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Original article

Endemic Papilionoideae of the Caatinga: a contribution to the palynological knowledge of Leguminosae

José Orlando Bispo dos Santos[1], Adriele Santana da Silva Oliveira Carneiro[2], Ilana Marques de Souza Dias[1], Marileide Dias Saba[1], Luciene Cristina Lima e Lima[2] and Francisco Hilder Magalhães-e-Silva[1*]

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ABSTRACT

Papilionoideae is the most diverse subfamily of Leguminosae, representing approximately 39 % of its species. Although it is particularly species rich and diverse in the Caatinga, the main phytophysiognomy of the semiarid Northeast Region of Brazil, little is known about the pollen morphology of the species that occur there. This study analyzes and describes the pollen morphology of the 27 species of Papilionoideae endemic to the Caatinga. Pollen grains were acetolyzed, mounted on permanent slides, measured, described and photomicrographed using light and scanning electron microscopy (LM and SEM, respectively). The main variable morphological features were pollen grain size, shape, amb, apertural type and exine ornamentation under LM (microreticulate, reticulate, psilate or finely scabrate) and SEM (microreticulate, reticulate, rugulate, granulate, fossulate and perforate). High intergeneric morphological heterogeneity was observed while morphological variation within each genus was subtle. Nonetheless, size, endoaperture shape, fastigium presence/absence and exine ornamentation were found to be important characteristics for the identification of some species.

Keywords: pollen morphology, semiarid, Leguminosae, LM, SEM.

Introduction

Leguminosae is considered the third largest family of angiosperms with 19,500 species and 770 genera currently distributed among six subfamilies: Cercidoideae, Detarioideae, Duparquetioideae, Dialioideae, Papilionoideae and Caesalpinioideae (LPWG 2013; LPWG 2017).

Papilionoideae is the most species rich and diverse subfamily within Leguminosae with approximately 14,000 species and 500 genera distributed among 28 tribes (Lewis *et al.* 2005; LPWG 2017). The subfamily is distributed worldwide and includes representatives of varied habits. Flower morphology is the most specialized among Leguminosae and is mainly related to pollination by bees (Judd *et al.* 2009). Papilionoideae accounts for a significant portion of the taxonomic diversity of the Caatinga biome in Brazil, representing approximately 39 % of the species of Leguminosae found there (Queiroz 2009).

1 Laboratório de Estudos Palinológicos, Departamento de Educação, Universidade do Estado da Bahia, Senhor do Bonfim, 48970-000, BA, Brazil
2 Laboratório de Estudos Palinológicos, Departamento de Ciências Exatas e da Terra, Universidade do Estado da Bahia, 48040-210, Alagoinhas, BA, Brazil

* Corresponding author: fhsilva@uneb.br

The Caatinga is an exclusively Brazilian biome that covers most of the Northeast Region of the country and has a rich diversity of plants, fungi and animals (Silva *et al.* 2003; Forzza *et al.* 2010). The vegetation has peculiar characteristics that help them adapt to the physical characteristics of the environment such as high temperatures and lack of water (Costa *et al.* 2010). Physiognomies of the Caatinga include subshrubs, shrubs and short deciduous trees, with crooked trunks, thorns and wax, in addition to some succulent species and annual herbs (Leal *et al.* 2003). There is also significant flower diversity and a high number of endemic species in the biome (Giulietti *et al.* 2002).

The family Leguminosae is known for its richness and diversity and is the angiosperm family with the greatest number of species in the Caatinga (Forzza *et al.* 2010). Papilionoideae is the most species rich subfamily of Leguminosae (Queiroz 2009), however, little is known about the pollen morphology of the endemic species of the subfamily.

Palynological studies of legumes describe the family as generally a eurypalynous group (Salgado-Labouriau 1973; Miranda & Andrade 1990; Roubik & Moreno 1991; Silvestre-Capelato & Melhem 1997; Souza *et al.* 2004; 2014; Buril *et al.* 2011; Silva *et al.* 2016) and that it may provide the basis for important investigations within the area of applied palynology, thus confirming the need to better understand its morphopalynous characteristics.

In general, the pollen grains of Papilionoideae are dispersed in monads that vary from small to large and among 3-colpate, 3-colporate or rarely porate with a microreticulate, reticulate, psilate or scabrate exine and the presence/absence of a fastigium or costa (Salgado-Labouriau 1973; Miranda & Andrade 1990; Buril *et al.* 2011). However, detailed palynological research is scarce for species of Leguminosae, especially those that are endemic to the Caatinga. Silva *et al.* (2016) analyzed and described the pollen morphology of 144 species in an area of caatinga in Canudos, state of Bahia, out of which 30 belong to Leguminosae and 11 to the subfamily Papilionoideae. Buril *et al.* (2011) studied palynous typification in an area of caatinga in the state of Pernambuco and provided palynological descriptions for 35 species of Papilionoideae. These authors reported that the subfamily is palynologically homogenous but pointed out some variation with respect to apertures, shape, scope and exine stratification. Nonetheless, systemic studies of endemic taxa of the Caatinga are still insufficient. Therefore, this study aimed to reduce this knowledge gap by analyzing and describing the pollen morphology of species of Papilionoideae that are endemic to the Caatinga.

Material and methods

Twenty-seven species of five tribes (Brongniartieae, Crotalarieae, Dalbergieae, Phaseoleae, Sophoreae) of the subfamily Papilionoideae, family Leguminosae, considered endemic to the Caatinga by Forzza *et al.* (2010) were subjected to palynological analysis. Floral buds in preanthesis (from three specimens whenever possible) were acquired from the following herbaria: Universidade Federal de Feira de Santana (HUEFS), Herbário Prisco Bezerra (EAC) and Herbário RADAMBRASIL (HRB) (Tab. 1).

The acetolysis method of Erdtman (1960) was used to prepare pollen grains of all species for light microscopy (LM). After chemical preparation, the pollen grains were mounted in glycerinated gelatin on slides with a coverslip, sealed with paraffin, and measured, described and photomicrographed using a Zeiss Axioskop microscope. The slides were deposited in the pollen collection of the Laboratory of Palynological Studies of Universidade do Estado Bahia, Senhor do Bonfim - BA. For analysis by scanning electron microscopy (SEM), fresh (with little polyniferous material) and acetolyzed pollen grains were rinsed in an ethanol series up to 100%, pipetted onto specimen stubs. After ethanol evaporation, the stubs were coated with gold by vacuum evaporation and photographed using either a Zeiss LEO 1430 VP microscope (SEMLab, Biological Sciences Department, Universidade Estadual de Feira de Santana) or a Jeol JSM-6390LV microscope (Instituto Gonçalo Muniz - IGM / Fiocruz, Salvador, Bahia).

Pollen grains were described based on the glossaries of Punt *et al.* (2007) and Hesse *et al.* (2009). Measurements of the main morphometric parameters (equatorial and polar diameters) were made, whenever possible, on 25 pollen grains within eight days after mounting (Salgado-Labouriau 1973). Other parameters (diameter of apertures and thickness of exine) were measured on 10 randomly-chosen pollen grains. Quantitative data were submitted to statistical analyses adequate for the sample size. The arithmetic mean (χ), standard deviation ($S\chi$), standard error (σ), 95 % confidence interval (CI) and coefficient of variation (CV) were calculated for all of the diameters of the pollen grains with a sample size of 25, while only the arithmetic mean was calculated for parameters with a sample size of less than 25.

Results

Palynological analysis revealed morphopalynous heterogeneity to the level of genus among the studied Papilionoideae, with subtle intrageneric morphological variation (Tabs. 2, 3) (Figs. 1-5). Thus, the subfamily Papilionoideae can be considered eurypalynous, but not the genera of this study. The main variable morphological features detected among the pollen grains were related to size (small, medium and large), shape (prolate to suboblate), amb (subcircular to subtriangular), apertural type (colpi and colpori) and exine ornamentation under light microscopy (microreticulate, reticulate, psilate or finely scabrate) and under SEM (microreticulate, reticulate, rugulate, granulate, fossulate, perforate).

Table 1. Specimens investigated in the morphological analysis of pollen grains of Papilionoideae (Leguminosae).

