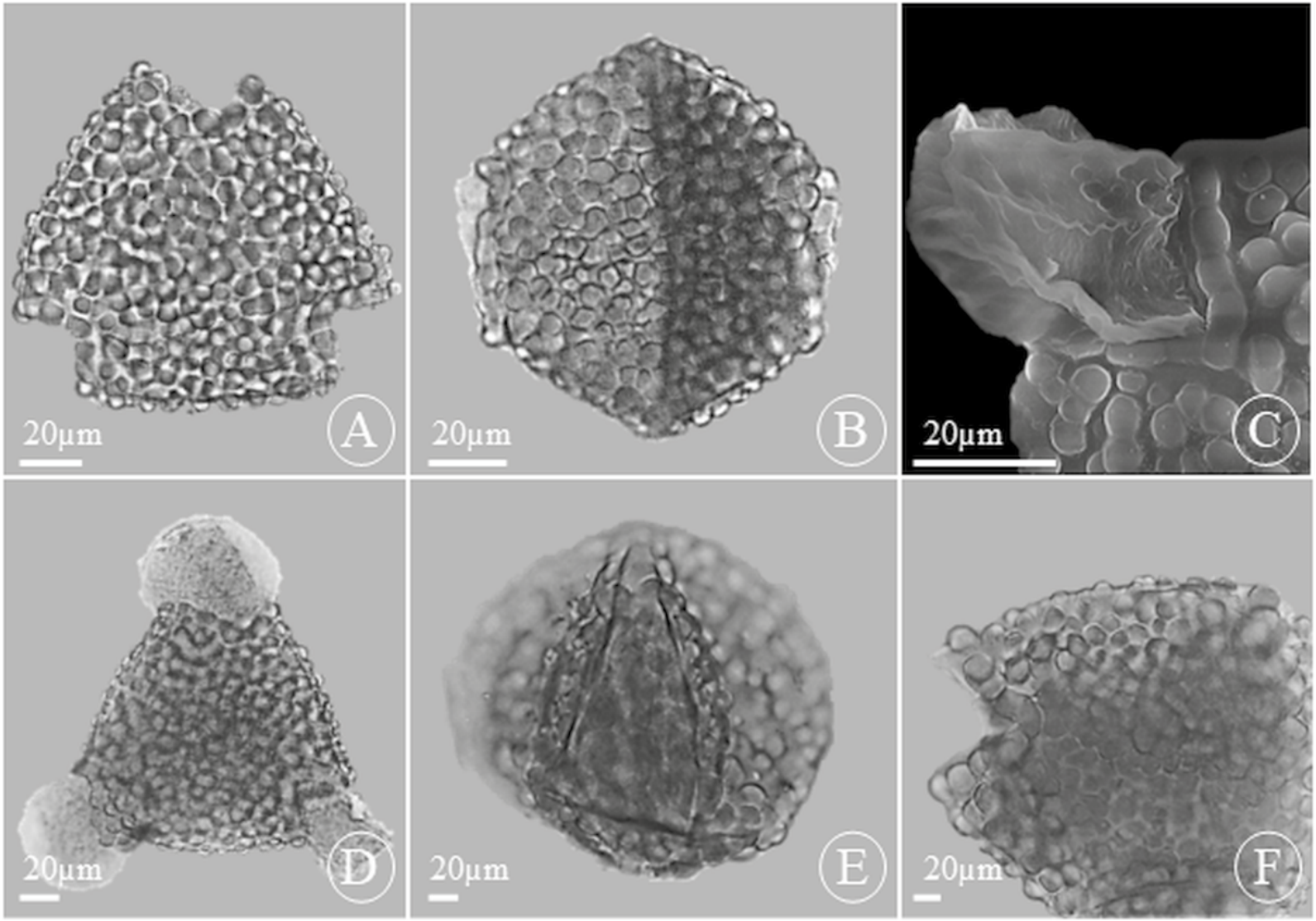

Figure 3. Photomicrographs (ML) and electromicrographs (SEM) of pollen grains of the studied species. A–C. *Stachytarpheta ratteri*. A. Polar view (ML - acetolized). B. Equatorial view (ML - acetolized). C. Details of the membrane and margin (SEM - non-acetolized). D–F. *S. rizzoi*. D. Polar view (ML - non-acetolized). E. Equatorial view (ML - acetolized). F. Details of the margin (ML - acetolized).

