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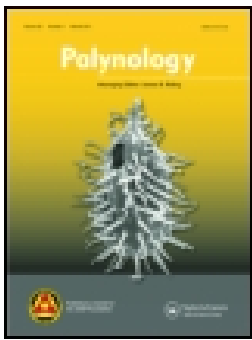
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Pollen morphology of 25 species in the family Apocynaceae from the Adolpho Ducke Forest Reserve, Amazonas, Brazil

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ABSTRACT

The Apocynaceae family consists of approximately 4555 species worldwide, distributed among 415 genera. In Brazil, this family is represented by 90 genera and 850 species. The Adolpho Ducke Forest Reserve, part of the Instituto Nacional de Pesquisas da Amazônia (INPA) since 1963, comprises 100 km² of non-isolated continuous primary forest, and 40 species and 16 genera of Apocynaceae have been recorded in this area. Pollen grains, collected from flower buds and/or flowers of voucher specimens deposited at the INPA Herbarium collection, were processed using the acetolysis method, and measured, described and photographed by light microscopy and scanning electron microscopy. The study included 25 Apocynaceae species distributed among 14 genera. The pollen grains were porate to colpiate, with exine ornamentation varying from psilate or scabrate to microreticulate. Other morphological characteristics were pores with granules at the base of the annulus in *Odontadenia*, a distinct margo forming arches around the colpus in *Geissospermum*, and the presence of a thick endocingulum in the equatorial region in species of *Tabernaemontana*.

KEYWORDS

pollen morphology;
Apocynaceae; Amazon;
Ducke Reserve; Brazil

1. Introduction

Apocynaceae Juss. belongs to the Order Gentianales, which includes four other families, namely Gelsemiaceae, Gentianaceae, Loganiaceae and Rubiaceae (APG III 2009). The Apocynaceae family can be divided into five subfamilies: Rauvolfioideae (cosmopolitan; 10 tribes/83 genera; 915 species), Apocynoideae (cosmopolitan; eight tribes/80 genera; 822 species), Periplocoideae (Old World; 33 genera), Secamonoideae (Old World; eight genera) and Asclepiadoideae (cosmopolitan; four tribes/172 genera) (Endress & Bruyns 2000; Endress et al. 2007; Middleton 2007).

Apocynaceae has been the subject of several taxonomic, evolutionary and phylogenetic analyses (Endress & Stevens 2001; Endress et al. 2007; Ionta & Judd 2007; Simões et al. 2007; Wyatt & Lipow 2007; Rapini 2012), many of which have described pollen grain morphological characteristics (Nilsson 1990; Nilsson et al. 1993; Verhoeven & Venter 1998; Furness 2007; Van Der Ham et al. 2010; Van der Weide & Van der Ham 2012). The earliest pollen record for Apocynaceae appears in the Paleocene of Borneo (Muller 1981). In Amazonia, Apocynaceae pollen fossil was also found in the Miocene as *Psilastephanoporites herngreenii* (Hoorn 1993) and *Ctenolophonidites suigeneris* (Silva-Caminha et al. 2010).

Approximately 100 genera and 1500 species of this family can be found in the American tropics (Rapini 2004), of which 90 genera and 850 species are present in Brazil (Souza & Lorenzi 2005).

The Adolpho Ducke Forest Reserve (RFAD) is located in Brazilian Amazonia and it contains 16 genera and 40 species, including trees, treelets, lianas and some shrubs such as *Tabernaemontana*, many of which have great economic and medicinal value. These species have white or brownish latex; opposite, verticillate or alternate leaves; bisexual flowers; simple fruits, berries, drupes, capsules or follicles; and single or multiple seeds. The flowers are pollinated by butterflies and bees and fruits dispersed by monkeys and birds (Ribeiro et al. 1999).

The objective of this study was to assess pollen morphology in 25 Apocynaceae species and compare them with others described in the literature. Plant taxonomy of RFAD was previously performed by Ribeiro et al. (1999) in the *Identification guide of the vascular plants of a terra-firme forest in the Central Amazon – Ducke Flora Reserve*. This study will contribute to the Amazon Palynologic Database of the National Institute of Amazonian Research (Instituto Nacional de Pesquisas da Amazônia – INPA) and will be used to expand the INPA Pollen Collection, as well as to provide information for future taxonomic analyses.

2. Materials and methods

The taxonomy of the species described in this study was determined by collaborators from the Ducke Flora Reserve Project, who are specialists in the Apocynaceae family (Hopkins 2005). It was not possible to describe all (40) of the species studied in this project due to the absence of voucher specimens with flower buds for pollen collection.

2.1. The Adolpho Ducke Forest Reserve (RFAD)

The RFAD was classified as a Biological Reserve in 1963. It is located to the north of Manaus city, at kilometre 26 along the AM-010 highway (Manaus-Itacoatiara) at, according to the mean geographic coordinate, 02°57'50.63"S, 59°57'18.21"W (Figure 1) and covers an area of 10,073 ha. The climate is classified as the Af group (humid equatorial) of Köppen, and the average yearly precipitation is 2000 mm, with two distinct seasons: rainy (November to May) and dry (June to October) (Köppen 1948). With a total area of approximately 100 km², the reserve consists of terra-firme forests divided into plateau, slope, lowland and campinarana types, distributed according to soil type and topography. The species described in this study can be found in each of these vegetation types (Ribeiro et al. 1999).

2.2. Sample preparation and morphological descriptions

Pollen grains were collected from the flower buds of voucher specimens deposited in the INPA herbarium collection. The grains were treated using the acetolysis method (Erdtman

1960) and mounted on slides with glycerinated gelatine, and cover slips were affixed to the slides with paraffin. The slides were deposited in the INPA Pollen Collection, in Manaus (Amazonas). The pollen grains were measured, described and photomicrographed (Canon A620 digital camera) using a light microscope (Zeiss) at 1000× magnification. Measurements were taken of 25 pollen grains, randomly, in polar and equatorial views in the isopolar grains and diameter 1 (D1) and diameter 2 (D2) in the apolar grains. For the other measurements, 10 grains were measured (exine thickness, equatorial diameter in polar view, apocolpium side and apertures pollen grains). For the equatorial views, the polar and equatorial diameters were measured, and the shape of the grain was established based on the relationship between the polar and equatorial mean diameters (P/E). Pollen size was characterised according to the method of Erdtman (1952) and using the terminology of Punt et al. (2007). Statistical analyses were based on calculations of the arithmetic mean (\bar{x}), standard deviation (s_x), sample standard deviation (s), coefficient of variation (CV%), 95% confidence interval (95% CI; $n = 25$) and range of variation (RV).

Regarding the variation in the pore, the following sizes should be considered: very small: 1.0–2.0 μm ; small: 3.0–5.6 μm ; large: 6.2–11.7 μm ; very large: up to 20 μm .

To obtain electron micrographs using scanning electron microscopy (SEM), non-acetolysed pollen grains were spread on the surface of double-sided carbon tape covering numbered aluminium stands. The samples were transferred to a vacuum pump, metalised with a fine palladium-gold layer (ca. 150 Å thick), and then analysed using a ZEISS DS M960 device in the

Table 1. Measurements (μm) of Apocynaceae pollen grains found in the Adolpho Ducke Forest Reserve. P: polar diameter; E: equatorial diameter; \bar{x} : arithmetic mean; $S\bar{x}$: mean standard deviation; s : standard deviation of sample; CV: coefficient of variation; VR: variation range; CI: 95% confidence interval ($n = 25$).

