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Pollen morphology of the Brazilian species of *Bernardia* Houst. ex Mill. and *Tragia* L. (Euphorbiaceae, Acalyphoideae)

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ABSTRACT

Bernardia and *Tragia* are the largest genera of the tribes Bernardieae and Plukenetieae (Euphorbiaceae,), with 68 and 125 species, respectively. Very few palynological studies have focused specifically on these genera in spite of the great pollen diversity observed in the family. The present study analyzed the pollen morphology of the Brazilian species of *Bernardia* and *Tragia* to identify diagnostic characteristics that could aid in their taxonomic circumscription. The pollen grains of 11 species of *Bernardia* and five of *Tragia* were obtained from specimens deposited in the HRB, HUEFS, PEUFR, RB and UFP herbaria, and were analyzed using light and scanning electron microscopy. The analyzed species had small- to medium-sized pollen grains in monads, with shapes from prolate to subprolate. The most significant differences were observed in aperture type and exine ornamentation. The pollen grains of *Bernardia* are tricolporate, with aperture margins varying from narrow to wide or with just slightly evident margins, and the exine varying between microreticulate and microreticulate-perforate. The pollens of *Tragia* are tricolpate with the exine varying between intectate pilate and verrucate. Some species of both genera could be diagnosed based on unique pollen characters, and four distinct morphological groups of *Bernardia* and two of *Tragia* were observed.

Keywords: Bernardieae, pollen grains, Plukenetieae, plant taxonomy, Tragiinae

Introduction

Euphorbiaceae *s.s.* comprises 219 genera and approximately 6,300 species distributed globally (with the exception of the coldest regions, such as the Arctic), being found predominantly in tropical and subtropical regions (Wurdack & Davis 2009). The family is represented in Brazil by ca. 950 species (634 of them endemics), distributed in 64 genera, and they are most common in the Cerrado Biome (395 spp.) (Flora do Brasil 2020 [under construction] 2017).

As it displays many pollen types, Euphorbiaceae is considered a euripollinic family (Salgado-Labouriau 1973). The grains can have three or more apertures, with a thin or thick exine, the sexine tectate or not, pilate, reticulate, with a *Croton* pattern, smooth or with spines (Salgado-Labouriau 1973). Punt (1962; 1967; 1972; 1980; 1987), Nowicke *et al*. (1999), Takahashi *et al*. (2000), and Nowicke & Takahashi (2002) examined representatives of Euphorbiaceae for pollinic features that could aid in taxonomic studies, and morphopollinic characters were used in the phylogenetic studies of Gillespie (1994b), Takahashi *et al*. (1995), and Cardinal-McTeague & Gillespie (2016) to better understand the phylogenetic relationships of the family.

Bernardia and *Tragia* belong to the subfamily Acalyphoideae and are placed in the tribes Bernardieae

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and Plukenetieae, respectively (Webster 1994). *Bernardia* comprises 68 species distributed in neotropical forests, principally from Brazil to Mexico (Webster 1994; Govaerts *et al*. 2000). It is represented in Brazil by 22 species that are distributed mostly in the Southeast, in areas of Cerrado (neotropical savanna) and Atlantic Forest (Carrión 2018). *Tragia* comprises 125 species with pantropical distribution, but its principal centers of diversity in the Americas and Africa (Webster 2014). Sixteen species occur in Brazil, distributed throughout the country, although more common in the Atlantic Forest (Flora do Brasil 2020 [under construction] 2017).

The two genera can be distinguished by their habits, breeding systems, types of trichomes, presence or absence of leaf glands, and the type of inflorescence. *Bernardia* species are herbs or shrubs, monoecious or dioecious, with simple or stellate trichomes, leaves generally with basilaminar glands, and unisexual inflorescences (Webster & Burch 1967; Webster 1994). *Tragia* species are herbaceous vines, monoecious, with simple, urticating trichomes and capitate glands, leaves lacking basilaminar glands, and bisexual inflorescences (Urtecho 1996; Cardinal-McTeague & Gillespie 2016).

In spite of their large numbers of species and broad geographic distributions, there have been relatively few palynological examinations of representatives of *Bernardia* and *Tragia* (Punt 1962; Gillespie 1994b; Nowicke *et al*. 1999; Suarez-Cervera *et al*. 2001; Nowicke & Takahashi 2002; Cardinal-McTeague & Gillespie 2016). The first observations of the pollen morphology of species of *Tragia* were performed by Punt (1962), who examined species of *Tragia* sect. *Bia*. That author separated them into types and subtypes based on the shapes of the pollen grains and their apertures and ornamentation. Gillespie (1994b) analysed 21 species of *Tragia* from various countries and described seven pollen types based on the morphology of their apertures and of their exine structure. Nowicke *et al*. (1999) examined the morphology of the pollen grains of nine species of *Bernardia*, which was described as having tricolpate grains with a well-developed exine. Nowicke & Takahashi (2002) analysed the pollen grains of 14 species of *Tragia* occurring in North America, South America, and Africa, and described three pollen types. Cardinal-McTeague & Gillespie (2016) studied the phylogeny and palynology of tribe Plukenetieae, including three species of *Bernardia* and 50 species of *Tragia*. Their results indicated that the pollen characters examined supported the monophyly of each subtribe of Plukenetieae.

The morphological characteristics of pollen grains of *Bernardia* and *Tragia* can be useful characters in phylogenetic and taxonomic studies. However, there are few palynological studies of those two genera. Thus, the present work was designed to describe the pollen grains of the some species of *Bernardia* and *Tragia* that occur in Brazil, using light (LM) and scanning electron microscopy (SEM), to identify diagnostic characteristics that will aid in delimiting the genera and their species.

Materials and methods

Sixteen species were analyzed, 11 of *Bernardia* Houst. ex. Mill. and five of *Tragia* L. The species were selected according to the material available for analysis of pollen grains. Whenever possible, three specimens of each species were analyzed, totaling 31 specimens. All species occur in Brazil and 12 of them are endemics. The non-endemic species are also found in Argentina (*T. geraniifolia* Klotzsch ex Baill.), Bolivia (*B. paraguariensis* Chodat & Hassl., *B. pulchella* [Baill.] Müll. Arg.), or in Costa Rica, Guatemala, Guyana, Mexico, Nicaragua, and Venezuela (*B. sidoides* [Klotzsch] Müll. Arg.) (Cervantes 2006). A list of the taxa examined, with voucher information, is provided below. The palynological analyses of *Bernardia* and *Tragia* were performed using both optical (MO) and Scanning Electron Microscopy (SEM). The pollen material was obtained from specimens deposited in the HRB, HUEFS, PEUFR, RB, and UFP herbaria (acronyms according to Thiers 2018). The palynological descriptions and terminology follow Punt *et al*. (2007).

The pollen grains were prepared for optical microscopic examination using the classic method of acetolysis (Erdtman 1960), with the simple adaptation of using a warm water bath to reduce the exposure time from two minutes to 30 seconds for *Tragia* species, as the pollen grains of that genus are fragile. The slides were mounted with glycerinated gelatin and sealed with melted paraffin. The grains were measured using the principal morphometric parameters, within seven days of treatment (Salgado-Labouriau 1973). Measurements of the smallest and largest diameters were made for 25 pollen grains, while exine measurements were made for ten pollen grains, all chosen at random.