Species	Code	Voucher	Specimen number	Herbaria
Aeschynomene carvalhoi G.P.Lewis	Aes.car.	Conceição, A.A. 3067	144631	HUEFS
		Queiroz, L.P. de 1955A	8390	HUEFS
		Conceição, A.S. 778	112624	HUEFS
Aeschynomene lewisiana Afr.Fern	Aes.lew.	Fernandes, A. s.n.	24307	EAC
		Lewis, G.P. *et al.*, s.n.	16790	EAC
Aeschynomene monteiroi A.Fern. & P.Bezerra	Aes.mon.	Fernandes, A. s.n.	125166	HUEFS
		Queiroz, L.P. de 7352	63839	HUEFS
		Queiroz, L.P. de 7332	63819	HUEFS
Aeschynomene sabulicola L.P.Queiroz & D.B.O.S.Cardoso	Aes.sab.	Cardoso, D. 2944	160780	HUEFS
		Queiroz, L.P. de 14675	161729	HUEFS
Aeschynomene soniae G.P.Lewis.	Aes.son.	Hatschbach, G. 67599	34239	HUEFS
		Atkins, S. PCD5088	28860	HUEFS
Cratylia mollis Mart. ex Benth.	Cra.mol.	Queiroz, L.P. de 7159	60869	HUEFS
		Souza, E.R. de 123	59018	HUEFS
		Giulietti, A.M. 2143	64999	HUEFS
Crotalaria bahiensis Windler & S.G.Skinner	Cro.bah.	Córdula, E. 258	124677	HUEFS
		Queiroz, L.P. de 12166	106851	HUEFS
		Souza, E.B. 1585	107101	HUEFS
Crotalaria brachycarpa Benth.	Cro.bra.	Melo, E. 5232	128412	HUEFS
		França, F. 4090	68990	HUEFS
		Queiroz, L.P. de, 12151	106836	HUEFS
Crotalaria harleyi Windler & S.G.Skinner.	Cro.har.	Queiroz, L.P. de 5363	36932	HUEFS
		Queiroz, L.P. de 14573	156591	HUEFS
		Castro, R.M. 995.	83310	HUEFS
Crotalaria holosericea Nees & Mart.	Cro.hol.	Guedes, M.L.S. 8180	55333	HUEFS
		Moraes, M.V. 537	68779	HUEFS
Dioclea grandiflora Mart. ex Benth.	Dio.gra.	Oliveira, R. P. 1492	140193	HUEFS
		Queiroz, L.P.de 12892	118389	HUEFS
		Funch, L.S. FCD166	60530	HUEFS
Dioclea marginata Benth.	Dio.mar.	Conceição, A.A. 1525	103926	HUEFS
		Souza, E.B. 1596	107878	HUEFS
		Miranda, E.B. 926	106959	HUEFS
Discolobium hirtum Benth.	Dis.hir.	Guedes, M.L.S. 7853	55151	HUEFS
		Queiroz, L.P. de 5916	43663	HUEFS
		Queiroz, L.P. de 5757	43504	HUEFS
Galactia remansoana Harms	Gal.rem.	Queiroz, L.P. de 7190	60900	HUEFS
		Fonseca, M.R.1344	65243	HUEFS
		Ribeiro, T. 61	47418	HUEFS
Harpalyce riparia São-Mateus, L.P.Queiroz & D.B.O.S.Cardoso	Har.rip.	Funch, R. 45	100957	HUEFS
Luetzelburgia auriculata (Allemão) Ducke	Lue.aur.	Fernandes, A. s.n.	127698	HUEFS
		Duarte, A.P. 1245	130642	HUEFS
		Andrade-Lima, 2126	144599	HUEFS
Luetzelburgia bahiensis Yakovlev.	Lue.bah.	Nunes, T.S. 977	59450	HUEFS
		Nunes, T.S. 366	53803	HUEFS
Platymiscium pubescens subsp. ***zehntneri*** (Harms) Klitgaard.	Pla.pub.	Melo, E. 6921	157273	HUEFS
Poecilanthe ulei (Harms) Arroyo & Rudd	Poe.ule.	Melo, E. 10581	178120	HUEFS
Pterocarpus ternatus Rizzini	Pte.ter.	Harley, R.M. 55267	92346	HUEFS
		Harley, R.M. 55351	92430	HUEFS
Pterocarpus villosus (Mart. ex Benth.) Benth.	Pte.vil.	Martins, P. s.n.	139537	HUEFS
		Martins, P. s.n.	139539	HUEFS
		Sarmento, A.C. 636	6170	HRB

Table 1. Cont.

Species	Code	Voucher	Specimen number	Herbaria
Pterocarpus zehntneri Harms.	Pte.zeh.	Castro, A.S.F 2131	44556	EAC
Stylosanthes seabrana B.L.Maass & 't Mannetje	Sty.sea.	Hind, D.J.N. 50275	27136	HUEFS
		Harley, R.M. 53582	37771	HUEFS
		Melo, A.C. 39	111629	HUEFS
Zornia echinocarpa (Moric. ex Meisn.) Benth.	Zor.ech.	Miranda, E.B. 846	98999	HUEFS
		Queiroz, L.P. de 7265	60974	HUEFS
Zornia gardneriana Moric.	Zor.gar.	Assis, J.S. 398	41801	HRB
Zornia harmsiana Standl.	Zor.har.	Queiroz, L.P. de 9654	89031	HUEFS
		Queiroz, L.P. de 10074	93070	HUEFS
		Queiroz, L.P. de 6615	52969	HUEFS
Zornia tenuifolia Moric.	Zor.ten.	Queiroz, L.P. de 13200	128724	HUEFS
		Nascimento, J.G.A. do 270	91401	HUEFS
		Queiroz, L.P. de 12137	106822	HUEFS

Table 2. Morphopolinic characteristics of species of Leguminosae (Papilionoideae) endemic to the Caatinga. (S= small, M= medium, L= large, Pr= prolate, PS= prolate spheroidal, Sp= subprolate, Sb= suboblate, OS= oblate spheroidal).

Species	Size	Shape	Apertural type	Aperture	Fastigium	Exine ornamentation
Aeschynomene carvalhoi	S	PS-Sp	3-colporate	long to vary long ectoapertures; circular to lolongate endoapertures	+	Microreticulate, heterobrochate. Granulate aperture membrane
Aeschynomene lewisiana	S	PS	3-colporate	long ectoapertures; circular to lalongate endoapertures	+	Microreticulate, heterobrochate
Aeschynomene monteiroi	S	Sp-Pr	3-colporate	long to vary long ectoapertures; circular to lolongate endoapertures	-	Microreticulate, heterobrochate. Granulate aperture membrane
Aeschynomene sabulicola	S	PS-Sp	3-colporate	long ectoapertures; circular to lalongate endoapertures	+	Reticulate, heterobrochate
Aeschynomene soniae	S	OS-PS	3-colporate	long ectoapertures; circular to lolongate endoapertures	-	Reticulate, heterobrochate
Cratyllia mollis	M	Sb-PS	3-colporate	long ectoapertures; lalongate endoapertures	-	Microreticulate, heterobrochate
Crotalaria bahiensis	M	Sp-Pr	3-colporate	long ectoapertures; lalongate endoapertures	+	Microreticulate, heterobrochate Psilate margo (SEM)
Crotalaria brachycarpa	M	Pr	3-colporate	long ectoapertures; lalongate endoapertures	-	Microreticulate, heterobrochate (LM), microreticulate-rugulate (SEM)
Crotalaria harleyi	M	Sp	3-colporate	long ectoapertures; lalongate endoapertures	+	Microreticulate, heterobrochate
Crotalaria holosericea	M	Sp-Pr	3-colporate	long ectoapertures; lalongate endoapertures	+	Microreticulate, heterobrochate
Dioclea grandiflora	L	Sb	3-colporate	long ectoapertures; lalongate endoapertures	-	Psilate
Dioclea marginata	L	-	3-colporate	long ectoaperture; lalongate endoapertures	-	Psilate
Discolobium hirtum	M	OS-PS	3-colporate	Short ectoapertures	-	Microreticulate-rugulate
Galactia remansoana	M	PS-Sp	3-colporate	long ectoapertures; lalongate endoapertures	-	Reticulate, heterobrochate. Psilate margo (SEM)
Harpalyce riparia	L	Pr	3-colpate	vary long ectoapertures	-	Finely microreticulate
Luetzelburgia auriculata	M	PS-Sp	3-(4)-colporate	long ectoapertures; lalongate endoapertures	-	Microreticulate, heterobrochate
Luetzelburgia bahiensis	M	Sp	3-colporate	long ectoapertures, constricted in the middle region; lalongate endoapertures	-	Microreticulate, heterobrochate

Table 2. Cont.