Species	P					E					P/E
	VR	$\bar{x} \pm s\bar{x}$	s	CV (%)	CI	VR	$\bar{x} \pm s\bar{x}$	s	CV (%)	CI	
<i>Ambelania acida</i>	(30–39)	34.6 \pm 0.48	2.4	6.9	(33.7–35.6)	(38–45)	40.7 \pm 0.29	1.5	3.6	(40.1–41.3)	0.85
<i>Couma guianensis</i>	(23–30)	27.1 \pm 0.35	1.7	6.4	(26.4–27.8)	(29–38)	33.7 \pm 0.46	2.3	6.8	(32.7–34.6)	0.81
<i>Couma utilis</i>	(29–37)	34.0 \pm 0.44	2.2	6.4	(33.1–34.9)	(35–40)	37.1 \pm 0.28	1.4	3.7	(36.6–37.7)	0.91
<i>Forsteronia acouci</i>	(17–30)	24.7 \pm 0.74	3.7	15.0	(23.2–26.3)	(19–33)	27.8 \pm 0.74	3.7	13.4	(26.2–29.3)	0.89
<i>Forsteronia gracilis</i>	(17–22)	19.5 \pm 0.29	1.4	7.4	(18.9–20.1)	(20–27)	23.3 \pm 0.34	1.7	7.3	(22.6–24.0)	0.84
<i>Geissospermum argenteum</i>	(44–56)	49.3 \pm 0.70	3.5	7.1	(47.9–50.8)	(40–48)	43.0 \pm 0.43	2.2	5.0	(42.1–43.9)	1.15
<i>Himatanthus bracteatus</i>	(35–45)	39.6 \pm 0.52	2.6	6.5	(38.5–40.7)	(41–53)	46.6 \pm 0.59	2.9	6.3	(45.4–47.9)	0.85
<i>Himatanthus stenophyllus</i>	(33–42)	37.2 \pm 0.58	2.9	7.8	(36.1–38.4)	(40–46)	43.2 \pm 0.36	1.8	4.2	(42.5–44.0)	0.86
<i>Himatanthus sucuba</i>	(33–43)	38.0 \pm 0.53	2.7	7.0	(36.9–39.1)	(38–48)	42.7 \pm 0.43	2.2	5.1	(41.8–43.6)	0.89
<i>Lacmellea arborescens</i>	(35–55)	42.3 \pm 0.85	4.3	10.1	(40.5–44.0)	(35–48)	41.0 \pm 0.70	3.5	8.5	(39.6–42.5)	1.03
<i>Lacmellea gracilis</i>	(25–39)	32.3 \pm 0.69	3.5	10.7	(31.6–33.0)	(13–23)	17.2 \pm 0.55	2.8	16.0	(16.1–18.4)	1.87
<i>Macoubea sprucei</i>	(36–48)	40.9 \pm 0.59	2.9	7.2	(39.5–42.4)	(31–44)	37.2 \pm 0.68	3.4	9.2	(35.7–38.6)	1.10
<i>Odontadenia perrotettii</i>	(36–65)	40.7 \pm 1.47	7.4	18.1	(37.7–43.8)	(43–74)	55.8 \pm 1.59	8.0	14.3	(52.5–59.1)	0.73
<i>Parahancornia fasciculata</i>	(30–51)	38.6 \pm 0.85	4.3	11.0	(36.8–40.4)	(24–48)	36.7 \pm 0.94	4.6	12.5	(34.8–38.6)	1.05
<i>Rauvolfia sprucei</i>	(87–118)	98.9 \pm 1.43	7.1	7.2	(95.9–101.8)	(100–112)	106.8 \pm 0.81	4.1	3.8	(105.1–108.5)	0.93
<i>Rhigospira quadrangulares</i>	(30–40)	37.0 \pm 0.60	3.0	8.2	(35.8–38.3)	(28–41)	36.5 \pm 0.68	3.4	9.3	(35.1–38.0)	1.01
<i>Secondatia duckei</i>	(19–29)	24.8 \pm 0.57	2.9	11.5	(23.6–26.0)	(16–27)	20.6 \pm 0.48	2.4	11.7	(19.6–21.6)	1.20
<i>Tabernaemontana angulata</i>	(45–75)	57.7 \pm 1.65	8.3	14.3	(54.3–61.1)	(31–44)	37.2 \pm 0.70	3.5	9.3	(35.8–38.7)	1.55
<i>Tabernaemontana flavicans</i>	(50–100)	83.4 \pm 2.46	12.3	14.8	(78.3–88.4)	(38–78)	64.0 \pm 1.76	8.8	13.8	(60.4–67.7)	1.30
<i>Tabernaemontana macrocalyx</i>	(75–105)	91.0 \pm 1.64	8.2	9.0	(87.6–94.3)	(38–55)	46.0 \pm 0.88	4.4	9.6	(44.1–47.8)	1.98
<i>Tabernaemontana muricata</i>	(46–68)	57.6 \pm 1.08	5.4	9.4	(55.4–59.9)	(36–53)	44.4 \pm 0.91	4.6	10.3	(42.5–46.3)	1.30
<i>Tabernaemontana undulata</i>	(52–75)	61.5 \pm 1.10	5.5	8.9	(59.2–63.8)	(36–48)	41.6 \pm 0.62	3.1	7.4	(40.4–42.9)	1.48

Species	D1					D2					
	VR	$\bar{x} \pm s\bar{x}$	s	CV%	CI	VR	$\bar{x} \pm s\bar{x}$	s	CV%	CI	
<i>Mandevilla scabra</i> *	(110–165)	139.0 \pm 3.10	15.5	11.2	(132.6–145.4)	(107–170)	138.7 \pm 4.17	20.9	15.0	(130.1–147.3)	–
<i>Odontadenia punctulosa</i> *	(55–72)	62.9 \pm 1.09	5.5	8.7	(60.6–65.1)	(45–63)	54.8 \pm 1.04	5.2	9.5	(52.6–56.9)	–
<i>Odontadenia verrucosa</i> *	(37–65)	52.3 \pm 1.26	6.3	12.0	(49.7–54.9)	(32–56)	45.2 \pm 1.08	5.4	12.0	(43.0–47.4)	–

*Pollen grains pantoporate.

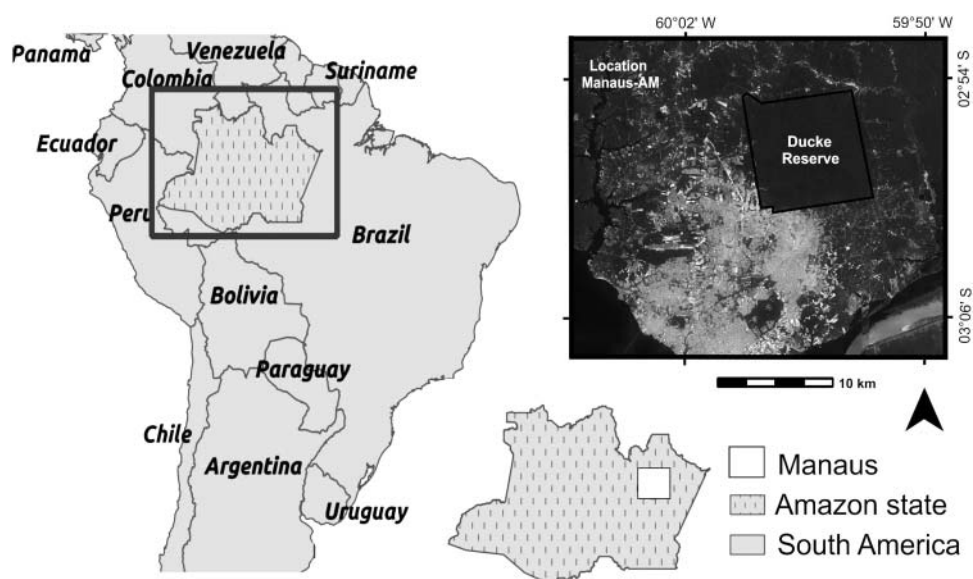


Figure 1. Adolpho Ducke Forest Reserve, located north of the city of Manaus, in the state of Amazon (Google Earth Satellite Image).

Hertha Meyer Laboratory of Cellular Ultrastructure, Institute of Biophysics, Federal University of Rio de Janeiro (Universidade Federal do Rio de Janeiro – UFRJ), or a Jeol 6390 LV device in the Scanning Electron Microscopy Centre of the Department of Invertebrates of the National Museum, UFRJ.