Stachytarpheta minasensis – Pollen grains very large (Tab. 2; Tab. 3), with triangular, subtriangular, quadrangular (Fig. 2K), and subcircular amb, prolate-spheroidal shape (Tab. 1); and 3-colpate or 4-colpate (Fig. 2K). Ornamentation with verrucae regularly distributed and distantly spaced (Fig. 2K-L). Double margin, the first with aligned verrucae, and the second presents verrucae fused and perforations(Fig. 2K-L). The apertural membrane is psilate (Fig. 2L).

Stachytarpheta ratteri – Pollen grains very large (Tab. 1), with triangular, subtriangular, and subcircular amb (Fig. 3A), prolate-spheroidal shape (Tab. 1), and 3-colpate (Fig. 3A). Ornamentation with verrucae regularly distributed (Fig. 3A–C). Simple margin, with aligned and fused verrucae (Fig. 3C). The apertural membrane is psilate, with occasional perforations and striations (Fig. 3C).

Stachytarpheta rizzoi – Pollen grains very large (Tab. 2; Tab. 3), with triangular (Fig. 3D), subtriangular, and subcircular amb, oblate-spheroidal shape (Tab. 1), and 3-colpate (Fig. 3D) or 4-colpate. Ornamentation with verrucae regularly distributed (Fig. 3D-F). Double margin, both ornamented with aligned and fused verrucae (Fig. 3E-F). The apertural membrane is perforate.

Multivariate analysis

The PCA (Table 5) showed that components 1 and 2 explain 80.1 % of the data, with 55.8 % explained by component 1 and 24.3 % by component 2 (Table 5). The scree plot (Fig. 4) confirms the importance of components 1 and 2.

Based on the PCA results (Fig. 5), it was possible to observe that the mean values of the standard material measurements allowed for the differentiation of the species in *Stachytarpheta longispicata*. The most relevant variables for this separation were polar diameter, equatorial diameter, largest diameter, nexine, and aperture width. *Stachytarpheta longibracteata* has the largest mean measurements of the standard material in polar diameter (128.38 µm) and equatorial diameter (130.30 µm) (Fig. 5), standing out from the other species analyzed. *Stachytarpheta longipedicellata* is positively positioned in components 1 and 2, presenting the largest mean measurements of the standard material in nexine (5.95 µm) and aperture width (103.12 µm) (Fig. 5). *Stachytarpheta rizzoi* is negatively positioned in components 1 and 2, presenting the smallest measurements of the standard material averages in the polar diameter (103.61 µm), equatorial diameter (106.90 µm) and largest diameter (86.52 µm) (Fig. 5). The species *S. brevibracteata*, *S. ratteri*, *S. longispicata*, and *S. minasensis* are close to each other, negatively positioned in component 1 by the measurements of the standard material averages in the largest diameter, being respectively 133.29 µm, 129.07 µm, 125.91 µm, and 124.02 µm (Fig. 5). The present results enable the distinction of the species in an identification key, presented below.

Table 5. Multivariate analysis (PCA) component data.

PC	Eigenvalue	% variance
1	393.817	55.797
2	171.228	24.26
3	84.0365	11.906
4	45.4275	6.4363
5	9.12391	1.2927
6	2.1714	0.30765

Palynological identification key for the *Stachytarpheta longispicata* group

1. Pollen grains that may have 4 colpi 2
1'. Pollen grains always 3-colpate 3
2. Pollen grains with membrane having occasional granules ..... *S. longibracteata*
2'. Pollen grains with membrane having occasional perforations and striations ..... *S. ratteri*
3. Pollen grains with double margins 4
3'. Pollen grains with simple margins 5
4. Pollen grains with a psilate membrane ..... *S. minasensis*
4'. Pollen grains with a perforate membrane ..... *S. rizzoi*
5. Pollen grains with margin presenting aligned and fused verrucae ..... *S. brevibracteata*
5'. Pollen grains with margin presenting fused verrucae 6
6. Pollen grains with margin measuring >10 µm ..... *S. longipedicellata*
6'. Pollen grains with margin measuring <10 µm ..... *S. longispicata*