The statistical analyses were performed using Excel, calculating the arithmetic means ($\overline{X}$), the standard deviations of the samples (s), the standard deviations of the means ($S_{\overline{X}}$), and the coefficient of variation (CV) for the measurements of the pollinic parameters, with sample sizes equal to 25; for the measurements with samples sizes equal to 10, only the arithmetic means were calculated. The microscopic analyses were undertaken in the Laboratório de estudos palinológicos (LAEP) in the Departamento de Ciências Exatas e da Terra (DCET) of the Universidade do Estado da Bahia (UNEB), *Campus* II, Alagoinhas, Bahia State.

The scanning electron microscopic analyses were performed using pollen grains that were dehydrated but not acetolized. Anthers from pre-anthesis flower buds were macerated and mounted on metal stubs with two-sided carbon adhesive tape and subsequently sputter-coated with gold under high vacuum. Electron micrographs of the pollen grains were captured using a Quanta 250 (FEI Company) scanning electron microscope at the Centro de

Microscopia Eletrônica (CME) at the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia State.

Material examined: *Bernardia axillaris* (Spreng.) Müll. Arg. - Brasil. Rio de Janeiro: Cabo Frio, 30/IX/1997, *Farney C. et al.* 3603 (RB); Armação de Búzios, 21/VIII/1998, *Farney C. et al.* 3816 (RB). *B. celastrinea* (Baill.) Müll. Arg. - Rio de Janeiro: Rio de Janeiro, 08/X/1946, *Duarte A. P.* 356 (RB); idem, III/1960, *Duarte A. P.* 5203 (RB). *B. crassifolia* Müll. Arg. - Bahia: Salvador, 04/07/2002, *Pereira-Silva G. et al.* 6553 (HUEFS); Minas Gerais: Serra do Cipó, 26/X/1961, *Duarte A. P.* 6422 (RB); Santana do Riacho, 09/I/1981, *Henrique M. C. et al.* s/n (UFP – 7471). *B. gambosa* Müll. Arg. - Bahia: Amargosa, 29/I/2006, *Costa M. A. A. et al.* 223 (HRB). *B. hirsutissima* (Baill.) Müll. Arg. - Goiás: Campo Alegre de Goiás, 08/IX/1998, *Souza V. C. et al.* 21322 (RB). *B. paraguariensis* Chodat & Hassl. - Mato Grosso do Sul: Caracol, 30/X/2003, *Hatschbach G. et al.* 76533 (HUEFS). *B. pulchella* (Baill.) Müll. Arg. - Paraná: Curitiba, 08/XII/1993, *Cordeiro J. & Soares A. A.* 1132 (HUEFS); Campo Mourão, 02/II/2006, *Geraldino H. C. L.* 311 (HUEFS). *B. scabra* Müll. Arg. - Bahia: Una, 12/VIII/1999, *Mattos-Silva L. A. et al.* 4020 (HUEFS); Bahia: Buerarema, 15/VI/2002, *Mattos-Silva L. A.* 4535 (HUEFS); Camacan, 31/VIII/2008, *Amorim A. M. et al.* 7694 (HUEFS). *B. sidoides* (Klotzsch) Müll. Arg. - Bahia: Abaré, 30/III/2007, *Oliveira M. et al.* 2790 (UFP); Pernambuco: Ilha Fernando de Noronha, 08/IV/1999, *Miranda A. M.* 3220 (HUEFS); Piauí: Teresina, 10/VII/1999, *Santos-Filho F. S.* 71 (PEUFR). *B. similis* Pax & K. Hoffm. - Bahia: Una, s.d., *Sobral M. et al.* 5799 (HRB); Minas Gerais: Conselheiro Mata, VI/1934, *Brade A. C.* 13590 (RB). *B. tamanduana* (Baill.) Müll. Arg. - Bahia: Conceição da Feira, 31/VII/1980, *Noblick L. R.* s/n (HUEFS – 00343); idem, X/1980, *Noblick L. R.* s/n (HUEFS – 01398); Jacobina, 23/VI/1999, *França F. et al.* 3054 (HUEFS). *Tragia bahiensis* Müll. Arg. - Minas Gerais: Monte Azul, 22/IV/2006, *Carneiro-Torres D. S. et al.* 715 (HUEFS); Pernambuco: Ouricuri, 10/III/1982, *Lima V. C. et al.* 49 (PEUFR). *T. cearensis* Pax & K. Hoffm. - Rio Grande do Norte: Serra Negra do Norte, 14/IV/2006, *Queiroz R. T.* 677 (HUEFS). *T. chlorocaulon* Baill. - Minas Gerais: Carangola, 10/VII/2009, *Marcolino F. & Pereira R. S.* 131 (RB). *T. geraniifolia* Klotzsch ex Müll. Arg. - Argentina. Corrientes: Estancia Yacare, 02/XII/1998, *Arbo M. M. et al.* 8202 (HUEFS). *T. volubilis* L. - Bahia: Bom Jesus da Lapa, 09/II/2000, *Queiroz L. P. et al.* 5798 (HRB); Catu, 26/IV/2017, *Santos M. O. & Costa M. M.* 30 (HUNEB); Rio de Janeiro: Rio de Janeiro, 10/XI/1971, *Sucre D.* 7905 (RB).

Results

The species studied produced pollen grains in small to medium-sized monads (Tabs. 1, 2; Figs. 1–5). The smallest grains were encountered in *Bernardia pulchella* (16.8 µm diam) and the largest in *Tragia geraniifolia* (26.6 µm diam) (Tab. 2). Most of the species had grains with shapes that varied between subprolate to prolate.

All of the species analysed showed isopolar grains with three apertures, colporate in *Bernardia* and colpate in *Tragia*. Some species of *Bernardia* demonstrated variations in their aperture margins from inconspicuous (*B. axillaris, B. crassifolia, B. sidoides, B. similis*; Figs. 1C, I, 3C, F) to narrow (*B. scabra*; Fig. 2L) to wide (*B. celastrinea*, *B. gambosa*, *B. hirsutissima*, *B. paraguariensis*, *B. pulchella*, *B. tamanduana*; Figs. 1F, L, 2C, 2F, I, 3I).

Exine ornamentation could be microreticulate (*Bernardia axillaris*, *B. celastrinea*, *B. crassifolia*, *B. gambosa*, *B. paraguariensis*, *B. pulchella*, *B. scabra*, *B. sidoides*, *B. similis*; Fig. 5A-D, F- J) or microreticulate-perforate (*B. hirsutissima*, *B. tamanduana*; Fig. 5E, K) in species of *Bernardia,* and intectate and pilate (*Tragia bahiensis*, *T. chlorocaulon*, *T. volubilis*; Fig. 5L, N, P) or verrucate (*T. cearensis*, *T. geraniifolia*; Fig. 5M, O) in species of *Tragia*. The sexines and nexines had equivalent

Table 1. Synopses of the morphopollinic characters of the some Brazilian species of *Bernardia* and *Tragia*.