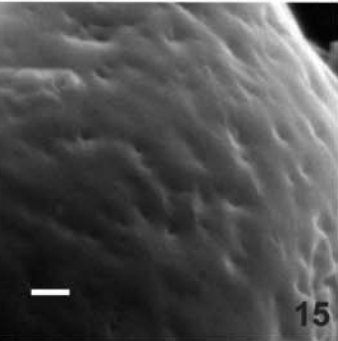
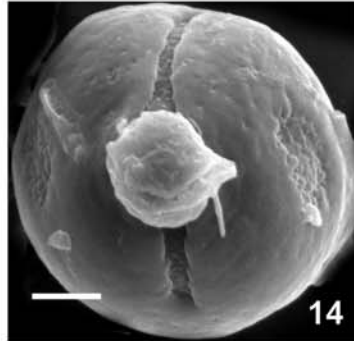
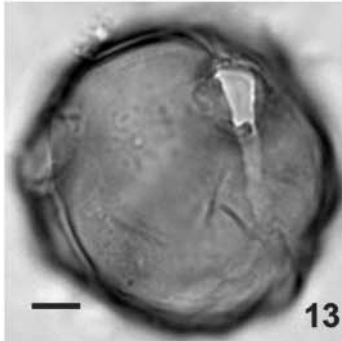
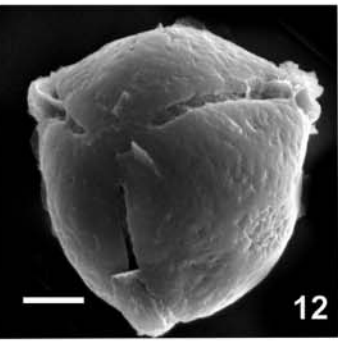
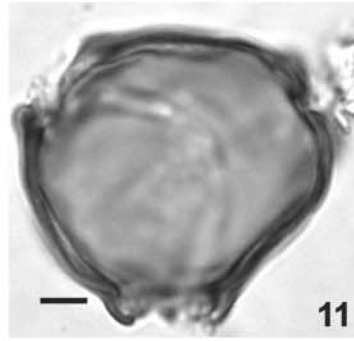
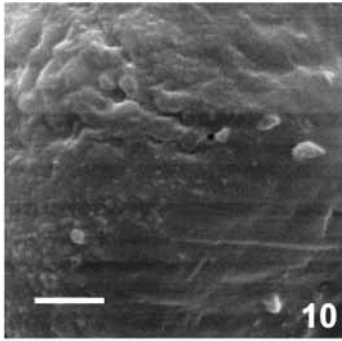
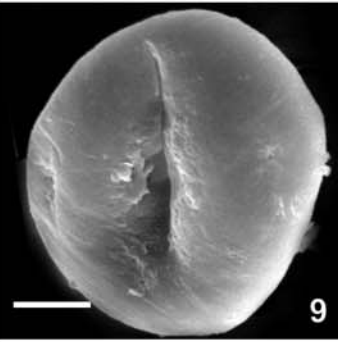
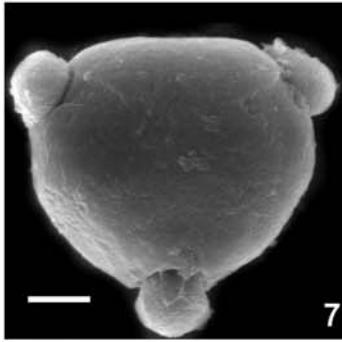
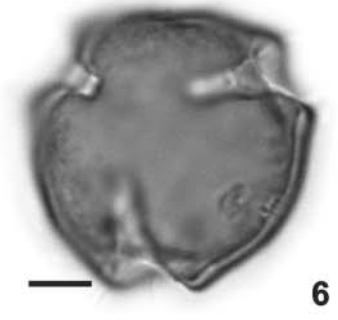
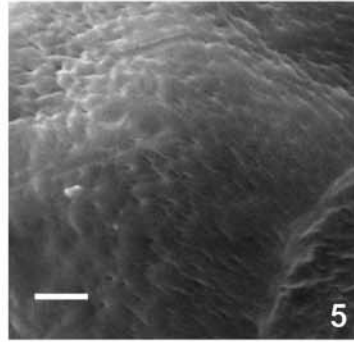
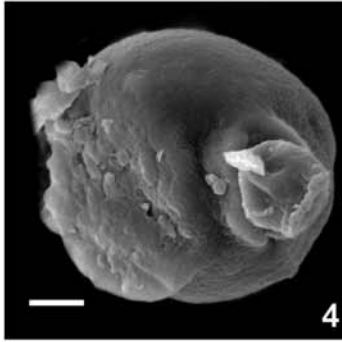
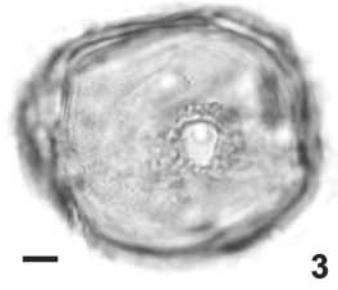
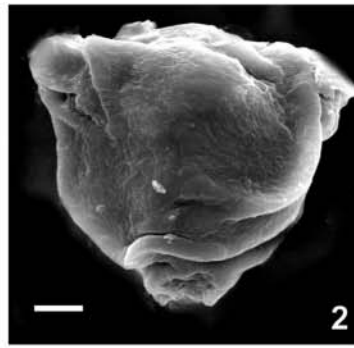
2.3. Analysed material

Ambelania acida Aublet: Brazil. Amazonas: Manaus, Reserva Florestal Ducke, 19 July 1963, fl., Rodrigues, W. 5380. 14049

(INPA). *Couma guianensis* Aublet: Brazil. Amazonas: Manaus, Reserva Florestal Ducke, fl., Assunção, P.A.C.L. 221 (INPA). *Couma utilis* (Mart.) Müll. Arg.: Brazil. Amazonas: Manaus, Reserva Florestal Ducke, 07 August 1957, fl., Rodrigues, W. 468. 228015 (INPA). *Forsteronia acouci* (Aublet) A. DC.: Brazil. Amazonas: Manaus-Itacoatiara, km 26 Reserva Florestal Ducke. 30 July 1997, fl., Assunção, P.A.C.L. & Silva, C.F. da 578. 191271 (INPA). *Forsteronia gracilis* (Benth.) Müll. Arg.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 27 November 1997, fl., Assunção, P.A.C.L. & Sothers, C.A. 723. 191674 (INPA).

Table 2. Arithmetic average (μm) of the measures of EDPV (equatorial diameter in polar view), AS (apocolpium side) of the wall structures and apertures of Apocynaceae pollen grains species found in the Adolpho Ducke Forest Reserve. D: diameter ($n = 10$).

	Layers			EDPV	AS	Endocingulo	Colpus		Endoaperture		Pore	
	Nexine	Sexine	Exine				Length	Width	D1	D2	D1	D2
<i>Ambelania acida</i>	–	–	1.4	–	–	–	–	–	–	–	7.3	7.1
<i>Couma guianensis</i>	–	–	1.2	31.4	9.4	–	21.7	7.6	11.2	6.2	–	–
<i>Couma utilis</i>	–	–	1.3	37.2	9.3	–	26.2	2.6	8.0	7.1	–	–
<i>Forsteronia acouci</i>	0.5	0.5	1.0	–	–	–	–	–	–	–	2.9	3.2
<i>Forsteronia gracilis</i>	–	–	1.0	–	–	–	–	–	–	–	1.9	2.0
<i>Geissospermum argenteum</i>	0.8	0.8	1.5	47.5	14.2	–	24.0	5.9	–	–	–	–
<i>Himatanthus bracteatus</i>	–	–	2.8	47.3	14.9	–	27.5	1.9	7.2	7.7	–	–
<i>Himatanthus stenophyllus</i>	1.1	2.1	3.2	44.2	12.8	–	22.7	2.1	4.9	2.1	–	–
<i>Himatanthus sucuba</i>	0.9	1.9	2.8	43.4	14.9	–	25.8	1.5	7.6	7.5	–	–
<i>Lacmellea arborescens</i>	–	–	1.6	40.2	28.8	–	8.2	1.9	2.4	2.4	–	–
<i>Lacmellea gracilis</i>	–	–	1.4	–	–	–	–	–	–	–	–	3.6
<i>Macoubea sprucei</i>	–	–	1.0	–	–	–	–	–	–	–	11.7	5.9
<i>Mandevilla scabra</i>	–	–	1.5	–	–	–	–	–	–	–	20.5	21
<i>Odontadenia perrotettii</i>	–	–	0.5	–	–	–	–	–	–	–	4.4	4.4
<i>Odontadenia puncticulosa</i>	–	–	1.4	–	–	–	–	–	–	–	6.2	6.2
<i>Odontadenia verrucosa</i>	–	–	2.0	–	–	–	–	–	–	–	9.0	9.0
<i>Parahancornia fasciculata</i>	–	–	1.7	–	–	–	–	–	–	–	7.6	7.6
<i>Rauvolfia sprucei</i>	–	–	1.1	83.8	63.2	–	17.8	6.6	8.7	8.7	–	–
<i>Rhigospira quadrangulares</i>	–	–	1.4	–	–	–	–	–	–	–	5.6	5.6
<i>Secondatia duckei</i>	–	–	1.2	–	–	–	–	–	–	–	2.0	2.0
<i>Tabernaemontana angulata</i>	–	–	1.5	–	–	7.7	20.4	0.8	–	–	–	–
<i>Tabernaemontana flavicans</i>	–	–	1.0	–	–	13.4	34.3	4.5	–	–	–	–
<i>Tabernaemontana macrocalyx</i>	–	–	3.2	–	–	9.4	30.3	1.8	–	–	–	–
<i>Tabernaemontana muricata</i>	–	–	2.3	–	–	10.3	23.4	2.8	–	–	–	–
<i>Tabernaemontana undulata</i>	–	–	3.3	–	–	4.6	26.4	0.7	–	–	–	–