Species	Size	Shape	Apertural type	Aperture	Fastigium	Exine ornamentation
Platymiscium pubescens* subsp. *zehntneri	P	PS	3-colporate	long ectoapertures; lalongate endoapertures	+	Finely microreticulate
Poecilanthe ulei	P	Sp	3-colpate	long ectoapertures, slightly constricted in the middle region	-	Microreticulate (LM); fossulate-perforate (SEM)
Pterocarpus ternatus	P	Sp-Pr	3-colporate	long ectoapertures; circular endoapertures	-	Microreticulate, heterobrochate. Perforate margo (SEM)
Pterocarpus villosus	P	Sp	3-colporate	long ectoapertures; circular to lolongate endoapertures	-	Microreticulate, homobrochate
Pterocarpus zehntneri	P	Sp	3-colporate	vary long ectoapertures, constricted in the middle region; circular to lalongate endoapertures	+	Microreticulate, heterobrochate
Stylosanthes seabrana	M	Pr	3-colpate	Sincolpate and operculate ectoapertures	-	Reticulate, heterobrochate
Zornia echinocarpa	M	Sp	3-colpate	vary long ectoapertures	-	Reticulate, heterobrochate. Finely scabrate margo, granulate aperture membrane (SEM)
Zornia gardneriana	P	PS	3-colpate	vary long and operculate ectoapertures	-	Microreticulate, heterobrochate. Finely scabrate margo
Zornia harmsiana	P	Sp-Pr	3-colpate	vary long and operculate ectoapertures	-	Microreticulate, heterobrochate. Finely scabrate margo
Zornia tenuifolia	M	Sp-Pr	3-colpate	long ectoapertures	-	Reticulate, heterobrochate. Finely scabrate margo, granulate aperture membrane

Table 3. Morphometric pollen data for species of Leguminosae (Papilionoideae) endemic to the Caatinga. (P= polar axis, E= equatorial axis, Epv= equatorial axis in polar view, R= range, AI= polar area index, Ecto= Length x width of the ectoaperture, Endo= Width x height of the endoaperture, SEX= sexine, NEX= nexine).

Species / specimens	P (µm)		E (µm)		Epv (µm)		P/E	AI	Ecto (µm)	Endo (µm)	SEX (µm)	NEX (µm)
	$\bar{x} \pm S\bar{x}$	R	$\bar{x} \pm S\bar{x}$	R	$\bar{x} \pm S\bar{x}$	R						
Aeschynomene carvalhoi												
A.A. Conceição 3067	20.5±0.2	18-23	18.6±0.2	17-20	19.3±0.1	18-21	1.10	0.24	16.7x2.7	7.0x5.4	1.5	0.5
L.P. de Queiroz 1955	20.9±0.3	18-24	19.3±0.3	16-22	18.2±0.2	16-20	1.08	0.25	17.7x2.7	9.3x8.0	1.1	0.5
A.A. Conceição 778	20.5±0.3	18-25	17.7±0.4	15-21	18.3±0.2	15-20	1.16	0.27	17.1x2.8	6.4x5.8	0.9	0.5
Aeschynomene lewisiana												
E. Nunes, 24307	22.5±0.1	21-24	21.1±0.2	18-23	20.6±0.2	19-22	1.07	0.31	17.4x2.9	8.5x9.3	0.8	0.7
G.P. Lewis *et al.*, s.n.	20.9±0.1	20-22	18.7±0.3	16-21	19.3±0.2	17-22	1.11	0.30	-	7.0x7.2	1.0	0.6
Aeschynomene monteiroi												
A Fernandes – (HUEFS)	16.8±0.2	15-18	10.9±0.1	10-12	12.9±0.2	11-15	1.53	0.24	12.5x1.2	5.2x4.8	0.6	0.4
L.P. de Queiroz 7352	17.5±0.1	16-19	12.5±0.2	11-15	13.5±0.2	12-16	1.39	0.31	13.1±1.2	5.9x4.8	0.7	0.5
L.P. de Queiroz 7332	16.7±0.1	16-18	13.4±0.1	12-15	14.3±0.1	13-16	1.24	0.33	13.8±1.6	6.2x5.0	0.7	0.3
Aeschynomene sabulicola												
D. Cardoso 2944	17.9±0.2	16-20	16.9±0.2	14-18	15.4±0.1	15-17	1.05	0.26	13.6x3.3	6.3x6.8	0.9	0.4
L.P. de Queiroz 14675	18.9±0.2	17-20	16.3±0.1	15-18	15.4±0.1	14-18	1.15	0.28	14.9x2.7	6.3x6.3	0.8	0.4
Aeschynomene soniae												
G. Hatschbach 67599	19.2±0.5	15-23	16.8±0.3	14-21	16.4±0.4	13-20	1.14	0.29	13.4x2.1	6.4x5.6	0.7	0.4
S. Atkins 5088	17.7±0.2	15-20	18.2±0.3	15-20	15.9±0.2	15-17	0.96	0.30	13.0x2.9	5.6x6.8	0.7	0.5
Cratylia mollis												
L.P. de Queiroz 7159	35.0±0.7	30-45	38.5±0.9	29-42	34.7±0.4	30-38	0.90	0.39	23.7x -	7.2x15.1	1.8	0.8
E. R. de Souza 123	42.0±0.4	37-46	40.1±0.6	35-45	37.3±0.6	30-43	1.04	0.35	26.8x -	-	1.4	0.9
A, M. Giulietti 2143	39.7±0.9	31-49	46.1±1.6	32-55	40.8±0.5	35-47	0.86	-	24.7x -	5.8x22.6	1.9	0.7
Crotalaria bahiensis.												
E. Córdula 258	38.9±0.5	33-44	26.0±0.4	21-30	-	-	1.49	-	31.8x -	-	0.8	0.4
L.P. de Queiroz 12166	28.0±0.1	25-32	22.7±0.3	22-44	21.5±0.2	20-23	1.23	0.32	22.3x -	-	1.1	0.8
E.B. Souza 1585	31.9±0.4	28-36	24.4±0.4	17-27	-	-	1.30	-	26.0x -	-	1.0	0.6
Crotalaria brachycarpa												
E. Melo 5232	31.8±0.4	24-35	23.1±0.3	20-25	23.5±0.3	21-26	1.37	0.30	26.3x -	-	1.1	0.5

Table 3. Cont.