Genera/Species	Size	Shape	Amb	Aperture Type	Exine ornamentation (LM and SEM)
Bernardia axillaris	Small/Medium	Prolate spheroid to Prolate	Subcircular	Colporate	Microreticulate
B. celastrinea	Small	Subprolate	Subcircular	Colporate	Microreticulate
B. crassifolia	Small/Medium	Subprolate to Prolate	Subcircular	Colporate	Microreticulate
B. gambosa	Small	Subprolate	Subcircular	Colporate	Microreticulate
B. hirsutissima	Small	Subprolate	Subcircular	Colporate	Microreticulate-perforated
B. paraguariensis	Small	Subprolate	Subcircular	Colporate	Microreticulate
B. pulchella	Medium	Prolate	Subcircular	Colporate	Microreticulate
B. scabra	Medium	Subprolate to Prolate	Subcircular	Colporate	Microreticulate
B. sidoides	Small	Subprolate	Subcircular	Colporate	Microreticulate
B. similis	Medium	Subprolate	Subcircular	Colporate	Microreticulate
B. tamanduana	Medium	Prolate	Subcircular	Colporate	Microreticulate-perforated
Tragia bahiensis	Medium	Subprolate to Prolate	Triangular	Colpate	Intectate, pilate
T. cearensis	Small	Subprolate	Subtriangular	Colpate	Verrucate
T. chlorocaulon	Medium	Subprolate	Subtriangular	Colpate	Intectate, pilate
T. geraniifolia	Medium	Prolate	Subcircular	Colpate	Verrucate
T. volubilis	Small/Medium	Subprolate to Prolate	Subtriangular	Colpate	Intectate, pilate

Table 2. Morphometric data of the pollen grains of the some species of *Bernardia* and *Tragia*.

Species/Specimen	PD		ED		EDp		P/E	Ecto	Endo	PAI	Sex	Nex
	$\bar{X} \pm S_{\bar{X}}$	Fv	$\bar{X} \pm S_{\bar{X}}$	Fv	$\bar{X} \pm S_{\bar{X}}$	Fv						
Bernardia axillaris												
Farney C. et al. 3603 (RB)	23.2 ± 1.6	20.0-27.0	20.7 ± 2.1	16.0-27.0	20.2 ± 1.2	17.0-23.0	1.13	14.7	2.7 x 5.0	0.18	1.0	1.4
Farney C. et al. 3816 (RB)	26.9 ± 2.4	21.0-31.0	19.8 ± 1.2	18.0-23.0	19.4 ± 1.6	16.0-22.0	1.35	22.7	3.5 x 5.5	0.20	1.0	1.0
B. celastrinea												
Duarte A. P. 356 (RB)	23.7 ± 2.6	20.0-28.0	17.8 ± 2.3	14.0-22.0	17.6 ± 2.5	14.0-22.0	1.33	20.9	2.0 x 2.9	0.22	0.9	0.9
Duarte A. P. 5203 (RB)	22.3 ± 1.5	19.0-25.0	18.4 ± 2.5	14.0-23.0	20.4 ± 2.2	17.0-26.0	1.23	18.2	-	0.29	1.0	1.0
B. crassifolia												
Pereira-Silva G. et al. 6553 (HUEFS)	29.0 ± 3.3	22.0-33.0	23.0 ± 2.8	17.0-27.0	21.9 ± 2.9	17.0-28.0	1.26	24.9	2.0 x 3.4	0.12	1.0	1.0
Duarte A. P. 6422 (RB)	22.4 ± 0.9	21.0-25.0	16.8 ± 1.1	14.0-19.0	18.0 ± 1.8	16.0-24.0	1.33	18.6	3.5 x 6.4	0.24	1.0	1.1
Henrique M. C. et al. s/n (UFP - 7471)	28.2 ± 1.2	26.0-31.0	21.0 ± 2.8	17.0-29.0	20.7 ± 1.7	18.0-25.0	1.35	23.7	2.7 x 4.8	0.10	1.0	1.0
B. gambosa												
Costa M. A. A. et al. 223 (HRB)	24.2 ± 1.6	20.0-26.0	20.2 ± 1.4	17.0-23.0	20.9 ± 0.9	19.0-23.0	1.20	18.8	3.0 x 4.4	0.37	1.0	1.1
B. hirsutissima												
Souza V. C. et al. 21322 (RB)	23.4 ± 2.3	20.0-30.0	20.1 ± 2.2	17.0-27.0	21.2 ± 2.6	16.0-26.0	1.16	21.7	4.9 x 6.6	0.44	1.0	1.0
B. paraguariensis												
Hatschbach G. et al. 76533 (HUEFS)	20.7 ± 2.6	17.0-28.0	17.5 ± 2.4	15.0-26.0	18.1 ± 1.9	15.0-22.0	1.19	16.1	3.0 x 5.1	0.16	1.0	1.0
B. pulchella												
Cordeiro J. & Soares A. A. 1132 (HUEFS)	27.5 ± 1.6	23.0-29.0	16.8 ± 0.9	15.0-19.0	18.1 ± 1.4	15.0-21.0	1.63	25.7	7.3 x 9.2	0.35	1.0	1.0
Geraldino H. C. L. 311 (HUEFS)	28.2 ± 2.0	22.0-31.0	18.7 ± 1.7	15.0-23.0	18.8 ± 1.6	16.0-21.0	1.51	24.7	5.4 x 6.5	0.32	1.0	1.4
B. scabra												
Mattos-Silva L. A. et al. 4020 (HUEFS)	30.5 ± 2.5	26.0-35.0	22.5 ± 2.5	18.0-27.0	23.3 ± 2.5	18.0-29.0	1.36	28.5	5.0 x 10.6	0.25	0.9	1.0
Mattos-Silva L. A. 4535 (HUEFS)	29.8 ± 2.3	26.0-35.0	21.8 ± 1.4	20.0-26.0	22.6 ± 1.6	20.0-26.0	1.36	27.5	3.7 x 9.5	0.36	1.0	1.0
Amorim A. M. et al. 7694 (HUEFS)	27.8 ± 1.8	25.0-31.0	22.2 ± 1.5	20.0-26.0	26.3 ± 1.6	22.0-30.0	1.25	24.2	2.0 x 4.2	0.19	1.0	1.8
B. sidoides												
Oliveira M. et al. 2790 (UFP)	20.2 ± 2.0	17.0-25.0	17.6 ± 2.3	14.0-22.0	18.2 ± 1.8	15.0-22.0	1.15	14.4	2.5 x 4.8	0.13	1.0	1.0
Miranda A. M. 3220 (HUEFS)	20.8 ± 2.6	17.0-25.0	17.0 ± 2.5	12.0-21.0	19.5 ± 2.0	13.0-22.0	1.24	19.6	2.7 x 6.1	0.28	0.7	0.7
Santos-Filho F. S. 71 (PEUFR)	20.8 ± 2.6	17.0-25.0	17.4 ± 2.4	13.0-21.0	17.2 ± 1.9	13.0-22.0	1.20	17.5	2.6 x 4.7	0.16	1.0	1.0
B. similis												
Sobral M. et al. 5799 (HRB)	25.6 ± 2.1	21.0-30.0	20.3 ± 1.6	17.0-23.0	21.9 ± 1.7	18.0-25.0	1.27	22.7	3.2 x 6.0	0.24	0.6	0.6
Brade A. C. 13590 (RB)	27.6 ± 1.3	25.0-30.0	20.7 ± 1.1	19.0-23.0	21.6 ± 1.4	19.0-25.0	1.33	22.6	2.9 x 6.5	0.14	1.0	1.0
B. tamanduana												
Noblick L. R. s/n (HUEFS - 00343)	28.8 ± 4.1	20.0-35.0	20.0 ± 2.3	15.0-24.0	23.6 ± 2.9	17.0-28.0	1.44	23.4	3.2 x 6.6	0.13	0.5	0.5
Noblick L. R. s/n (HUEFS - 01398)	27.0 ± 1.2	24.0-29.0	17.7 ± 1.2	16.0-21.0	18.7 ± 1.6	16.0-23.0	1.53	25.4	2.5 x 6.7	0.16	0.7	0.7
França F. et al. 3054 (HUEFS)	28.7 ± 2.2	24.0-32.0	19.3 ± 1.6	17.0-23.0	19.2 ± 1.0	17.0-21.0	1.49	26.6	3.6 x 6.3	0.19	1.0	1.0
Tragia bahiensis												
Carneiro-Torres D. S. et al. 715 (HUEFS)	27.6 ± 2.8	22.0-32.0	21.5 ± 2.4	15.0-26.0	29.4 ± 4.5	21.0-42.0	1.29	23.4	-	0.20	0.6	0.6
Lima V. C. et al. 49 (PEUFR)	27.0 ± 3.3	22.0-34.0	19.3 ± 3.9	13.0-28.0	24.0 ± 2.9	20.0-30.0	1.44	24.6	-	0.31	0.5	0.5
T. cearenses												
Queiroz R. T. 677 (HUEFS)	23.8 ± 2.4	21.0-30.0	18.6 ± 1.9	16.0-23.0	23.7 ± 5.0	18.0-32.0	1.28	23.7	-	0.24	0.5	0.5
T. chlorocaulon												
Marcolino F. & Pereira R. S. 131 (RB)	33.1 ± 3.0	28.0-39.0	25.4 ± 3.6	18.0-33.0	35.2 ± 3.0	30.0-40.0	1.32	23.9	-	0.27	0.6	0.6
T. geraniifolia												
Arbo M. M. et al. 8202 (HUEFS)	37.0 ± 7.7	26.0-58.0	26.6 ± 7.0	18.0-45.0	38.2 ± 8.3	21.0-57.0	1.42	25.6	-	0.18	0.7	0.7
T. volubilis												
Queiroz L. P. et al. 5798 (HRB)	25.5 ± 2.5	19.0-32.0	18.8 ± 2.5	15.0-25.0	25.7 ± 3.2	21.0-32.0	1.37	21.5	-	0.28	0.5	0.5
Santos M. O. & Costa M. M. 30 (HUNEB)	24.2 ± 2.8	20.0-31.0	18.8 ± 2.2	14.0-23.0	21.4 ± 2.8	15.0-26.0	1.29	22.9	-	0.34	0.5	0.5
Sucre D. 7905 (RB)	23.7 ± 2.1	20.0-27.0	16.9 ± 2.6	11.0-21.0	22.8 ± 3.3	17.0-29.0	1.43	21.4	-	0.31	0.5	0.5