Geissospermum argenteum Woodson: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 19 August 1994, fl., Nascimento, J.R. & Silva, C.F. da 578. 180470 (INPA). *Himatanthus bracteatus* (A. DC.) Woodson var. *bracteatus*: Brazil. Amazonas: Manaus, Reserva Florestal Ducke, 05 October 1965, fl., Loureiro, A. 16151 (INPA). *Himatanthus stenophyllus* Plumel: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 28 November 1994, fl., Nascimento, J.R. & Silva, C.F. da 667. 180471 (INPA). *Himatanthus sucubus* (Spruce ex Müll. Arg.) Woodson: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 01 December 1994, fl., Assunção, P.A.C.L. 102. 180453 (INPA). *Lacmellea arborescens* (Müll. Arg.) Markgr.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 01 August 1995, fl., Oliveira, A.A. & Assunção, P.A.C.L. 2804. 180475 (INPA). *Lacmellea gracilis* (Müll. Arg.) Markgr.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 12 December 1995, fl., Souza, M.A.D. de & Silva, C.F. da 180. 189617 (INPA). *Macoubea sprucei* (Mull. Arg.) Markgr.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 09 August 1995, fl., Sothers, C.A. & Nee, M., Silva, C.F. da, Assunção, P.A.C.L., Pereira, E. da C. 547. 181862 (INPA). *Mandevilla scabra* (Roem. & Schult.) K. Schum.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke. 21 April 1998, fl., Souza, M.A.D., Pereira, E. da C., Martins, L.H.P. 675 192611 (INPA). *Odontadenia perrottetii* (A. DC.) Woodson: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke, 14 September 1994, fl., Ribeiro, J.E.L.S., Vicentini, A., Silva, C.F. da, Pereira, E. da C., Athaide, S. 1413 (INPA). *Odontadenia punctulosa* (L. C. Rich.) Pulle: Brazil. Amazonas: Manaus-Itacoatiara km 50, Reserva Florestal Ducke, 05 October 1995, fl., Vicentini, A., Bonatto, F., Pereira, E. da C. 1069 (INPA). *Odontadenia verrucosa* (Willd. ex Roem. & Schult.) K. Schum. ex Markgr.: Brazil. Amazonas: Manaus-Itacoatiara km 50, Reserva Florestal Ducke, 14 September 1994, fl., Ribeiro, J.E.L.S. & Vicentini, A., Silva, C.F. da, Pereira, E. da C., Athaide, S. 1410 (INPA). *Parahancornia fasciculata* (Poir.) Benoist: Brazil. Amazonas: Manaus-Itacoatiara km 50, Reserva Florestal Ducke, fl., Sothers, C.A. 613 (INPA). *Rauvolfia sprucei* Mull. Arg.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 21 July 1994, fl., Vicentini, A. & Assunção, P.A.C.L., Nascimento, J.R. 637. 180511 (INPA). *Rhigospira quadrangularis* (Müll. Arg.) Miers: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 16 September 1994, fl., Vicentini, A. & Ribeiro, J.E.L.S., Athayde, S.F. de, Silva, C.F. da, Pereira, E. da C. 697. 180513 (INPA). *Secondatia duckei* Markgr.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 15 September 1994, fl., Vicentini, A. & Ribeiro, J.E.L.S., Athayde, S.F. de, Silva, C.F. da, Pereira, E. da C. 692. 180512 (INPA). *Tabernaemontana angulata* Mart. ex Müll. Arg.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 25 April 1996, fl., Sothers, C.A. & Assunção, P.A.C.L., Pereira, E. da C. 854. 189613 (INPA). *Tabernaemontana flavicans* Willd. ex Roem. & Schult.: Brazil. Amazonas: Manaus, Reserva Ducke, 13 November 2007, fl., Koch, I.M. & Simões, A.O., Paula-Souza, J., Obando, S.,

Mesquita, J.R. 19. 228020 (INPA). *Tabernaemontana macrocalyx* Mull. Arg.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 25 August 1994, fl., Sothers, C. A. & Pereira, E. da C., Silva, C.F. da, Assunção, P.A.C.L. 135 180499 (INPA). *Tabernaemontana muricata* Spruce ex Müll. Arg.: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°5'W). 01 August 1995, fl., Oliveira, A. A. & Assunção, P. A.C.L. 2809. 180477 (INPA). *Tabernaemontana undulata* Vahl: Brazil. Amazonas: Manaus-Itacoatiara km 26, Reserva Florestal Ducke (02°53'S, 59°58'W). 31 October 1995, fl., Souza, M.A.D. & Pereira, E. da C. 134. 182009 (INPA).

3. Results

Information on the habit, habitat, geographic distribution, reproductive characteristics (Ribeiro et al. 1999) and detailed palynological descriptions of 25 Apocynaceae species from 14 genera are given below. Measurement of the grains are given in Tables 1 and 2.

Ambelania Aublet

Ambelania acida Aublet (Plate 1, figures 1–5)

Characteristics: tree, 15 m tall, 15 cm in diameter, cream flowers, rare, distributed in the Central and Eastern Amazon and in the Guianas; collected in clay soil, terra-firme forest.

Palynological description: monad, isopolar, average size ($P = 34.6 \pm 0.48 \mu\text{m}$; $E = 40.7 \pm 0.29 \mu\text{m}$), $P/E = 0.85$, suboblate, amb circular, tricolporate. The colpi are relatively short (ca. $20 \mu\text{m}$ in length), thick margin (ca. $2 \mu\text{m}$), acute extremities (Plate 1, figures 1, 2, 4), endoaperture lalongate (ca. $3\text{--}6 \mu\text{m}$), with granulated surroundings (Plate 1, figure 3); exine thin ($1.4 \mu\text{m}$), sexine scabrate with perforations (Plate 1, figure 5), best observed under SEM.

Species variations: stephanocolporate grains (tetracolporate) were also found.

Comparisons: Nilsson (1990) described the same species with densely perforated ornamentation.

Couma Aublet

Couma guianensis Aublet (Plate 1, figures 6–10)

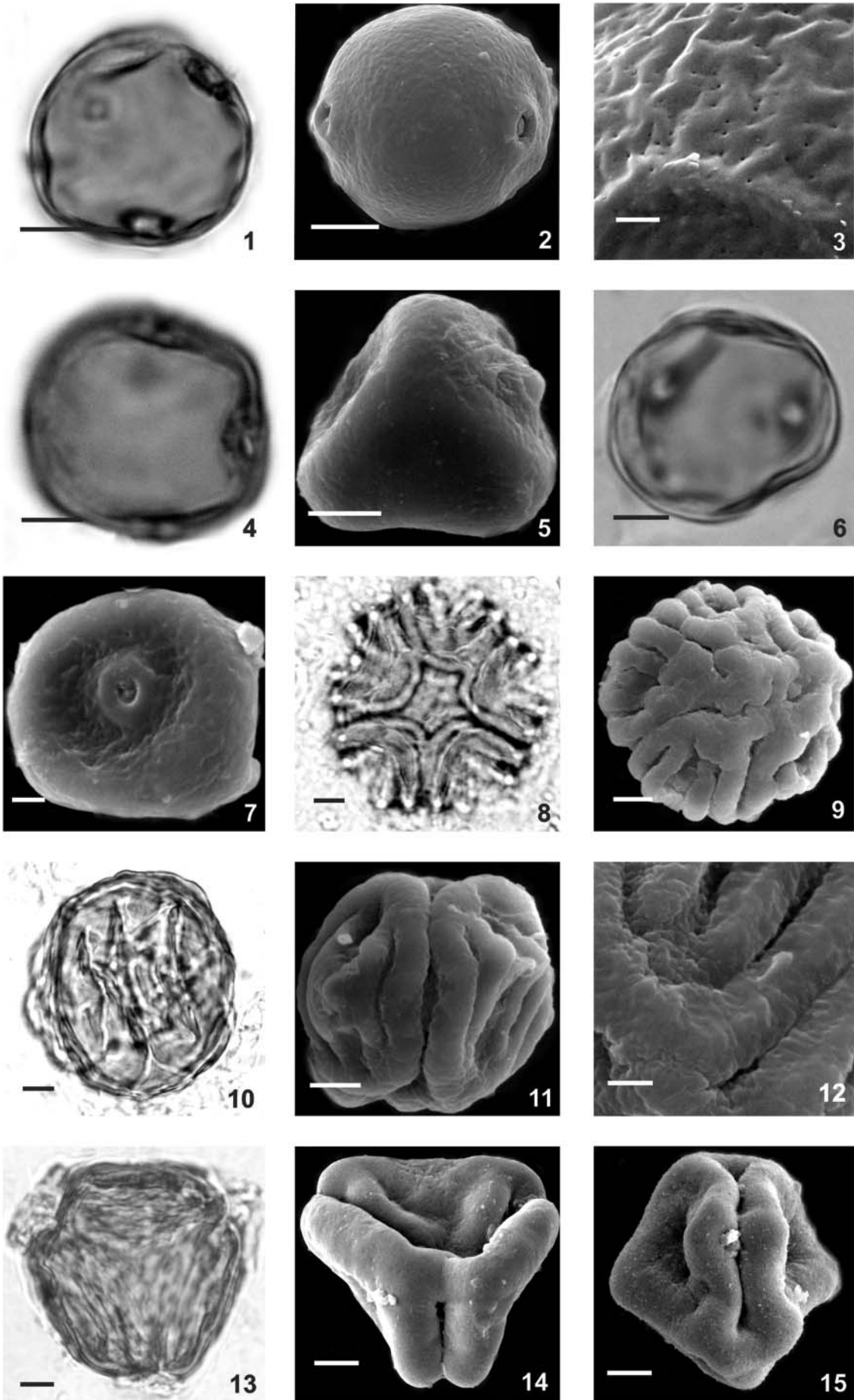
Characteristics: canopy tree, diameter at breast height (DBH) = 29.0 cm, pink flower, common, distributed in Central America and northern South America.