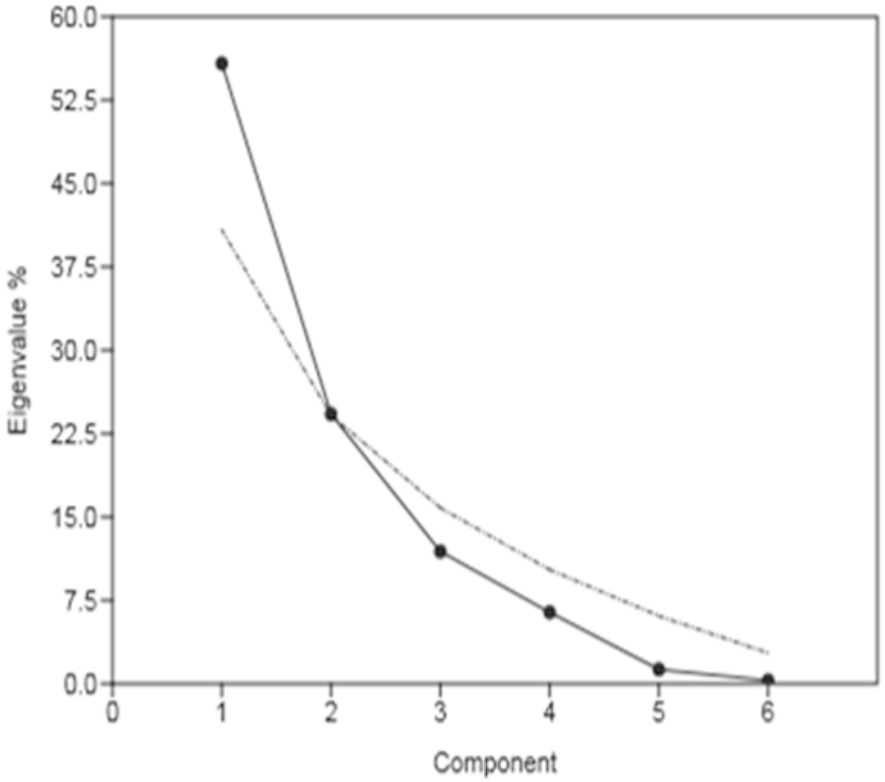

Figure 4. Graph showing the scree plot of the principal component analysis.

Discussion

Although palynological studies on *Stachytarpheta* are still incipient (Erdtman, 1963; Raj, 1983; Atkins, 1991; 2004; 2005; Adedeji, 2010; Patrício *et al.*, 2024; Cardoso *et al.*; 2025), research on related genera within Verbenaceae, such as *Lantana* L. and *Phyla* Lour. (see Atkins, 2004; O'Leary & Múlgura, 2012), suggests that characters like ornamentation and number of colpi have similar diagnostic value, reinforcing the applicability of this type of analysis within the family. Raj (1983) and Atkins (1991) described the pollen grains of *Stachytarpheta longispicata* as 3-colpate, oblate to peroblate. Although employing distinct terminology, Atkins (1991) reported variations in amb that are corroborated by the present study, as well as the verrucate ornamentation. Atkins (1991) noted that Erdtman's (1960) acetolysis method caused some pollen grains to collapse and that measurements were taken from only seven pollen grains. In the present study, lactic acetolysis (Raynal & Raynal, 1971) was employed, a method previously used for *Stachytarpheta* pollen grains by Patrício *et al.* (2024). The different preparation techniques used for pollen grains may account for the size variations observed when comparing the present data with those reported by Atkins (1991), particularly in equatorial diameter.