Note: PD = Polar Diameter; ED = Equatorial diameter; EDp = Equatorial diameter in polar view; P/E = Ratio between polar diameter and equatorial diameter; Ecto = Colpus length; Endo = Height x width of the endoaperture; PAI = Polar area index; Sex = Sexine; Nex = Nexine; $\bar{X}$ = Arithmetic mean; $S_{\bar{X}}$ = Standard deviation of the mean; Fv = Range variation; measurements in μm, and indices in absolute numbers.

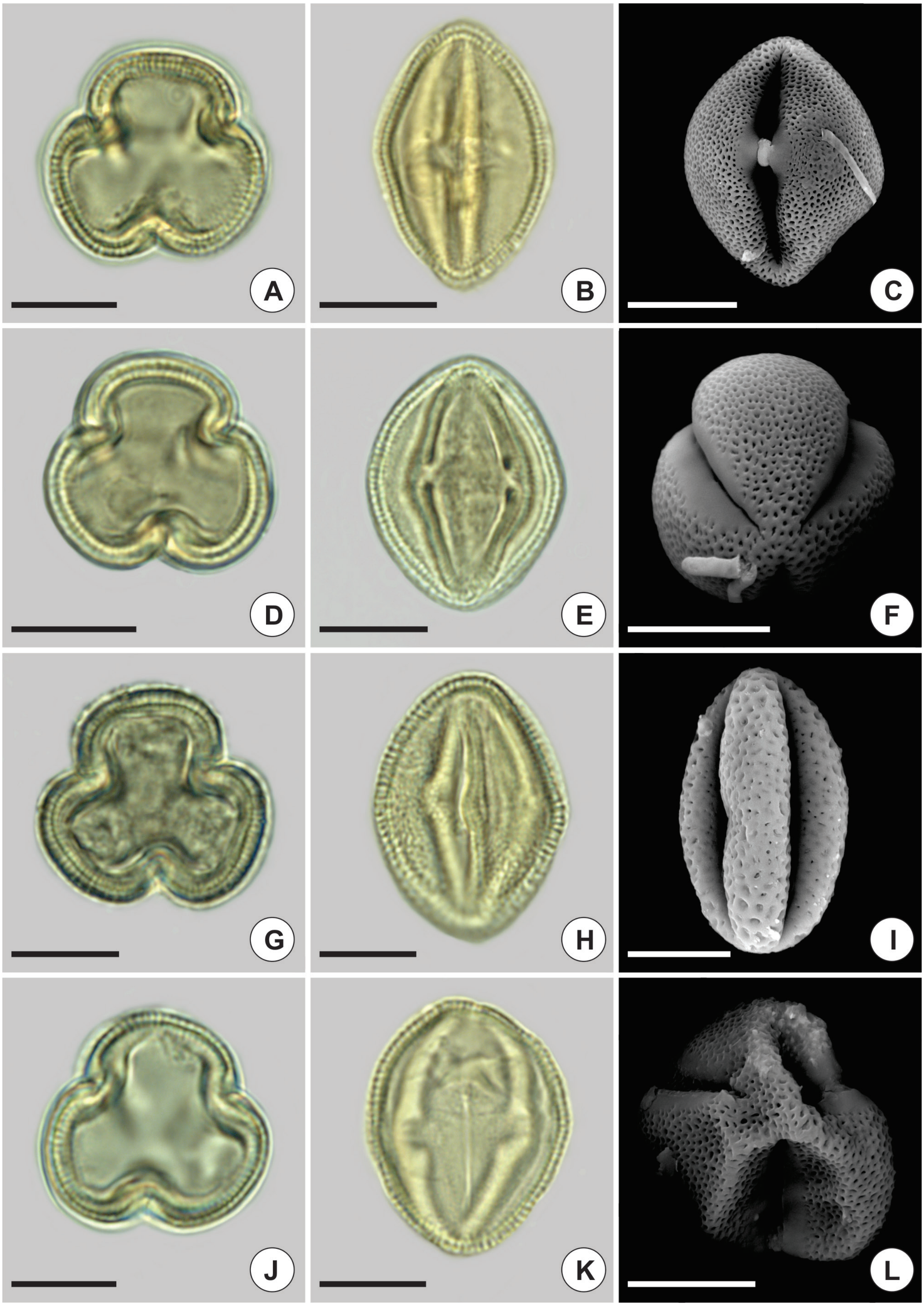

Figure 1. A-C: *Bernardia axillaris*. **A.** Polar view; **B.** Equatorial view; **C.** Aperture (SEM); **D-F:** *B. celastrinea*. **D.** Polar view; **E.** Equatorial view; **F.** Aperture margins (SEM); **G-I:** *B. crassifolia*. **G.** Polar view; **H.** Equatorial view; **I.** Apertures (SEM); **J-L:** *B. gambosa*. **J.** Polar view; **K.** Equatorial view; **L.** Aperture margins (SEM). Scales = 10 µm.