Palynological description: monad, isopolar, average size ($P = 27.1 \pm 0.35 \mu\text{m}$; $E = 33.7 \pm 0.46 \mu\text{m}$), $P/E = 0.81$, suboblate, amb subcircular (Plate 1, figures 6, 7), tectate, tricolporate. The colpi ($21.7 \times 7.6 \mu\text{m}$) are long, margin undifferentiated, truncated extremities (Plate 1, figures 8, 9), endoaperture lalongate ($11.2 \times 6.2 \mu\text{m}$); exine thin ($1.2 \mu\text{m}$), sexine scabrate (Plate 1, figure 10), best observed under SEM.

Species variations: number of colpi, tetracolporate grains were also observed.

Couma utilis (Mart.) Müll. Arg. (Plate 1, figures 11–15)

Plate 1. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Ambelania acida*: figure 1, polar view, general view (LM); figure 2, general view (SEM); figure 3, equatorial view, aperture (LM); figure 4, aperture (SEM); figure 5, surface detail (SEM). *Couma guianensis*: figure 6, polar view, general view (LM); figure 7, general view (SEM); figure 8, equatorial view, aperture (LM); figure 9, general view (SEM); figure 10, surface detail (SEM). *Couma utilis*: figure 11, polar view, optical cross section (LM); figure 12, general view (SEM); figure 13, equatorial view, aperture (LM); figure 14, aperture (LM); figure 15, surface detail (SEM). Scale bars: 1–4, 6–9, 11–14 = $5 \mu\text{m}$; 2, 5, 10 = $2 \mu\text{m}$; 15 = $1 \mu\text{m}$.



Characteristics: Understorey tree, elliptic leaves attenuate at the base, cultivated in the Reserve, distributed in Central and Western Amazon and the Guianas.

Palynological description: monad, isopolar, average size ($P = 34.0 \pm 0.44 \mu\text{m}$; $E = 37.1 \pm 0.28 \mu\text{m}$), $P/E = 0.91$, oblate-spheroidal, amb circular, tricolporate. The colpi are very long ($26.2 \times 2.6 \mu\text{m}$), undifferentiated margin, acute extremities (Plate 1, figure 12), membrane ornamented, with a fastigium (Plate 1, figure 11), endoaperture circular ($8.0 \times 7.1 \mu\text{m}$), exine thin ($1.3 \mu\text{m}$ in thickness), sexine with conspicuous perforations (Plate 1, figure 15). In the medial region of the mesocolpium there is a depression with granulated ornamentation (Plate 1, figures 13, 14), best observed under SEM.

Species variations: endoaperture shape varies from lalongate, circular to lolongate.

Forsteronia G.F.W. Meyer

Forsteronia acouci (Aublet) A. DC. (Plate 2, figures 1–3)

Characteristics: woody canopy liana, DBH = 8.0 cm; 25 m in height, yellow petals, brown anthers; stylus, stigma and ovary green with white indument, collected in slope forest, clay soil, infrequent, found in northern South America.

Palynological description: monad, isopolar, average size ($P = 24.7 \pm 0.74 \mu\text{m}$; $E = 27.8 \pm 0.74 \mu\text{m}$), $P/E = 0.89$, oblate-spheroidal, amb circular, tetraporate – hexaporate (Plate 2, figures 1, 2), exine rugulate-perforated. Pores are small, circular ($2.9 \times 3.2 \mu\text{m}$), annuli $1.0 \mu\text{m}$ thick, exine thin (ca. $1 \mu\text{m}$ in thickness), sexine rugulate, $0.5 \mu\text{m}$, with perforations in the regions between the rugulae (Plate 2, figure 3), best observed under SEM.

Species variations: tetraporate grains.

Forsteronia gracilis (Benth.) Müll. Arg. (Plate 2, figures 4–7)

Characteristics: woody liana, creeping or in the canopy, DBH = 1.5 cm; 3.0 m in height, white petals, greenish calyx, dark brown androecium and gynoecium, dark peduncle and fruits, collected in secondary forest as ‘capoeira’, infrequent, widely distributed in the Amazon and the Guianas.

Palynological description: monad, isopolar, small size ($P = 19.5 \pm 0.29 \mu\text{m}$; $E = 23.3 \pm 0.34 \mu\text{m}$), $P/E = 0.84$, suboblate, amb. circular, triporate – tetraporate (Plate 2, figures 4–6). Pores are very small, circular ($1.9 \times 2.0 \mu\text{m}$), annulate (ca. $1.0 \mu\text{m}$ in thickness), region around the annuli with grains, exine thin (ca. $1.0 \mu\text{m}$ in thickness), exine scabrate, perforated (Plate 2, figures 5, 7).

Species variations: stephanoporate pollen grains may also be observed.

Geissospermum Allemão

Geissospermum argenteum Woodson (Plate 2, figures 8–12)

Characteristics: tree, DBH = 35 cm, dark-gray buds, pedicels and sepals, beige flowers, petals free internally at the lobes

with white pilosity, collected in plateau forest, frequent, found in Central and Eastern Amazon and in the Guianas.

Palynological description: monad, isopolar, average size ($P = 49.3 \pm 0.70 \mu\text{m}$; $E = 43.0 \pm 0.43 \mu\text{m}$), $P/E = 1.15$, prolate-spheroidal, amb circular, stephanocolporate. Colpi are long ($24 \times 5.9 \mu\text{m}$), surrounded by two thick ‘cords’: the first one shorter and parallel to colpi and the second one surrounding both the aperture and the first thickening, margin with a thickness of $2 \mu\text{m}$. This set of margins makes it difficult to visualise and measure the aperture, giving the impression that it is only the colpus; exine thin ($1.6 \mu\text{m}$ in thickness) or thick (ca. $2 \mu\text{m}$ close to the aperture), scabrate, nexine $0.8 \mu\text{m}$, sexine $0.8 \mu\text{m}$. The apocolpium is formed by the connection between the two margin-forming ‘cords’.

Himatanthus Willd. ex Schult.

Himatanthus bracteatus (A. DC.) Woodson var. *bracteatus* (Plate 2, figures 13–15)

Characteristics: tree, 12 m in height, DBH = 10 cm, white flowers, infrequent, found in the Amazon-Atlantic Forest Disjunction.

Palynological description: monad, isopolar, average size ($P = 39.6 \pm 0.52 \mu\text{m}$; $E = 46.6 \pm 0.59 \mu\text{m}$), $P/E = 0.85$, suboblate, amb triangular (Plate 2, figure 13), tricolporate, exine psilate. Colpi are long ($27.5 \times 1.9 \mu\text{m}$) with truncated extremities, colpus with a thick margin (ca. $2.0 \mu\text{m}$), endoaperture lalongate $7.2 \times 7.7 \mu\text{m}$, hard to visualise and measure, exine thick ($3.5 \mu\text{m}$ in thickness), sexine (ca. $2.5 \mu\text{m}$) thicker than nexine (ca. $1 \mu\text{m}$), sexine psilate throughout most of the surface; in the mesocolpium region there is a depression with rugulate ornamentation (Plate 2, figures 14, 15).