The species limits within the *Stachytarpheta longispicata* group were clarified by Cardoso *et al.* (2022; 2023). Among these, *S. brevibracteata* and *S. minasensis* are the most similar, as both have shorter inflorescences and occur in the state of Minas Gerais (Cardoso *et al.*, 2022). In addition to the morphological traits distinguishing them, such as leaf size, indumentum, and calyx width (Cardoso *et al.*, 2022), *S. brevibracteata* presents oblate-spheroidal pollen grains, with three colpi, a single ornamented margin with aligned verrucae, and a membrane with occasional perforations. In contrast, *S. minasensis* has prolate-spheroidal pollen grains, with three to four colpi, a double margin, and a psilate membrane. In the multivariate analysis, the species are positioned on the same side of the graph due to the similarity in the measurements of the standard material for the largest diameter; however, they are separated by variation in the sexine measurement.

Cardoso *et al.* (2023) highlighted that *Stachytarpheta longibracteata*, found in the Jalapão region, state of Tocantins, is morphologically most similar to *S. longipedicellata*, which is frequently found in the *Chapada dos Veadeiros* region, state of Goiás. The pollen grains of these two species are very large in size and exhibit an oblate-spheroidal shape. However, they can be distinguished by amb, as well as by the ornamentation of the margin and membrane. In *S. longibracteata*, the amb ranges from triangular and subtriangular to quadrangular, the grain may present four colpi, the margin is composed of aligned and fused verrucae, and the membrane is psilate with occasional granules. In contrast, *S. longipedicellata* has triangular, subtriangular, or subcircular amb, a simple margin with fused verrucae, and a perforated membrane.