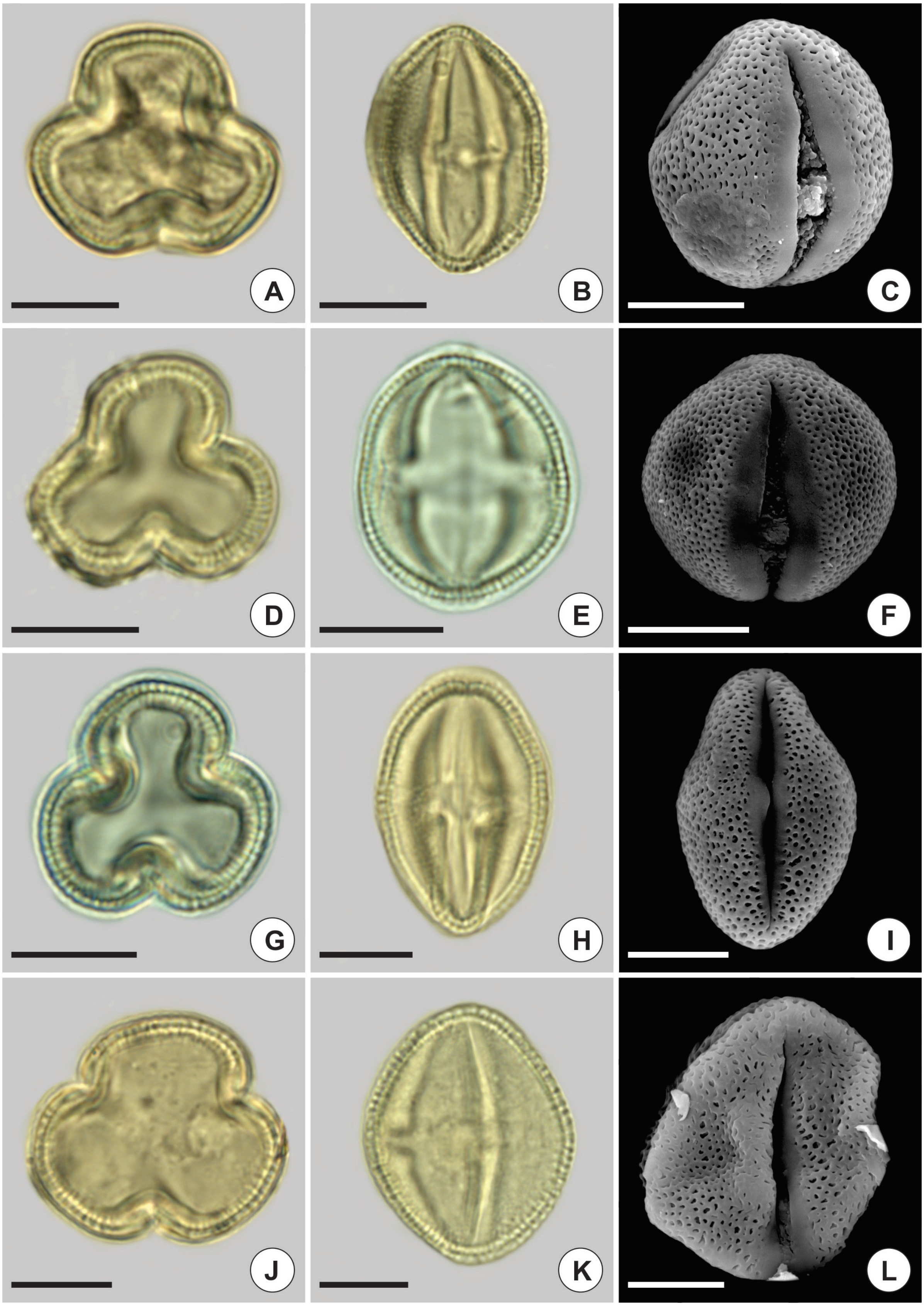

Figure 2. A-C: *Bernardia hirsutissima*. **A.** Polar view; **B.** Equatorial view; **C.** Aperture margin (SEM). **D-F:** *B. paraguariensis*. **D.** Polar view; **E.** Equatorial view; **F.** Aperture margin (SEM); **G-I:** *B. pulchella*. **G.** Polar view; **H.** Equatorial view; **I.** Aperture margin (SEM). **J-L:** *B. scabra*. **J.** Polar view; **K.** Equatorial view; **L.** Aperture margin (SEM). Scales = 10 μm.