Species / specimens	P (µm)		E (µm)		Epv (µm)		P/E	AI	Ecto (µm)	Endo (µm)	SEX (µm)	NEX (µm)
	x̄ ± Sx̄	R	x̄ ± Sx̄	R	x̄ ± Sx̄	R						
F. França 4090	31.1±0.4	28-35	21.6±0.3	19-25	-	-	1.43	-	25.6x -	-	1.0	0.5
L.P. de Queiroz 12151	32.5±0.3	30-35	22.7±0.2	20-24	23.7±0.2	22-26	1.43	0.35	27.3x -	-	1.0	0.5
Crotalaria harleyi												
L.P. de Queiroz 5363	38.6±0.8	30-50	23.9±0.5	18-30	-	-	-	-	28.5x -	-	0.8	0.7
L.P de Queiroz 14573	32.5±0.7	28-42	25.2±0.2	23-28	23.7±0.4	20-28	1.27	-	27.5x3.2	4.0x -	1.0	1.0
R.M. Castro 995	29.0±0.3	27-32	24.7±0.3	22-28	23.0±0.3	20-26	1.27	0.27	25.4x -	-	1.0	1.0
Crotalaria holosericea												
L.M. Guedes 8180	33.4±0.4	30-38	24.0±0.3	21-26	23.9±0.3	21-26	1.39	0.41	27.8x -	-	1.1	0.6
M.V. Moraes 537	31.3±0.8	27-36	25.0±0.2	23-27	23.5±0.6	20-26	1.25	0.31	25.0x -	-	1.0	0.6
Dioclea grandiflora												
R.P. Oliveira 1492	-	-	-	-	53.6±0.9	45-62	-	0.35	-	-	4.3	1.0
L.P. de Queiroz 12892	50.8±0.7	44-59	64.4±0.8	51-71	57.0±0.7	50-66	0.79	0.30	8.3x26.4	-	5.5	1.9
L.S. Funch 166	-	-	-	-	59.9±0.8	51-67	-	0.29	-	-	5.4	1.7
Dioclea marginata												
A.A. Conceição 1525	-	-	-	-	60.3±0.8	53-69	-	0.26	-	-	6.3	2.4
E.B. Souza 1596					59.4± -	56-63		0.31	-	-	4.6	3.0
E.B. Miranda 926	-	-	-	-	60.8± -	53-66	-	-	-	-	5.5	2.1
Discolobium hirtum												
M.L. Guedes 7853	31.5± -	24-35	31.2± -	23-38	31.3± -	30-45	1.01	0.66	-	-	1.2	0.9
L.P. de Queiroz 5916	31.2±0.3	28-34	33.3±0.4	29-38	33.0±0.6	30-40	0.93	-	-	-	1.1	0.8
L.P. de Queiroz 5757	32.1±0.4	29-38	32.8±0.5	29-42	35.0±0.7	30-42	0.94	0.62	-	-	1.1	0.6
Galactia remansoana												
L.P. de Queiroz 7190	29.2±0.6	23-36	24.7±0.5	18-30	23.1±0.4	18-27	1.18	0.29	-	-	0.7	0.6
M.R. Fonseca 1344	28.1±0.9	20-35	25.0±0.5	20-31	22.3±0.3	20-25	1.12	0.39	-	-	0.8	0.6
T. Ribeiro 61	21.8±0.4	19-29	21.3±0.3	19-25	18.4±0.3	15-22	1.32	0.37	-	-	0.8	0.7
Harpalyce riparia												
R. Funch 45	53.5±0.7	48-62	31.4± 0.8	23-38	48.8±0.8	41-60	1.70	0.20	-	-	0.7	0.6
Luetzelburgia auriculata												
A. Fernandes 127698	26.3±0.3	26-30	21.5±0.2	19-23	21.4±0.3	20-25	1.22	0.26	21.2x2.6	-	0.9	0.8
A. P. Duarte 1245	25.3±0.2	21-27	21.8±0.2	18-24	20.5±0.2	18-23	1.15	0.30	20.2x2.5	4.5x -	1.0	0.8
A. Ducke 2126	24.5±0.2	20-27	22.5±0.3	21-24	21.5±0.2	20-24	1.08	0.25	18.5x3.4	6.3x -	1.0	0.9
Luetzelburgia bahiensis												
T.S. Nunes 977	28.0±0.3	25-30	21.0±0.2	23-25	23.6±0.3	21-26	1.33	0.26	22.0x2.2	-	0.9	0.9
T.S. Nunes 366	28.6±0.2	26-30	21.6±0.2	20-24	22.8±0.3	20-25	1.32	0.29	-	-	1.0	0.9
Platymiscium pubescens subsp. *zehntneri*												
E. Melo 6921	17.0±0.3	15-21	15.1±0.2	13-18	15.2±0.2	11-18	1.12	0.34	-	-	0.8	0.5
Poecilanthe ulei												
E.Melo 10581	23.6±0.3	21-27	19.6±0.4	16-24	19.9±0.2	17-22	1.20	0.34	-	-	0.5	0.5
E.Melo 10570	23.2±0.5	20-28	19.5±0.5	15-25	20.3±0.4	17-25	1.19	0.29	-	-	0.5	0.5
Pterocarpus ternatus												
R.M. Harley 55267	23.4±0.5	19-29	18.2±0.3	15-21	18.6±0.3	16-21	1.28	0.35	18.5x2.6	-	0.9	0.7
R.M. Harley 55351	23.6±0.3	22-26	17.5±0.2	16-19	18.8±0.3	15-21	1.35	0.34	18.3x2.2	-	0.9	0.6
Pterocarpus villosus												
P. Martins s/n	22.3±0.3	20-26	16.9±0.3	14-20	17.3±0.2	15-20	1.32	0.30	17.6x -	-	0.6	0.6
P. Martins s/n	23.1± -	-	17.5± -	-	15.9± -	-	1.32	0.31	-	-	0.6	0.6
A.C. Sarmento 636	19.3± -	-	15.6± -	-	17.7± -	-	1.24	0.29	-	-	0.6	0.6
Pterocarpus zehntneri												
A.S.F. Castro 2131	22.4±0.2	21-25	18.0±0.2	16-20	18.8±0.2	17-21	1.24	0.22	18.1x -	-	0.8	0.5
Stylosanthes seabrana												
R.M. Harley 53582	45.4±2.8	40-53	27.5±1.9	22-31	-	-	1.65	-	38.6x4.1	-	1.1	0.6
Zornia echinocarpa												
E.B. Miranda 846	29.1±1.0	27-33	22.3±1.2	20-26	22.5±1.0	21-25	1.30	0.24	24.2x4.4	-	0.8	0.7
Zornia gardneriana												
J.S. Assis 398	21.5±0.2	20-24	19.3±0.2	16-21	18.7±0.2	17-20	1.11	1.11	18.2x3.7	-	0.6	0.6

Table 3. Cont.

Species / specimens	P (µm)		E (µm)		Epv (µm)		P/E	AI	Ecto (µm)	Endo (µm)	SEX (µm)	NEX (µm)
	x̄ ± Sx̄	R	x̄ ± Sx̄	R	x̄ ± Sx̄	R						
Zornia harmsiana												
L.P. de Queiroz 9654	22.1±1.1	20-25	18.2±1.0	15-21	19.1±1.2	17-22	1.21	0.26	17.0x4.1	-	0.8	0.7
L.P. de Queiroz 10074	21.5±1.1	20-25	16.9±0.7	15-19	16.9±0.8	15-20	1.26	0.28	16.8x3.2	-	0.7	0.5
L.P. de Queiroz 6615	24.0±2.3	20-28	16.8±0.8	15-20	17.2±0.8	15-19	1.42	0.28	18.8x3.2	-	0.7	0.5
Zornia tenuifolia												
L.P. de Queiroz 13200	29.0±1.3	26-32	20.6±1.3	18-24	21.1±1.0	18-23	1.40	0.27	22.5x3.3	-	0.8	0.6
J.G.A. do Nascimento 270	26.7±1.2	25-30	20.9±0.6	19-22	22.7±1.1	20-26	1.27	0.27	21.1x4.3	-	0.7	0.6
L.P. de Queiroz 12137	31.3±1.3	29-34	21.6±0.9	20-24	23.0±1.5	20-26	1.44	0.28	24.7x -	-	1.0	1.0

***Aeschynomene* L.**

Species included: *Aeschynomene carvalhoi* G.P.Lewis, *Aeschynomene lewisiana* Afr.Fern, *Aeschynomene monteiroi* A.Fern. & P.Bezerra, *Aeschynomene sabulicola* L.P.Queiroz & D.B.O.S.Cardoso, *Aeschynomene soniae* G.P.Lewis (Fig. 1A-L).

Pollen grains small, spheroidal prolate to prolate, amb predominantly subcircular; 3-colporate, ectoapertures long and narrow, endoapertures circular to lolongate (*A. carvalhoi, A. monteiroi* and *A. soniae* (Fig. 1 A-B, G-H, K-L)) or circular to lalongate (*A. lewisiana* and *A. sabulicola*). Under SEM, apertural membrane granulate with sparsely distributed granules and areolae in center of colpori in *A. carvalhoi* (Fig. 1B) and *A. monteiroi* (Fig. 1I); fastigium present in most species; exine microreticulate, reticulate in *A. sabulicola* and *A. soniae*, heterobrochate in most species; sexine predominantly thicker than nexine.

***Cratylia* Mart. ex Benth.**

Species included: *Cratylia mollis* Mart. ex Benth. (Fig. 2A-B).

Pollen grains medium in size, subprolate to prolate spheroidal, amb subtriangular; 3-colporate, ectoapertures wide, endoapertures lalongate, sometimes slightly constricted in the middle and thick on upper and lower parts. Under SEM, margins psilate; fastigium absent; exine microreticulate, heterobrochate; sexine thicker than nexine.

***Crotalaria* L.**

Species included: *Crotalaria bahiensis* Windler & S.G.Skinner, *Crotalaria brachycarpa* Benth., *Crotalaria harleyi* Windler & S.G. Skinner., *Crotalaria holosericea* Nees & Mart. (Fig. 2C-J).

Pollen grains medium in size, subprolate to prolate, amb subcircular; 3-colporate, ectoapertures long, narrow, sometimes constricted in the middle, endoapertures lalongate rectangular (*C. harleyi* and *C. holosericea*), lolongate (difficult to observe in *C. brachycarpa*). Under SEM, apertural membrane finely granular in *C. brachycarpa* and *C. harleyi*; fastigium present in most species; exine microreticulate, heterobrochate; sexine thicker than nexine, maybe equally thick in *C. harleyi*.

***Dioclea* Kunth**

Species included: *Dioclea grandiflora* Mart. ex Benth., *Dioclea marginata* Benth. (Figs. 2K-L, 3A-C).

Pollen grains large, suboblate in one specimen of *D. grandiflora*, amb subcircular; 3-colporate, ectoapertures long with fine, narrow extremities, endoapertures lalongate with a slight middle constriction in *D. grandiflora* (Fig. 2L); lens-shaped structure that is resistant to acetolysis is present beneath the apertures of the pollen grains (Fig. 3B). Under SEM, margins finely fossulate, fastigium absent; exine psilate under LM and SEM; sexine thicker than nexine; visibly long and fine columella in *D. marginata*.

***Discolobium* Benth.**

Species included: *Discolobium hirtum* Benth. (Fig. 3D-E).

Pollen grains medium in size, oblate spheroidal, amb subcircular; 3-colporate, ectoapertures long and very narrow, endoapertures fused laterally forming endocingulum; fastigium absent; exine microreticulate-rugulate under LM and SEM; sexine thicker than nexine.

***Galactia* P.Browne**

Species included: *Galactia remansoana* Harms (Fig. 3F-H).