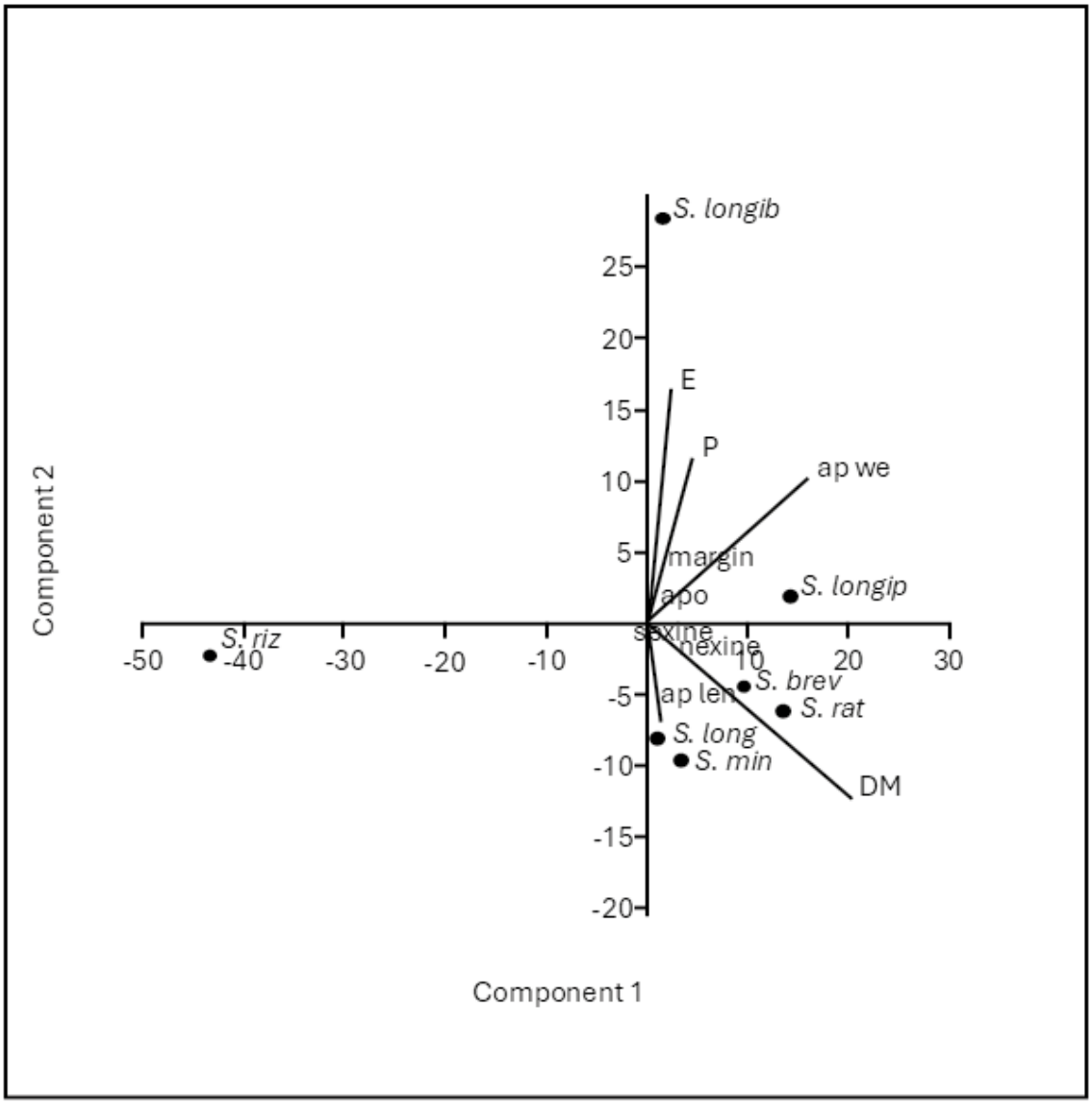

Figure 5. Graph showing principal component analysis: *S. brev* = *Stachytarpheta brevibracteata*. *S. longib* = *S. longibracteata*. *S. longip* = *S. longipedicellata*. *S. long* = *S. longispicata*. *S. min* = *S. minasensis*. *S. rat* = *S. ratteri*. *S. riz* = *S. rizzoi*.

In the multivariate analysis, the two species were differentiated based on the mean measurements of the standard material. *Stachytarpheta longibracteata* was distinguished mainly by its polar and equatorial diameters, whereas in *S. longipedicellata*, the most informative variables were nexine thickness and aperture width. Both species were positioned on the same side of the graph for components 1 and 2; however, all standard measurements differed between them, except for the margin measurement (10.25 µm).

Stachytarpheta longispicata and *S. ratteri* are morphologically similar and constitute the two most difficult species to distinguish from one another within the group when relying solely on herbarium material. The former is endemic to the municipality of Cristalina, in the state of Goiás, whereas the latter is the most frequently collected species of the group, common in Brasília, Distrito Federal, and surrounding areas (Cardoso *et al.*, 2022). However, their pollen grains can be distinguished based on the following characteristics: *S. longispicata* has pollen grains with a suboblate shape, fused verrucae on the margin, and a psilate membrane with occasional perforate; whereas in *S. ratteri*, the pollen grains have a prolate-spheroidal shape, aligned verrucae on the margin, and a psilate membrane with occasional perforations and striations. According to Cardoso

et al. (2023), *S. rizzoi* is also morphologically similar to *S. ratteri*. Nevertheless, the pollen grains of these two species differ in shape and margin type. In *S. rizzoi*, pollen grains are oblate-spheroidal, with a double margin and a perforated membrane. In the multivariate analysis, *S. longispicata* and *S. ratteri* clustered together in the scatter plot due to similar mean values of the largest diameter of the standard material. In contrast, *S. rizzoi* was separated from the other species, positioned negatively on components 1 and 2, mainly due to its polar diameter, equatorial diameter, and largest diameter measurements. The results of this study demonstrate that pollen morphology and PCA support the distinction among the seven species comprising the *Stachytarpheta longispicata* group. Therefore, this study reinforces the role of palynology as a useful tool for the taxonomy of *Stachytarpheta*, as also demonstrated by Patrício *et al.* (2024).

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Authors' Contributions
Conceptualization (Luizi-Ponzo A., Patrício PT and Cardoso PH); Investigation, data curation, and analysis (Luizi-Ponzo AP and Patrício PT), Project administration (Luizi-Ponzo AP, Patrício PT and Cardoso PH); Resources (Luizi-Ponzo AP and Cardoso PH); Figures (Luizi-Ponzo AP, Patrício PT and Cardoso PH); Writing – original draft (Luizi-Ponzo AP, Patrício PT and Cardoso PH); Writing – review and editing (Luizi-Ponzo AP, Patrício PT and Cardoso PH).

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