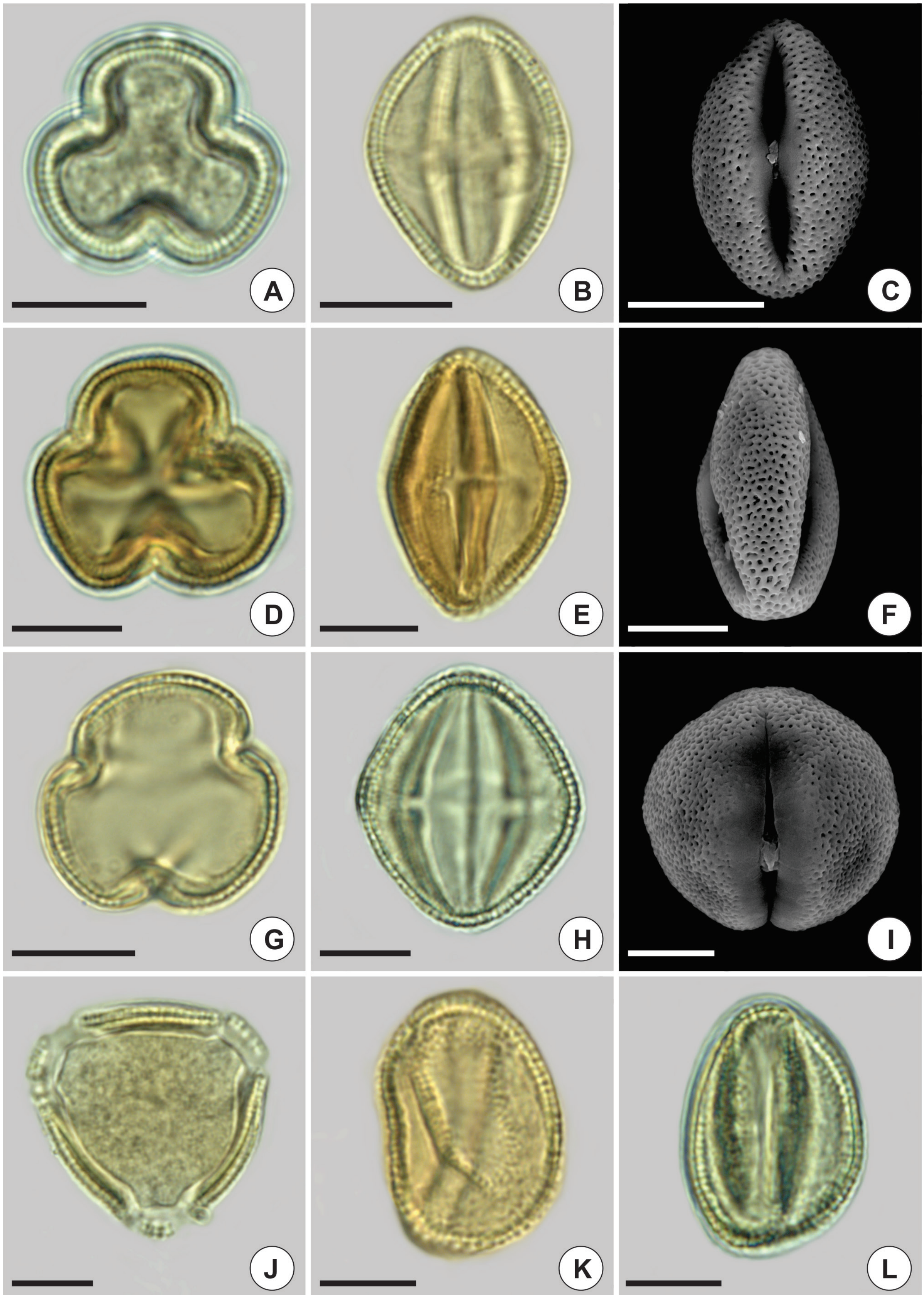

Figure 3. A-C: *Bernardia sidoides*. **A.** Polar view; **B.** Equatorial view; **C.** Aperture (SEM). **D-F:** *B. similis*. **D.** Polar view; **E.** Equatorial view; **F.** Apertures (SEM). **G-I:** *B. tamanduana*. **G.** Polar view; **H.** Equatorial view; **I.** Aperture margin (SEM). **J-L:** *Tragia bahiensis*. **J.** Polar view; **K.** Equatorial view; **L.** Aperture in equatorial view. Scales = 10 μm.

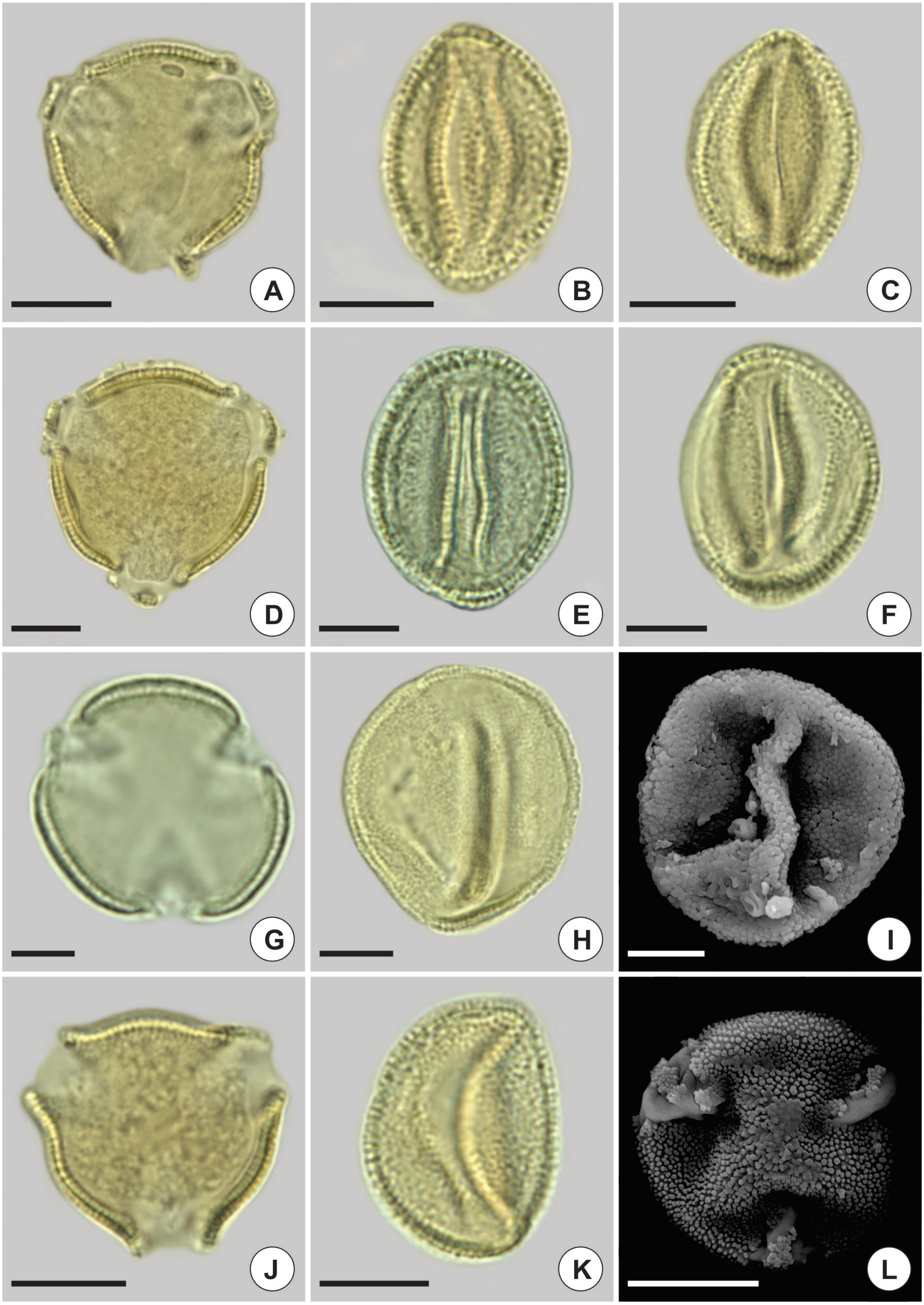

Figure 4. A-C: *Tragia cearensis*. **A.** Polar view; **B.** Equatorial view; **C.** LM analysis. **D-F:** *T. chlorocaulon*. **D.** Polar view; **E.** Equatorial view; **F.** Aperture in equatorial view. **G-I:** *T. geraniifolia*. **G.** Polar view; **H.** Equatorial view; **I.** Aperture (SEM). **J-L:** *T. volubilis*. **J.** Polar view; **K.** Equatorial view; **L.** Apertures with sexine islands (SEM). Scales = 10 µm.

thicknesses in most species, except in some specimens of *B. axillaris*, *B. crassifolia*, *B. gambosa*, *B. pulchella,* and *B. scabra*, where the nexine was thicker than the sexine (Tab. 2).

The variation observed in some characters of the *Bernardia* species analyzed allowed their separation into four morphological groups based on exine ornamentation and the aperture margins: (1) microreticulate with inconspicuous margins (*B. axillaris*, *B. crassifolia*, *B. sidoides*, *B. similis*; Figs. 1C, I, 3C, F); (2) microreticulate with narrow margins (*B. scabra*; Fig. 2L); (3) microreticulate, with wide margins (*B celastrinea*, *B. gambosa*, *B. paraguariensis*, *B. pulchella*; Figs. 1F, L, 2F, I); and (4) microreticulate-perforate, with wide margins (*B. hirsutissima*, *B. tamanduana*; Figs. 2C, 3I). We divided the five species of *Tragia* into two groups based on exine ornamentation: (1) exine intectate, pilate (*T. bahiensis*, *T. chlorocaulon*, *T. volubilis*; Fig. 5L, N, P); and, (2) exine verrucate (*T. cearensis*, *T. geraniifolia*; Fig. 5M, O).