Himatanthus stenophyllus Plumel (Plate 3, figures 1–4)

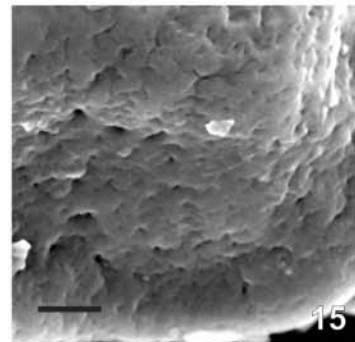
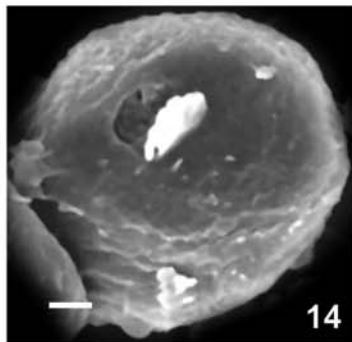
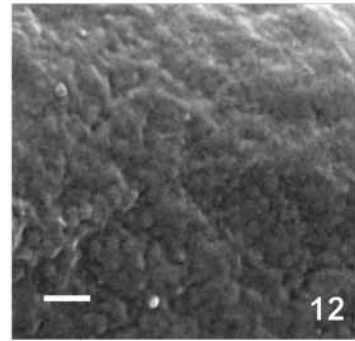
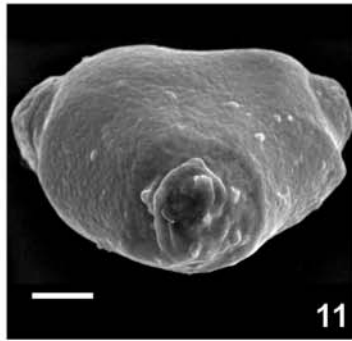
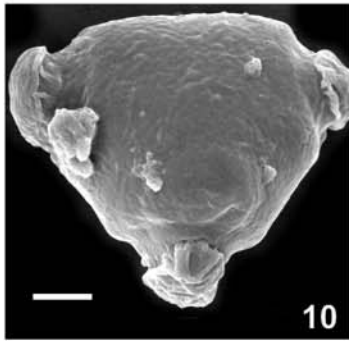
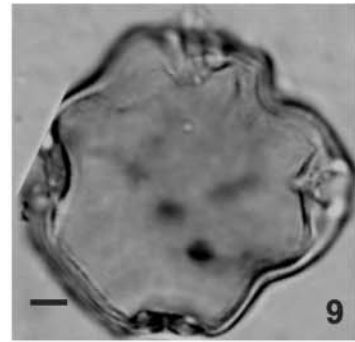
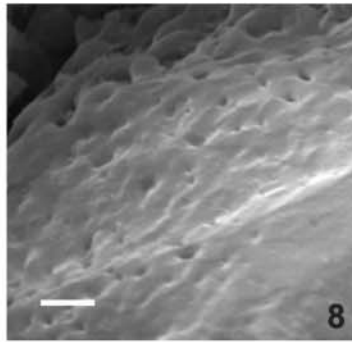
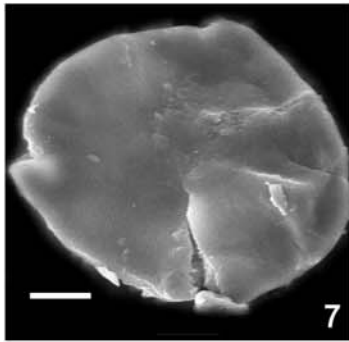
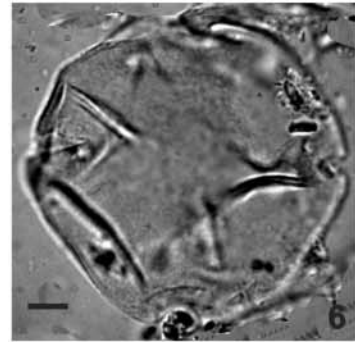
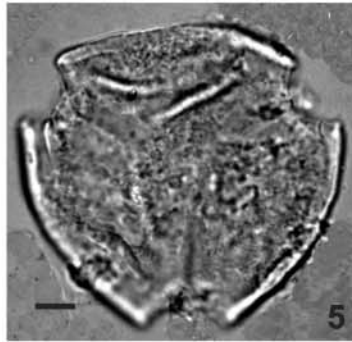
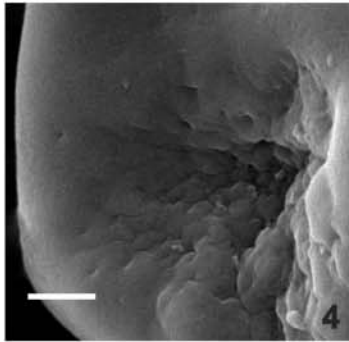
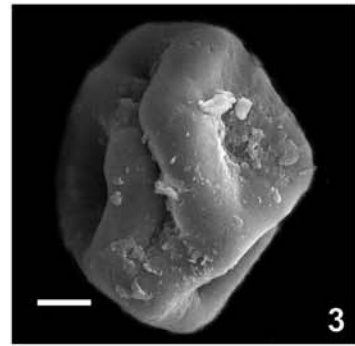
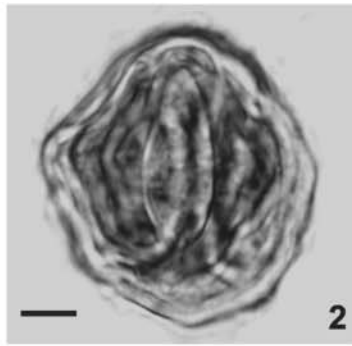
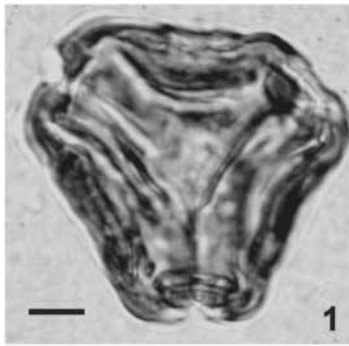
Characteristics: tree, 18 m in height, DBH = 19 cm, white flowers, collected in plateau forest, frequent, widely distributed in the Amazon.

Palynological description: monad, isopolar, average size ($P = 37.2 \pm 0.58 \mu\text{m}$; $E = 43.2 \pm 0.36 \mu\text{m}$), $P/E = 0.86$, suboblate, amb triangular (Plate 3, figure 1), tricolporate. Colpi are long ($22.7 \times 2.1 \mu\text{m}$), with truncated extremities (Plate 3, figures 2, 3), colpus with a thick margin (ca. $2–3 \mu\text{m}$), endoaperture lalongate ($4.9 \times 2.1 \mu\text{m}$), exine thick (ca. $3.2 \mu\text{m}$), sexine thicker than nexine (ca. $2.1 \mu\text{m}$), nexine (ca. $1.1 \mu\text{m}$), sexine psilate throughout most of the surface, ornamentation perforate (Plate 3, figures 3, 4).

Himatanthus sucuuba (Spruce ex Müll. Arg.) Woodson (Plate 3, figure 5)

Characteristics: tree, green sepals, white petals yellow at the base, collected in slope forest, sandy soil, frequent, found in tropical South America.

Plate 2. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Forsteronia acouci*: figure 1, polar view, general view (LM); figure 2, equatorial view, general view (SEM); figure 3, surface detail (SEM). *Forsteronia gracilis*: figure 4, polar view, general view (LM); figure 5, general view (SEM); figure 6: equatorial view, general view (LM); figure 7, aperture (SEM). *Geissospermum argenteum*: figure 8, polar view, general view (LM); figure 9, general view (SEM); figure 10, equatorial view, general view (LM); figure 11, general view (SEM); figure 12, surface detail (SEM). *Himatanthus bracteatus* var. *bracteatus*: figure 13 polar view, general view (LM); figure 14, general view (SEM); figure 15, equatorial view, surface detail (SEM). Scale bars: 1 = $10 \mu\text{m}$; 2, 4–6, 8–11, 13–15 = $5 \mu\text{m}$; 3 = $1 \mu\text{m}$; 7, 12 = $2 \mu\text{m}$.



Palynological description: monad, isopolar, average size ($P = 38.0 \pm 0.53 \mu\text{m}$; $E = 42.7 \pm 0.43 \mu\text{m}$), $P/E = 0.89$, oblate-spheroidal, amb subtriangular (Plate 3, figure 5), tricolporate. Colpi ($25.8 \times 1.5 \mu\text{m}$) have acute extremities; endoaperture circular ($7.6 \times 7.5 \mu\text{m}$); exine thick ($2.8 \mu\text{m}$), sexine psilate to finely perforated; sexine ($1.9 \mu\text{m}$) as thick as nexine ($0.9 \mu\text{m}$).

Species variations: tetracolporate grains were also observed.

Lacmellea H. Karst.