Pollen grains medium in size, spheroidal prolate to subprolate, amb (sub)triangular; 3-colporate, ectoapertures long, large, endoapertures lalongate. Under SEM, margins psilate, rare granules on apertural membrane; fastigium absent; exine reticulate, heterobrochate.

***Harpalyce* MOc. & Sessé ex DC.**

Species included: *Harpalyce riparia* São-Mateus, L.P.Queiroz & D.B.O.S.Cardoso (Fig. 3I-J).

Pollen grains large, prolate, amb subtriangular; 3-colpate, colpi long, large, with fine extremities; exine finely microreticulate, heterobrochate; sexine thicker than nexine; visibly long and fine columella.

***Luetzelburgia* Harms**

Species included: *Luetzelburgia auriculata* (Allemão) Ducke, *Luetzelburgia bahiensis* Yakovlev. (Figs. 3K-L, 4A-B).

Pollen grains medium in size, subprolate to spheroidal prolate, amb subcircular; 3-colporate (3-(4)colporate in *L. auriculata*), ectoapertures long, constricted in the middle (*L. bahiensis*), endoapertures lalongate (difficult to observe

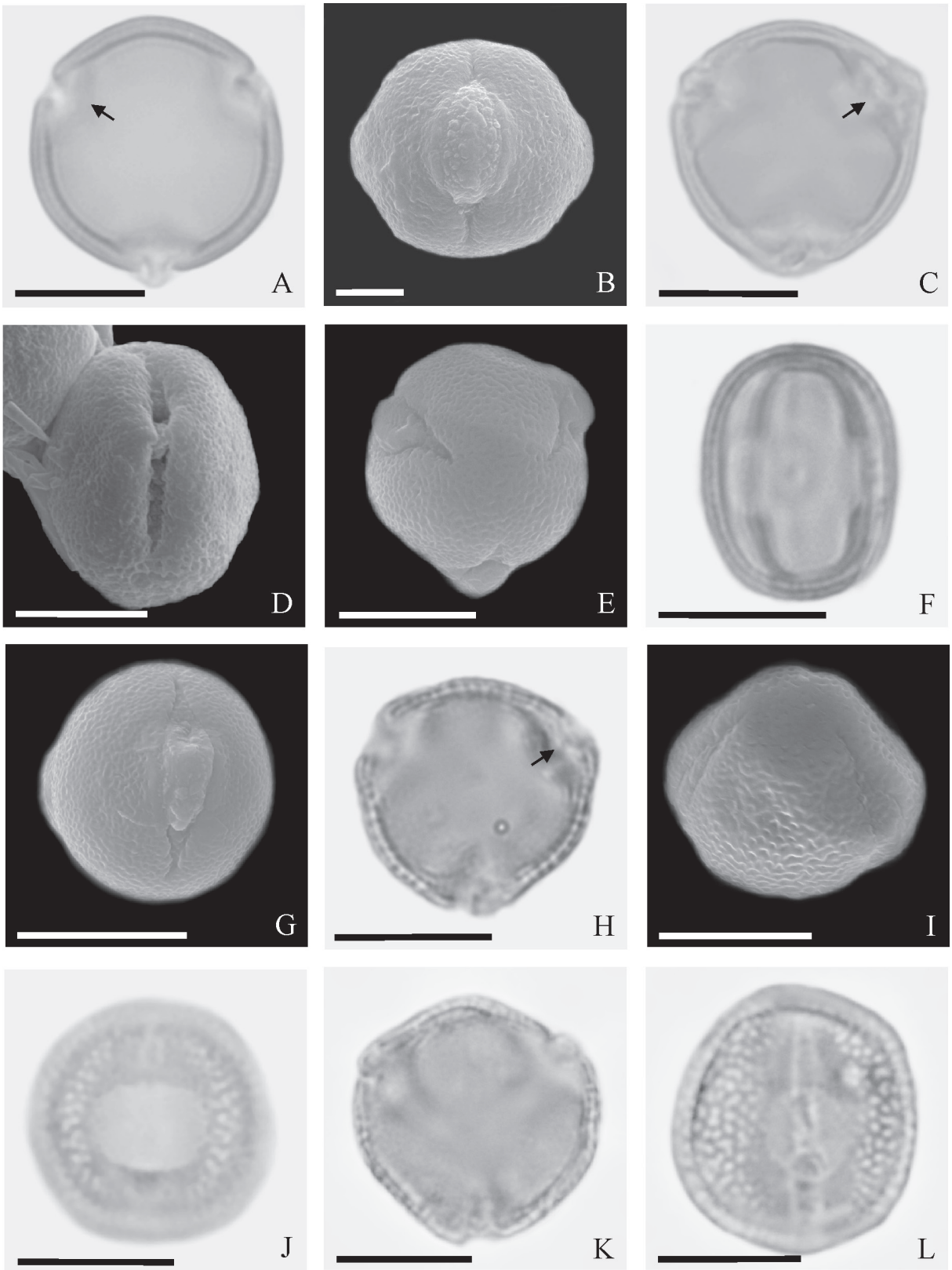

Figure 1. Pollen grains of species of Papilionoideae endemic to the Caatinga. **A-B**: *Aeschynomene carvalhoi* G.P.Lewis. **A**. Polar view; **B**. Equatorial view. **C-D**: *Aeschynomene lewisiana* Afr.Fern. **C**. Polar view; **D**. Equatorial view (SEM). **E-F**: *Aeschynomene marginata* var. *grandiflora* Benth. **E**. Polar view; **F**. Equatorial view. **G-H**: *Aeschynomene monteroi* A.Fern. & P.Bezerra. **G**. Polar view (SEM); **H**. Equatorial view (SEM). **I-J**: *Aeschynomene sabulicola* L.P.Queiroz & D.B.O.S.Cardoso. **I**. Equatorial view; **J**. Equatorial view (SEM). **K-L**: *Aeschynomene soniae* L.P.Queiroz & D.B.O.S.Cardoso. **K**. Polar view; **L**. Equatorial view. Arrow: Fastigium. Scales = 10 µm.