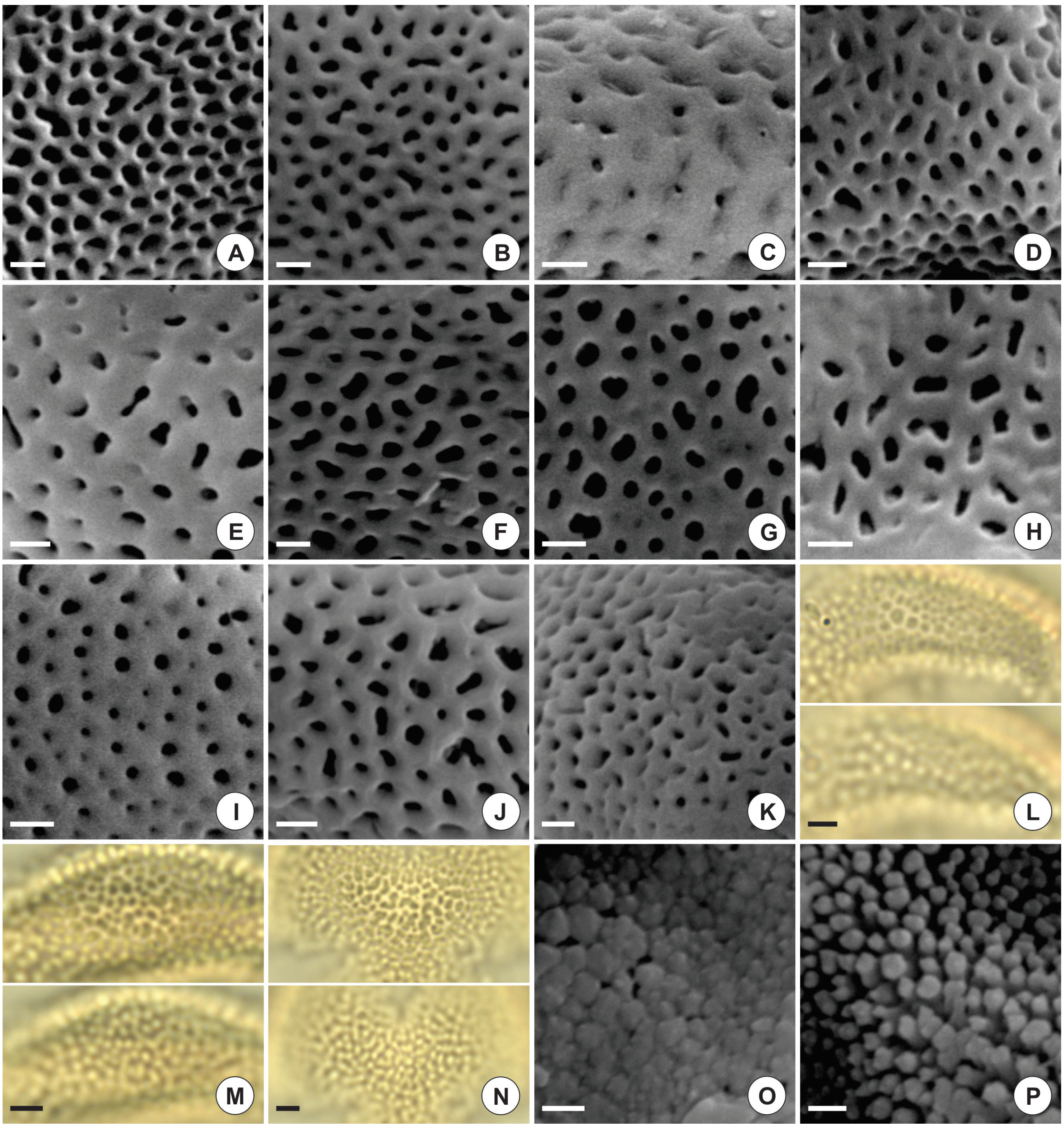

Figure 5. Detail of exine ornamentation. **A-K:** SEM analysis. **A.** *Bernardia axillaris*; **B.** *B. celastrinea*; **C.** *B. crassifolia*; **D.** *B. gambosa*; **E.** *B. hirsutissima*; **F.** *B. paraguariensis*; **G.** *B. pulchella*; **H.** *B. scabra*; **I.** *B. sidoides*; **J.** *B. similis*; **K.** *B. tamanduana*. **L-N:** LM analysis. L. *Tragia bahiensis*; **M.** *T. cearensis*; **N.** *T. chlorocaulon*. **O-P:** SEM analysis. **O.** *Tragia geraniifolia*; **P.** *T. volubilis*. Scales = 2 µm.

Sizes of the pollen grains

The pollen grains ranged in size between small and medium in both genera (Tab. 1). Considering their means, the smallest grains of *Bernardia* were observed in *B. pulchella* (16.8 µm diam), and the largest in *B. crassifolia* (23.0 µm diam). For *Tragia*, the smallest grains were encountered in *T. volubilis* (16.9 µm diam) and the largest in *T. geraniifolia* (26.6 µm diam; Tab. 2).

Shapes of the pollen grains

The shapes of the pollen grains in equatorial view (P/E) varied from subprolate to prolate, except for one specimen of *Bernardia axillaris* (*Farney C. et al.* 3603 - RB) that showed prolate-spheroid. The amb of the pollen grains in polar view were subcircular (*B. axillaris*, *B. celastrinea*, *B. crassifolia*, *B. gambosa*, *B. hirsutissima*, *B. paraguariensis*, *B. pulchella*, *B. scabra*, *B. sidoides*, *B. similis*, *B. tamanduana*, *Tragia geraniifolia*; Figs. 1A, D, G, J, 2A, D, G, J, 3A, D, G, 4G) and less frequently, triangular (*T. bahiensis*; Fig. 3J) or subtriangular (*T. cearensis*, *T. chlorocaulon*, *T. volubilis*; Fig. 4A, D, J).

Apertures

Grains with three apertures were observed in all of the species analyzed here, although there were variations in terms of the types of aperture in each genus (Tab. 2). In *Bernardia*, the grain apertures were of the colporus type, ectoapertures are long to very long with lalongate endoapertures. Some species showed psilate margins that could be wide (*B. celastrinea*, *B. gambosa*, *B. hirsutissima*, *B. paraguariensis*, *B. pulchella*, *B. tamanduana*; Figs. 1F, L, 2C, F, I, 3I) or narrow (*B. scabra*; Fig. 2L), while others showed inconspicuous margins (*B. axillaris*, *B. crassifolia*, *B. sidoides*, *B. similis*; Figs. 1C, I, 3C, F). In *Tragia*, the apertures were of the colpus type, the colpi 21.4–25.6 µm long with poorly defined margins and with exine fragments in the apertural membrane, visible only in polar view. The apertures, in polar view, were located at the corners of an angular amb (angulaperturate), with the exception of *Tragia geraniifolia* (Fig. 4G).

Exine

Exine ornamentation in *Bernardia* varied from microreticulate (*B. axillaris*, *B. celastrinea*, *B. crassifolia*, *B. gambosa*, *B. paraguariensis*, *B. pulchella*, *B. scabra*, *B. sidoides*, *B. similis*; Fig. 5A-D, F-J) to microreticulate-perforate (*B. hirsutissima*, *B. tamanduana*; Fig. 5E, K), the microreticulum with heterobrocate lumens. The species of *Tragia* analyzed here displayed ornamentation from intectate and pilate (*T. bahiensis*, *T. chlorocaulon*, *T. volubilis*; Fig. 5L, N, P) to verrucate (*T. cearensis*, *T. geraniifolia*; Fig. 5M, O). The proportions between the nexine and sexine varied only in some specimens of *Bernardia* (*B. axillaris*, *B. crassifolia*, *B. gambosa*, *B. pulchella*, *B. scabra*; Tab. 2), where the nexine was slightly thicker than the sexine. The nexines and sexines of the other species of *Bernardia*, and all of the species of *Tragia*, had equivalent thickness.