Lacmellea arborescens (Müll. Arg.) Markgr. (Plate 3, figures 6–8)

Characteristics: tree, leathery leaves, greenish flowers and buds, collected in plateau forest, rare, found in Central and Western Amazon.

Palynological description: monad, isopolar, average size ($P = 42.3 \pm 0.85 \mu\text{m}$; $E = 41.0 \pm 0.70 \mu\text{m}$), $P/E = 1.03$, prolate-spheroidal, amb circular (Plate 3, figures 6, 7), tetracolporate. Colpi are short ($8.2 \times 1.9 \mu\text{m}$) with acute extremities, with a costa, endoaperture circular ($2.4 \mu\text{m}$), exine thick ($1.6 \mu\text{m}$), sexine thicker than nexine, exine scabrate with perforations (Plate 3, figure 8).

Species variation: annuli thickness varying from 2 to $4 \mu\text{m}$.

Lacmellea gracilis (Müll. Arg.) Markgr. (Plate 3, figures 9–12)

Characteristics: tree, 7.0 m in height, DBH = 10.0 cm, green-whitish flowers, collected in plateau forest, understory, clay soil, frequent, found in Central and Western Amazon.

Palynological description: monad, isopolar, average size ($P = 32.3 \pm 0.69 \mu\text{m}$; $E = 17.2 \pm 0.55 \mu\text{m}$), $P/E = 1.87$, prolate, amb circular or triangular (Plate 3, figures 9, 10), tricolporate – tetracolporate (Plate 3, figures 9, 10). Colpi are short (ca. $10 \mu\text{m}$ in length), with fastigiate costa (Plate 3, figures 9–11), endoaperture circular (ca. $3.6 \mu\text{m}$), exine thin ($1.4 \mu\text{m}$), sexine scabrate with sparse perforations (Plate 3, figure 12).

Macoubea Aublet

Macoubea sprucei (Mull. Arg.) Markgr. (Plate 3, figures 13–15)

Characteristics: treelet, 7 m in height, DBH = 11.8 cm, green and yellow buds, yellow fragrant flowers, collected in campinarana forest (vegetation type that grow over pure leached white sand (Pires & Prance 1985), understory, sandy soil, frequent, found in Central Amazon to Central America.

Palynological description: monad, isopolar, average size ($P = 40.9 \pm 0.59 \mu\text{m}$; $E = 37.2 \pm 0.68 \mu\text{m}$), $P/E = 1.10$, prolate-spheroidal, amb circular, diporate. Pores ($11.7 \times 5.9 \mu\text{m}$) are large, have a thick annulus (ca. $3 \mu\text{m}$), exine thicker and scabrae more abundant close to the pore, exine $1.0 \mu\text{m}$ in thickness in the other regions of the pollen grain.

Mandevilla Lindl.

Mandevilla scabra (Roem. & Schult.) K. Schum. (Plate 4, figures 1–3)

Characteristics: prostrate herb, voluble, creeping, collected in secondary vegetation (capoeira), sandy soil.

Palynological description: monad, apolar, large size ($D1 = 139.0 \pm 3.10 \mu\text{m}$; $D2 = 138.7 \pm 4.17 \mu\text{m}$), amb circular, pantoporate (4–6 pores) (Plate 4, figure 1), exine rugulate. Pores are small, circular ($20.5 \times 21 \mu\text{m}$), with a thin annulus (Plate 4, figure 2), exine thin ($1.5 \mu\text{m}$), sexine with inconspicuous rugulae, best seen under SEM (Plate 4, figure 3).

Odontadenia Benth.

Odontadenia perrottetii (A. DC.) Woodson (Plate 4, figures 4–6)

General plant characteristics: tall liana in terra-firme forest, yellow flowers, rare, found in Central and Eastern Amazon.

Palynological description: monad, isopolar, large size ($P = 40.7 \pm 1.47 \mu\text{m}$; $E = 55.8 \pm 1.59 \mu\text{m}$), amb circular (Plate 4, figure 4), triporate – tetraporate, exine psilate. Pores are small ($4.4 \mu\text{m}$), circular, with thin annuli (ca. $3 \mu\text{m}$) (Plate 4, figure 5), exine thin ($0.5 \mu\text{m}$), sexine psilate to scabrate close to the pore (Plate 4, figure 6).

Odontadenia puncticulosa (L. C. Rich.) Pulle (Plate 4, figures 7–10)

Characteristics: woody liana, yellow flowers, collected in campinarana forest, frequent, found in southern Central America and northern South America.

Palynological description: monad, apolar, large size ($D1 = 62.9 \pm 1.09 \mu\text{m}$, $D2 = 54.8 \pm 1.04 \mu\text{m}$), amb circular, pantoporate (6–8 pores). Pores are large ($6.2 \mu\text{m}$), circular, with thin annuli (Plate 4, figures 7–9), exine thin ($1.4 \mu\text{m}$), sexine with conspicuous rugulae and sparse perforations (Plate 4, figure 10).

Odontadenia verrucosa (Willd. ex Roem. & Schult.) K. Schum. ex Markgr. (Plate 4, figures 11–15)

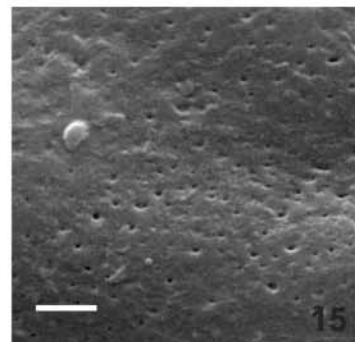
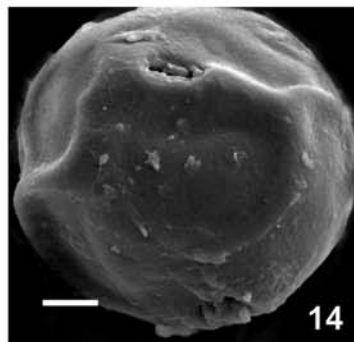
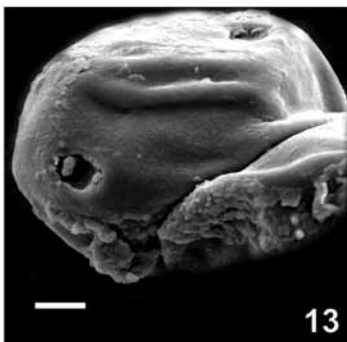
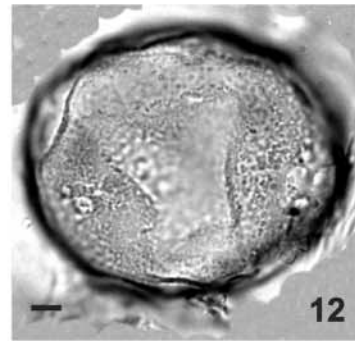
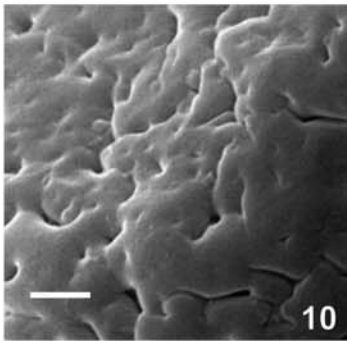
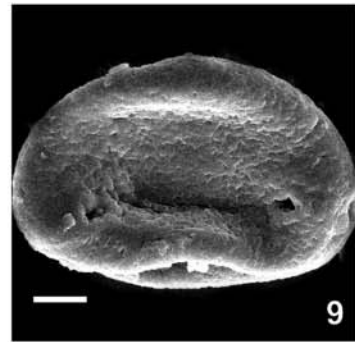
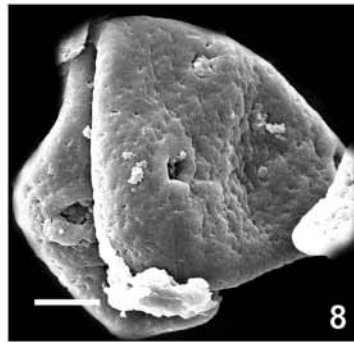
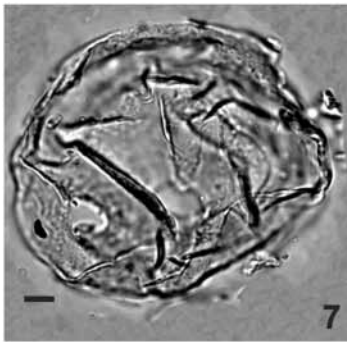
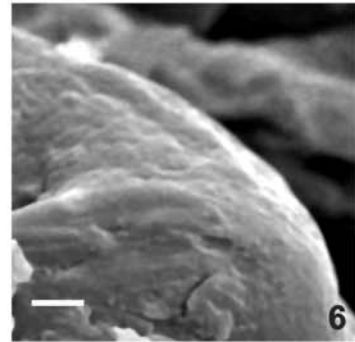
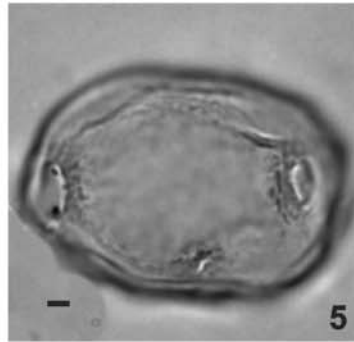
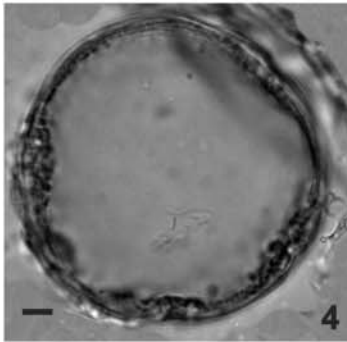
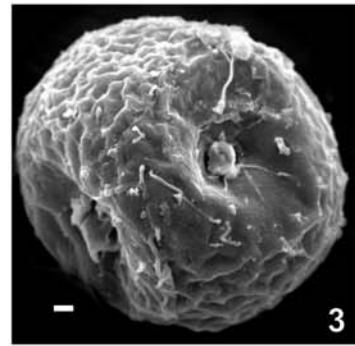
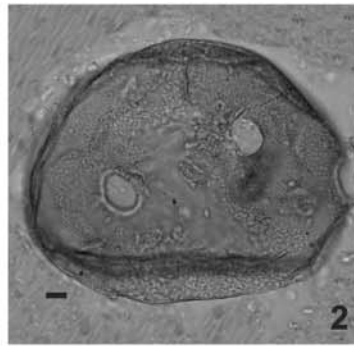
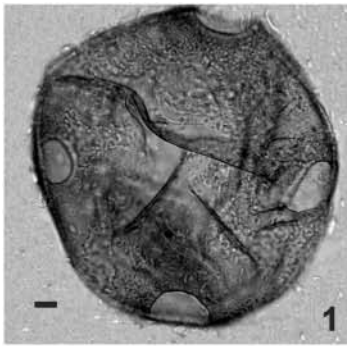
Characteristics: canopy liana, green sepals, golden-yellow petals, orange with red bands in the tube interior, very fragrant, collected in lowland forest, common, found in southern Central America and northern South America.