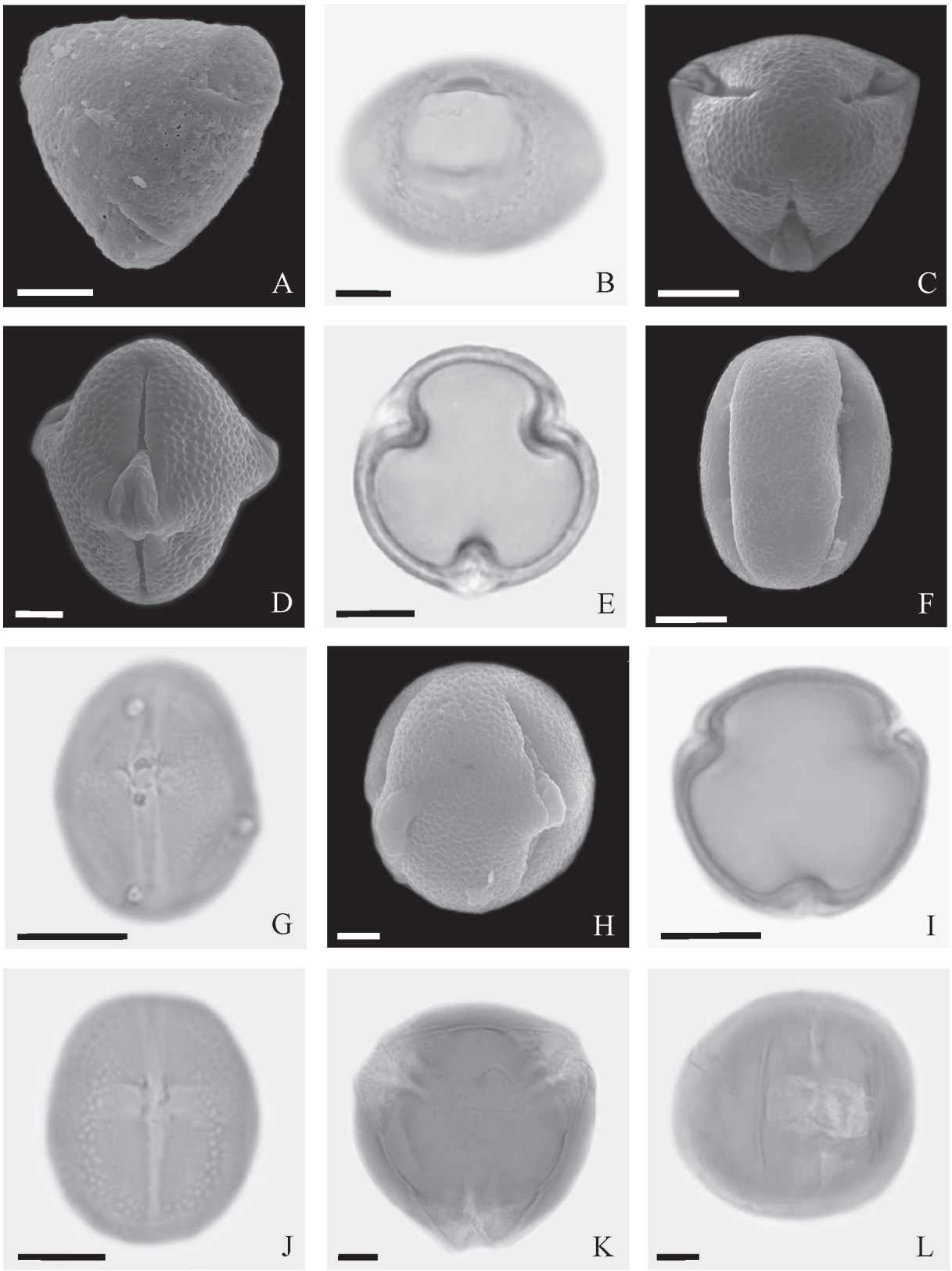

Figure 2. Pollen grains of endemic Papilionoideae of Caatinga. **A-B**: *Cratyllia mollis* Mart. ex Benth. **A**. Polar view (SEM); **B**. Equatorial view. **C-D**: *Crotalaria bahiensis* Windler & S.G.Skinner. **C**. Polar view (SEM); **D**. Equatorial view (SEM). **E-F**: *Crotalaria brachycarpa* Benth. **E**. Polar view; **F**. Equatorial view (SEM). **G-H**: *Crotalaria harleyi* Windler & S.G. Skinner. **G**. Equatorial view; **H**. Equatorial view (SEM). **I-J**: *Crotalaria holosericea* Nees & Mart. **I**. Polar view; **J**. Equatorial view. **K-L**: *Dioclea grandiflora* Mart. ex Benth. **K**. Polar view; **L**. Equatorial view. Scales = 10 μm.

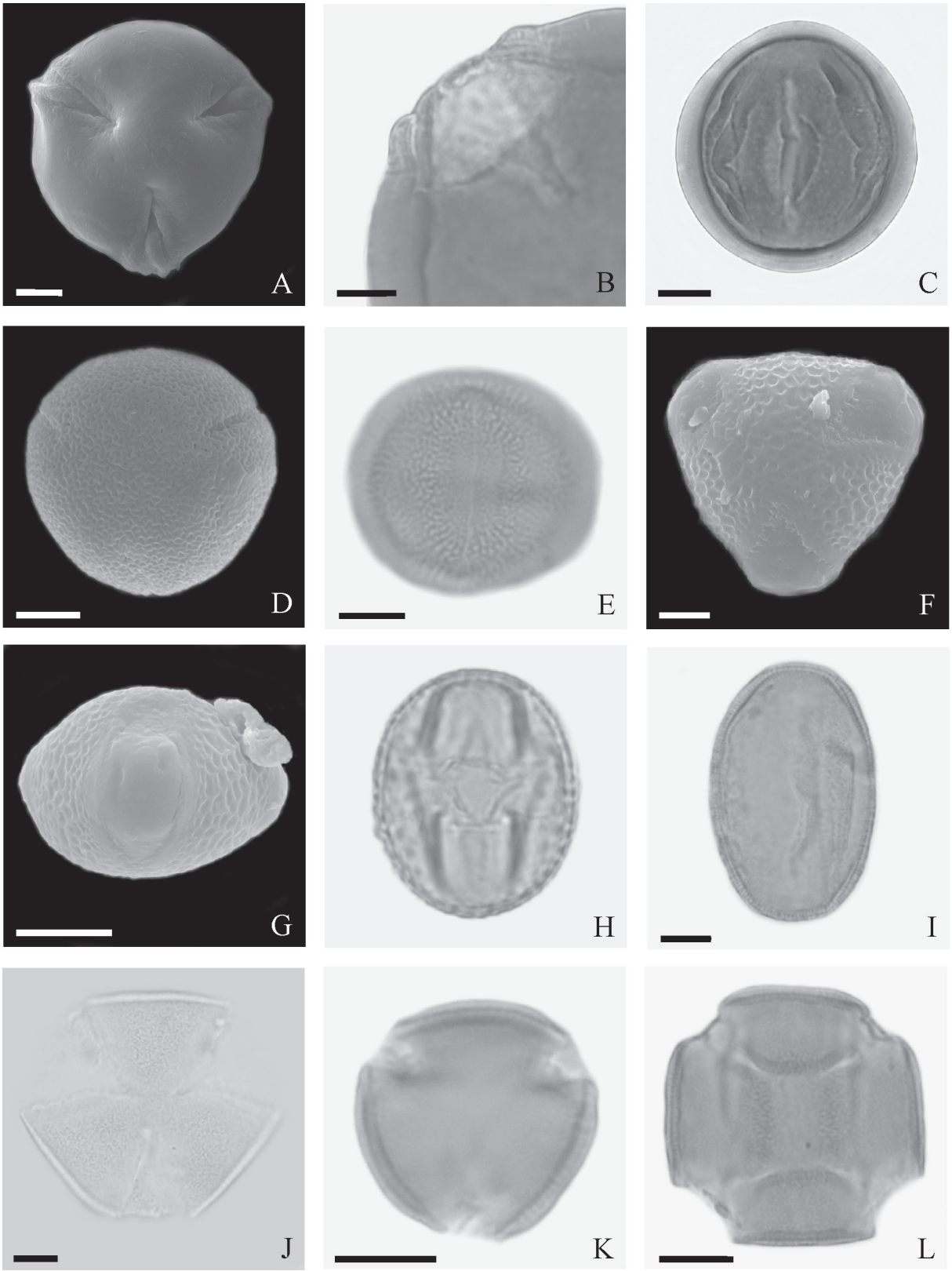

Figure 3. Pollen grains of species of Papilionoideae endemic to the Caatinga. **A-C**: *Dioclea marginata* Benth. **A**. Polar view (SEM); **B**. Lens-shaped structure, in polar view; **C**. Equatorial view. **D-E**: *Discolobium hirtum* Benth. **D**. Polar view; **E**. Equatorial view. **F-H**: *Galactia remansoana* Harms. **F**. Polar view (SEM); **G**. Equatorial view (SEM); **H**. Equatorial view. **I-J**: *Harpalyce riparia* São-Mateus, L.P.Queiroz & D.B.O.S.Cardoso. **I**. Equatorial view; **J**. Polar view. **K-L**: *Luetzelburgia auriculata* (Allemão) Ducke. **K**. Equatorial view, pollen grain 3-aperturate; **L**. Equatorial view. pollen grain 4-aperturate. Scales = 10 μm.

in *L. bahiensis*). Under SEM, apertural membrane finely granular, with sparse granules, and presence of rugulae in the central area of the colporus of *L. auriculata*; exine microreticulate, heterobrochate; sexine slightly thicker than nexine.

***Platymiscium* Vogel**

Species included: *Platymiscium pubescens* subsp. *zehntneri* (Harms) Klitgaard. (Fig. 4C-D).

Pollen grains small, prolate spheroidal, amb subcircular; 3-colporate, ectoapertures long and very narrow, endoapertures lalongate, rectangular to ovalate with middle constriction, fastigium present; exine finely microreticulate; sexine thicker than nexine.

***Poecilanthe* Benth.**

Species included: *Poecilanthe ulei* (Harms) Arroyo & Rudd (Fig. 4E-F).

Pollen grains small, subprolate, amb subtriangular; 3-colpate, slightly constricted in the middle; exine microreticulate, heterobrochate. Under SEM, exine fossulate-perforate; sexine thick as nexine.

***Pterocarpus* Jacq.**

Species included: *Pterocarpus ternatus* Rizzini, *Pterocarpus villosus* (Mart. ex Benth.) Benth., *Pterocarpus zehntneri* Harms (Fig. 4G-L).

Pollen grains small, subprolate to prolate, amb subcircular, subtriangular in *P. ternatus*; 3-colporate, ectoapertures long, with irregular outline in *P. ternatus*, constricted in the middle in *P. zehntneri*; endoapertures circular (difficult to observe in *P. ternatus*) circular to lolongate in *P. villosus*, circular to lalongate in *P. zehntneri*; fastigium present in *P. zehntneri*; exine microreticulate; exine in apertural area with same ornamentation as mesocolpium in *P. villosus* and *P. zehntneri*, whereas rugulate near the ectoapertures and microreticulate heterobrochate in the mesocolpium in *P. ternatus*; sexine thicker than nexine, but equally thick in *P. villosus*.

***Stylosanthes* Sw.**

Species included: *Stylosanthes seabrana* B.L.Maass & 't Mannetje (Fig. 5A-B).