Discussion

The morphological characteristics of the pollen grains of nine species of *Bernardia* (*B. axillaris, B. celastrinea, B. crassifolia, B. gambosa, B. paraguariensis, B. scabra, B. sidoides, B. similis, B. tamanduana*) and one species of *Tragia* (*T. cearensis*) are presented here for the first time.

In comparing our results of pollen morphology of *Bernardia* to other published accounts (*e.g.*, Punt 1962; Nowicke *et al*. 1999; Cardinal-McTeague & Gillespie 2016), some morphological differences can be observed in relation to the size and shape of the pollen grains and exine ornamentation. A study undertaken by Punt (1962), for example, which analyzed one of the same species (*B. pulchella*), described those grains as prolate spheroids with the exine ornamentation finely reticulate, while in the present study that species displayed prolate grains (Fig. 2H) with microreticulate exine ornamentation (Fig. 5G).

Similarities and differences were apparent between observations of two species (*Bernardia hirsutissima*, *B. pulchella*) analyzed by both Nowicke *et al*. (1999) and the present study. As for *B. hirsutissima*, the pollen grains were described in both studies as subprolate, but observations diverged in relation to their size and exine ornamentation, as those authors described them as medium-sized grains with the exine profoundly perforate (*vs*. small grains with a microreticulate-perforate exine as observed in the present study). As for *B. pulchella*, observations of the grains diverged in relation to their size and shape, as the above-cited authors described them as small and subprolate *vs*. medium-sized and prolate in the present study, although both groups agreed on microreticulate exine ornamentation. Additionally, the study by Nowicke *et al*. (1999) indicated that most of the species analyzed showed perforate to profoundly perforate exine ornamentation, with only one species being described as microreticulate; in the present study, 81.81 % of the species of *Bernardia* analyzed demonstrated microreticulate exine, while the others had microreticulate-perforate exine.

In a more recent study, Cardinal-McTeague & Gillespie (2016) described the pollen grains of *Bernardia* species as having tectate-perforate foveolate to semitectate finely reticulate exine ornamentation, which differed from the results presented here. The differences found in the shapes of the pollen grains were not especially relevant, as the terms used by those authors refer to the same class (prolate), being merely subdivisions (subprolate, prolate spheroid). According to Barth & Melhem (1988), the size and shape of pollen grains actually have very little diagnostic value, while the number, position, and shape of the apertures and exine ornamentation have much greater taxonomic value.

There are no published records describing differences between the margins of the pollen grains of the species of *Bernardia* as described here. The pollen grains of the *Bernardia* species studied by Nowicke *et al*. (1999) were too uniform to be used to define differences between the species. In the present study, however, it was possible to observe relevant differences in the aperture margins and exine ornamentation, and to separate the 11 species analyzed into four groups.

Tragia demonstrated enormously variable pollen characters, as observed also by Punt (1962), who analyzed eight species and separated them into four groups based on their apertures and exine ornamentation: (1) inaperturate, pilate; (2) tricolpate, intectate; (3) tricolporate, intectate, and reticulate; and (4) tricolpate, tectate, and psilate. Miller (1964) utilized the methodology of Wodehouse (1935) (instead of acetolysis following Erdtman, 1960), and described those grains as tricolpate, intectate, and pilate, and he stated that the tricolpate grains had opercula (which were not mentioned in regard to the species studied by Punt [1962]). Miller (1964) noted, however, the extreme difficulty encountered in deciding whether the grains were truly intectate, as the pila may be proximally fused.

Our results corroborate those of Miller (1964) in relation to the grains being tricolpate, intectate, and pilate, but the opercula mentioned by that author are described here as islands of sexine – a term utilized in more recent studies of *Tragia* (*e.g.*, Gillespie 1994a; b; Suarez-Cervera *et al*. 2001; Nowicke & Takahashi 2002; Cardinal-McTeague & Gillespie 2016). Those sexine islands are indications of the evolution of the aperture condition in the tribe Plukenetieae from an ancestral tricolporate condition (Gillespie 1994b). According to the latter author, no evidence for sexine islands was observed under either LM or SEM with acetolyzed grains. It was, however, possible to see persistent exine fragments on the colpus membrane in all of the species of *Tragia* analyzed in the present study (Figs. 3J, 4A, D, G, L).

Differences were also noted in terms of the size and shape of pollen grains in the present study as compared to the results presented by Gillespie (1994b), who analyzed two of the same species (*Tragia chlorocaulon* and *T. volubilis*). That author described medium and suboblate grains with baculate exine ornamentation, differing from our observations of the grains being small to medium, subprolate to prolate, and the exine intectate, pilate.

Differences from previously described exine ornamentation (*e.g.*, *Tragia volubilis*) were also noted. Suarez-Cervera *et al*. (2001) described the exine ornamentation of *T. volubilis* as tectate, baculate-clavate. Nowicke & Takahashi's (2002) observations disagreed with that characterization, however, describing the grains of *T. volubilis* as intectate with large columns on its surface. More recently, Cardinal-McTeague & Gillespie (2016) described grains with intectate-baculate exine in sections *Tragia*, *Leucandra*, and *Ratiga*. Our results do not corroborate those observations, as we observed those same sections to have the exine between intectate pilate and verrucate.

The fragility of *Tragia* pollen grains was mentioned by Miller (1964), who found it necessary to use an alternative method to visualize some of their structures; Nowicke & Takahashi (2002) also reported that many grains were found collapsed or ruptured after acetolysis. The grains of *Tragia* species analyzed here likewise proved to be fragile after acetolysis, requiringsome adjustments in the acetolysis protocols to be able to make accurate measurements. Even non-acetolyzed grains would rupture when the electron beam (SEM) approached the samples.

Some authors (*e.g.*, Punt 1962; Gillespie 1994b; Nowicke & Takahashi 2002) described broad morphological variation and various pollen types in *Tragia*. Most of the *Tragia* species analyzed in the present study belong to section *Tragia* and had morphologically more uniform grains. Still, it was possible to separate them into two groups based on ornamentation of the exine. Group 1, including *T. bahiensis*, *T. chlorocaulon* and *T. volubilis,* shows the exine intectate and pilate, which corresponds to one of the pollen subtypes established by the current authors. Group 2, including *T. cearensis* and *T. geraniifolia*, has verrucate exine, although Punt (1962) grouped *T. geraniifolia* in the same pollen type as *T. volubilis*, with exine intectate and pilate. We note that verrucate exine ornamentation was not reported by any of the authors in their studies with *Tragia*, indicating that a larger sampling is needed to reveal more fully the variations in their pollen characters.

The palynological data obtained in the present study demonstrated that the morphological characters of the pollen grains of the species analyzed were useful in separating the two genera, as both demonstrated significant differences in the numbers, positions, and shapes of the apertures, as well as in their exine ornamentation which, according to Barth & Melhem (1988) are the principal characters of taxonomic value in pollen grains. The species of *Bernardia* studied here had tricolporate grains with exine ornamentation varying from microreticulate to microreticulate-perforate, while the *Tragia* species showed tricolpate grains with intectate pilate or verrucate exine ornamentations. Some species of both genera could be diagnosed based on exine ornamentation, and the aperture margins and four and two distinct morphological groups were observed in *Bernardia* and *Tragia*, respectively.

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