Pollen grains medium in size, prolate, amb subcircular; 3-syncolpate, colpi long and narrow, finely microreticulate apertural membrane present. Under SEM, apertural membrane with irregular pattern; exine reticulate, heterobrochate; sexine thicker than nexine.

***Zornia* J.F.Gmel.**

Species included: *Zornia echinocarpa* (Moric. ex Meisn.) Benth., *Zornia gardneriana* Moric., *Zornia harmsiana* Standl., *Zornia tenuifolia* Moric. (Fig. 5C-L).

Pollen grains small and medium in size, subprolate to spheroidal prolate, amb circular, subcircular in *Z. echinocarpa* and *Z. tenuifolia*; 3-colpate, colpi long and large with fine extremities, margins finely scabrate, apertural membrane granulate-scabrate, however, the margins in *Z. echinocarpa* are finely rugulate and the apertural membranes are with conspicuous rugulae, with lateral fusions mainly in the central areas and sparse granules in the peripheral areas; exine microreticulate, heterobrochate with smaller lumina around the apertures in *Z. gardneriana* and *Z. harmsiana,* and exine reticulate, heterobrochate, also with reduced lumina around the apertures in *Z. echinocarpa* and *Z. harmsiana*. Under SEM, exine microreticulate or reticulate; sexine thicker than nexine.

Discussion

The palynological characteristics of the analyzed species of the genus *Aeschynomene* were homogeneous. However, the species, *A. sabulicola* and *A. soniae* can be separated from the others due to their possessing a reticulate exine. In general, the species of *Aeschynomene* endemic to the Caatinga exhibited morphopalynous characteristics similar to those reported in the literature for other species of the genus regarding size, aperture and exine stratification (Salgado-Labouriau 1973; Carreira *et al.* 1996; Buril *et al.* 2011; Silva *et al.* 2016). However, with the exception of Buril *et al.* (2011), other authors did not mention the presence of a fastigium for species of this genus.

The results here for *Cratyllia mollis* pollen grains were similar to those of Carreira *et al.* (1996) for *C. argentea*, differing only with regards to the amb, which they described as triangular. Miranda & Andrade (1990) also investigated species of *Cratyllia* and registered the presence of a psilate exine. Silva *et al.* (2016) studied the pollen of species that occur in the vegetation of Canudos, Bahia, and described *Cratyllia mollis*, which was corroborated by the present study.

Pollen grains of the genus *Crotalaria*, characterized as medium-sized 3-colporate, were relatively homogeneous, making it difficult to delimit species. However, *C. bahiensis*, *C. harleyi* and *C. holosericea* diverged from the others due to the presence of a fastigium, and *C. brachycarpa* due to a reticulate exine. The results obtained here for *Crotalaria* are generally in accordance with the literature for other species of the genus (Salgado-Labouriau 1973; Carreira *et al.* 1996; Melhem *et al.* 2003; Silva *et al.* 2010; Buril *et al.* 2011; Mouga & Dec 2012). However, Silva *et al.* (2010) described the exine of *C. micans* as perforated and Melhem *et al.* (2003) described the pollen grains of *C. bracystachia* as having a margin.

The pollen grains of *Dioclea* were large and with the sexine being twice as thick as the nexine. The results for *Dioclea grandiflora* were very similar to those found by Miranda & Andrade (1990) and Buril *et al.* (2011) for the same species. However, Miranda & Andrade (1990) considered the exine ornamentation as granular or psilate, whereas the present study found it psilate or finely scabrate.

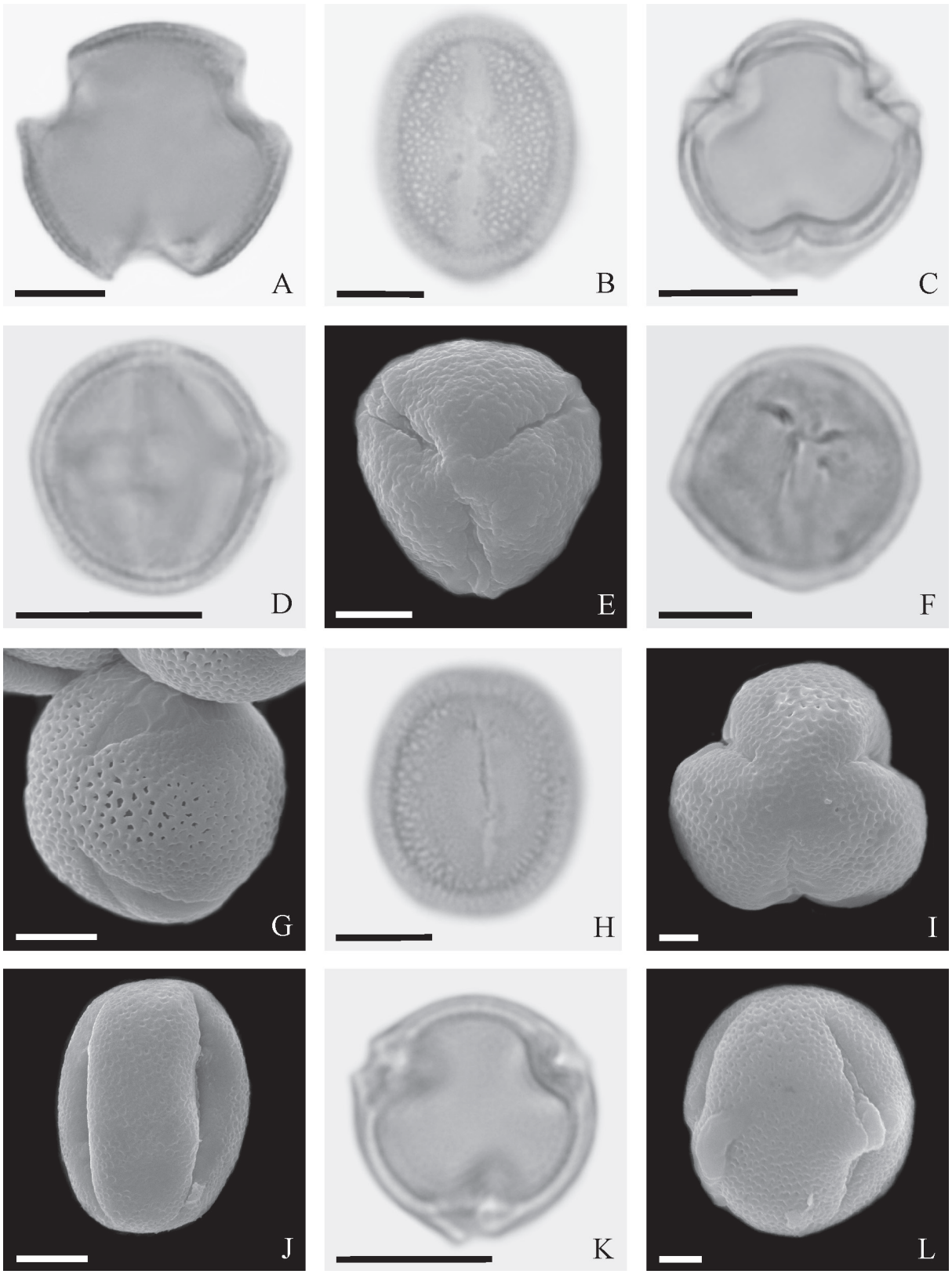

Figure 4. Pollen grains of species of Papilionoideae endemic to the Caatinga. **A-B**: *Luetzelburgia bahiensis* Yakovlev. **A**. Polar view; **B**. Equatorial view. **C-D**: *Platymiscium pubescens* subsp. *zehntneri* (Harms) Klitgaard. **C**. Polar view; **D**. Equatorial view. **E-F**: *Poecilanthe ulei* (Harms) Arroyo & Rudd. **E**. Polar view (SEM); **F**. Equatorial view. **G-H**: *Pterocarpus ternatus* Rizzini. **G**. General view (SEM); **H**. Equatorial view. **I-J**: *Pterocarpus villosus* (Mart. ex Benth.) Benth. **I**. Polar view (SEM); **J**. Equatorial view (SEM). **K-L**: *Pterocarpus zehntneri* Harms. **K**. Polar view; **L**. Equatorial view (SEM). Scales = 10 μm.

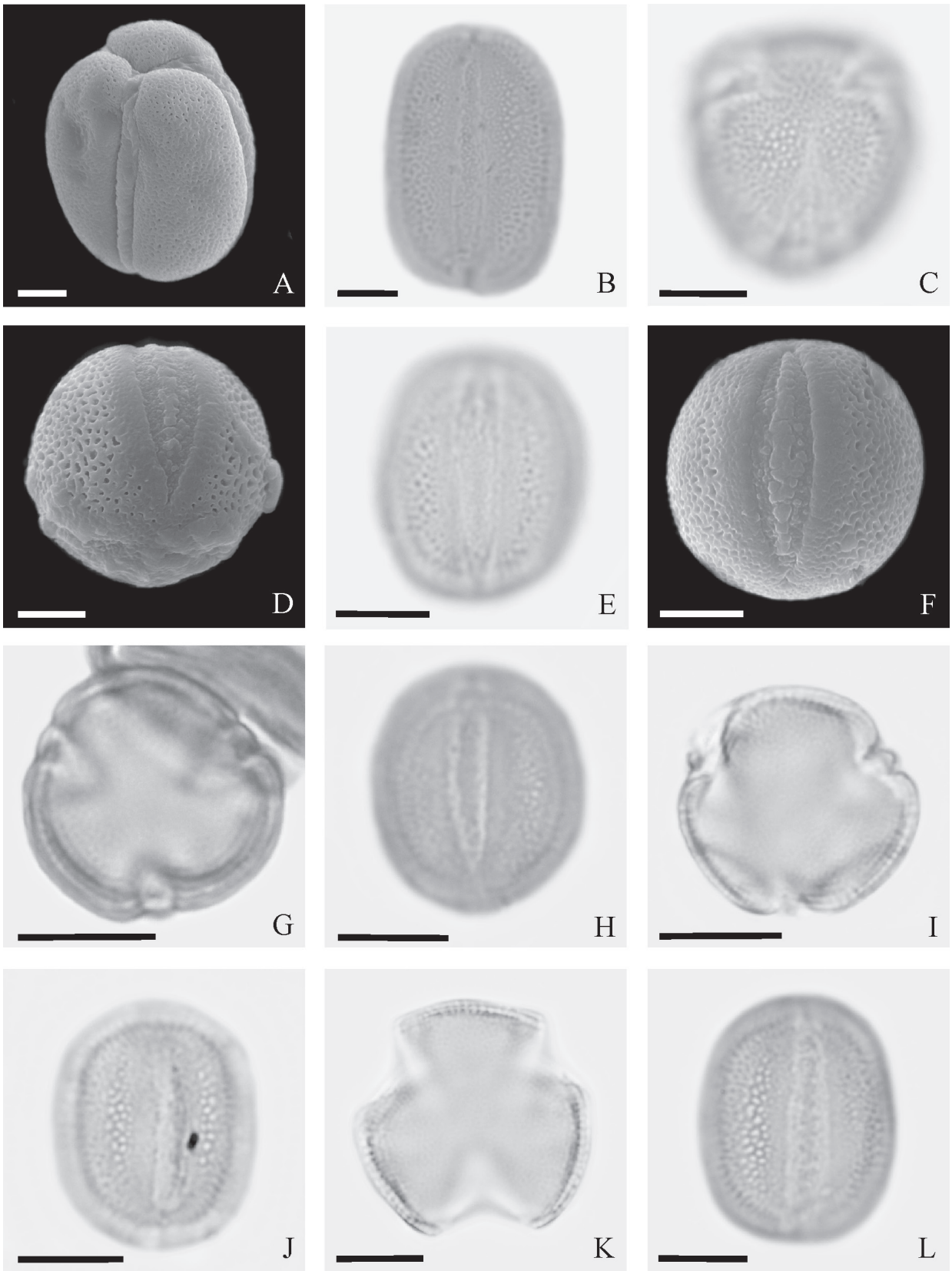

Figure 5. Pollen grains of species of Papilionoideae endemic to the Caatinga. **A-B**: *Stylosanthes seabrana* B.L.Maass & 't Mannetje. **A**. General view (SEM); **B**. Equatorial view. **C-F**: *Zornia echinocarpa* (Moric.) Benth. **C**. Polar view; **D**. General view (SEM); **E**. Equatorial view; **F**. Equatorial view (SEM). **G-H**: *Zornia gardneriana* Moric. **G**. Polar view; **H**. Equatorial view. **I-J**: *Zornia harmsiana* Standl. **I**. Polar view; **J**. Equatorial view. **K-L**: *Zornia tenuifolia* Moric. **K**. Polar view; **L**. Equatorial view. Scales = 10 µm.

There are no descriptions in the literature of the pollen grains of *D. marginata*, but here they were found to be very similar to those of *D. grandiflora*.

The pollen grains of *Discolobium hirtum* had general similarities with the other genera with regards to size and exine ornamentation. Given that no palynological reports were found in the literature for the species of this genus, the description provided here is apparentley new.

The results for *Galactia remansoana* were similar to those described by Moreti *et al.* (2007) for *Galactia glaucescens* and *Galactia striata*, and Buril *et al.* (2011) also for *G. striata*, with respect to size, type and number of openings and pollen grain shape. The amb, described here as subtriangular, is intermediate between the as circular and triangular descriptions by Moreti *et al.* (2007) and Buril *et al.* (2011), respectively. On the other hand, the endoaperture shape described here as lalongate differs from the lolongate described for *G. striata* by Buril *et al.* (2011). The reticulate and heterobrochate results for exine ornamentation were consistent only with Buril *et al.* (2011), since Moreti *et al.* (2007) described the exine of both species studied by them as microreticulate. The presence of margins and granules on the apertural membrane was not reported by either of these studies.

The pollen grains of *Harpalyce riparia* were relatively crumpled and scarce, which made it difficult to make measurements and describe them. However, it was possible to recognize some characteristics similar to those found by Salgado-Labouriau (1973) and Lozano-Garcia (1979) for *H. brasiliana* and *H. aboresencens*, respectively, differing only in size, which, in this study, was slightly larger.

The two species of *Luetzelburgia* analyzed here were homogeneous; however, one specimen of *L. auriculata* had 3(4)-colporate pollen grains, a character not observed in any other species of Papilionoideae endemic to the Caatinga. Buril *et al.* (2011), described the pollen grains of *L. auriculata* but did not mention 3(4)-colporate pollen grains.

The pollen grains of *Platymiscium pubescens* subsp. *zehntneri* showed similar morphology to those of the genera *Aeschynomene* and *Pterocarpus*; however, *P. pubescens* subsp. *zehntneri* could be separated by the presence of a very conspicuous fastigium, which was not evidenced by other authors who studied species of this genus. In general, the characteristics observed in here have also been reported for other species of the genus with respect to size, apertural type and exine stratification (Barth 1964; Roubik & Moreno 1991; Jiménez-B 1996; Klitgaard 2005). Nevertheless, Klitgaard (2005) described the presence of an operculum in pollen grains of *P. floribundum* var. *floribundum*, *P. lasiocarpum*, and *P. stipulare*, which was not evidenced in the present investigation.

Poecilanthe ulei had pollen grains that followed the general pattern for Papilionoideae — small, 3-aperturate with a microreticulate exine. The characters found here for *P. ulei* were very similar to the description of Souza *et al.* (2014).

The pollen grains of *Stylosanthes seabrana* had characteristics similar to those of species of the genus *Zornia*. Nevertheless, *S. seabrana* could be differentiated by the union of the colpi at their ends (syncolpate), which was not observed in *Zornia*. The results found here for *S. seabrana* were similar to those found by Silvestre-Capelato & Melhem (1997) for *S. guianensis* and *S. viscosa*, Carreira *et al.* (1996) for *S. hispida* and Carreira & Barth (2003) for *S. humilis*. However, there were some disagreements with Silva *et al.* (2016), who considered the pollen grains of *S. seabrana* to be 3-colporate with circular endoapertures.

The pollen grains of the species of *Zornia* analyzed here were very similar; however, *Z. harmsiana* and *Z. gardneriana* could be separated from the others by their small size and the latter also by exine ornamentation (homobrochate microrreticulate). The results found in the present work were in agreement with those of Silva *et al.* (2016) for *Z. echinocarpa*. However, that study analyzed pollen grains of *Z. brasiliensis* and *Z. sericea* and described them as 3-colporate, which was not observed for any of the species of the genus analyzed here.

In general, the pollen grains of species of Papilionoideae endemic to the Caatinga were small, spheroidal prolate to subprolate with subcircular to subtriangular amb, 3-colporate or 3-colpate and microreticulate or reticulate. These results were consistent with those found in the literature (Fergusson & Skvarla 1988; Silvestre-Capelato & Melhem 1997; Buril *et al.* 2011).

The results reported here corroborate the eurypalynous character of Papilionoideae as indicated by previous studies with representatives of the group (Barth 1964; Salgado-Labouriau 1973; Sowunmi 1973; Miranda & Andrade 1990; Roubik & Moreno 1991; Silvestre-Capelato & Melhem 1997; Buril *et al.* 2011; Silva *et al.* 2016). However, some genera were homogeneous, making it difficult to identify species from palynological analysis alone. On the other hand, pollen grain size, endoaperture shape, fastigium presence/absence and exine ornamentation were found to be important characteristics for the identification of some species.

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