Palynological description: monad, apolar, large size ($D1 = 52.3 \pm 1.26 \mu\text{m}$, $D2 = 45.2 \pm 1.08 \mu\text{m}$), amb circular (Plate 14, figure 11), pantoporate (6–8 pores). Pores are large, circular ($9.0 \mu\text{m}$), with thin annuli (ca. $1 \mu\text{m}$ in thickness), with granules in their surroundings (Plate 4, figures 12–14), exine scabrate with perforations, thin (ca. $2 \mu\text{m}$), up to $3 \mu\text{m}$ close to the pores (Plate 4, figure 15).

Parahancornia Ducke

Parahancornia fasciculata (Poir.) Benoist (Plate 5, figures 1–3)

Plate 3. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Himatanthus stenophyllus*: figure 1, polar view, general view (LM); figure 2, equatorial view, general view (LM); figure 3, general view (SEM); figure 4, surface detail (SEM). *Himatanthus sucuuba*: figure 5, polar view, general view (LM). *Lacmellea arborescens*: figure 6, polar view, general view (LM); figure 7, general view (SEM); figure 8, surface detail (SEM). *Lacmellea gracilis*: figure 9, polar view, general view (LM); figure 10, general view (SEM); figure 11, equatorial view, general view (SEM); figure 12, surface detail (SEM). *Macoubea sprucei*: figure 13, equatorial view, general view (LM); figure 14, general view (SEM); figure 15, surface detail (SEM). Scale bars: 1–3, 5–7, 9–11, 13 = $5 \mu\text{m}$; 4, 8, 14, 15 = $2 \mu\text{m}$, 12 = $1 \mu\text{m}$.



Characteristics: Canopy tree, 20.0 m in height, DBH = 25.0 cm; yellowish-green buttons, white petals, yellow sepals, collected in lowland forest, infrequent, widely distributed in the Amazon and the Guianas.

Palynological description: monad, isopolar, average size ($P = 38.6 \pm 0.85 \mu\text{m}$; $E = 36.7 \pm 0.94 \mu\text{m}$), $P/E = 1.05$, prolate-spheroidal, amb subcircular, triporate (Plate 5, figure 1). Pores are large, circular ($7.6 \mu\text{m}$), with an annulus (ca. $1 \mu\text{m}$), exine thin ($1.7 \mu\text{m}$), sexine psilate to scabrate in the mesopore and granules surrounding the pores (Plate 5, figures 2, 3).

Rauvolfia L.

Rauvolfia sprucei Mull. Arg. (Plate 5, figures 4–7)

Characteristics: treelet, flowers with a greenish tube and purple lobes, DBH = 6 cm, 10 m in height, collected in plateau forest, infrequent.

Palynological description: monad, isopolar, very large size ($P = 98.9 \pm 1.43 \mu\text{m}$; $E = 106.8 \pm 0.81 \mu\text{m}$), $P/E = 0.93$, oblate-spheroidal, amb subcircular, tricolporate. Colpi are short ($17.8 \times 6.6 \mu\text{m}$), with a margin (ca. $5.0 \mu\text{m}$), a distinct fastigium (Plate 5, figure 4); endoaperture circular ($8.7 \mu\text{m}$) (Plate 5, figures 5–7). Each aperture is located in a depression formed by two wide and thick margins, exine psilate and thin ($1.1 \mu\text{m}$).

Species variation: tetracolporate grains were also observed.

Rhigospira Miers

Rhigospira quadrangularis (Müll. Arg.) Miers (Plate 5, figures 8–10)

Characteristics: canopy tree, DBH = 25.0 cm; terminal yellowish-green inflorescence; petals yellowish-green in the tube and internally, free lobes of the petals white, collected in campinarana forest, infrequent, found in Central and Western Amazon.

Palynological description: monad, isopolar, average size ($P = 37.0 \pm 0.60 \mu\text{m}$; $E = 36.5 \pm 0.68 \mu\text{m}$), $P/E = 1.01$, prolate-spheroidal, amb subtriangular, tricolporate. Colpi are short (ca. $10 \times 14 \mu\text{m}$), endoaperture circular ($5.6 \mu\text{m}$), with a distinct fastigium (Plate 5, figures 8, 9), granules in the surroundings of the aperture (Plate 5, figure 10), exine psilate and thin ($1.4 \mu\text{m}$).

Secondatia A. DC.

Secondatia duckei Markgr. (Plate 5, figures 11, 12)

Characteristics: woody liana, leaves with a recurved margin, yellow flowers, collected in campinarana, sandy to sandy-clay soils, common, found in Central Amazon.

Palynological description: monad, isopolar, small size ($P = 24.8 \pm 0.57 \mu\text{m}$; $E = 20.6 \pm 0.48 \mu\text{m}$), $P/E = 1.20$, subprolate, amb circular, triporate – stephanoporate (Plate 5, figure 11). Pores are very small and circular ($2.0 \mu\text{m}$), with an annulus (ca. $2 \mu\text{m}$), exine thin ($1.2 \mu\text{m}$), sexine scabrate with perforations best seen under SEM (Plate 5, figures 12, 13) and granules surrounding the pores.

Tabernaemontana L.

Tabernaemontana angulata Mart. ex Müll. Arg. (Plate 5, figures 13, 14)

Characteristics: shrub, 2 m in height, calyx and corolla white, with a purple base, collected in plateau forest, common, found in Central and Eastern Amazon.

Palynological description: monad, isopolar, large size ($P = 57.7 \pm 1.65 \mu\text{m}$; $E = 37.2 \pm 0.70 \mu\text{m}$), $P/E = 1.55$, prolate, amb circular, tricolporate. Colpi are short ($20.4 \times 0.8 \mu\text{m}$), with a costa, endoaperture endocingulate ($7.7 \mu\text{m}$) (Plate 5, figure 14), exine thin ($2 \mu\text{m}$), sexine (ca. $1.5 \mu\text{m}$) thicker than nexine (ca. $0.5 \mu\text{m}$), sexine scabrate in the mesocolpium and rugulate with perforations on the pole (Plate 5, figure 15).

Species variation: verrucae were observed close to the endocingulum in some grains.

Tabernaemontana flavicans Willd. ex Roem. & Schult. (Plate 6, figures 1–4)

Characteristics: tree, DBH = 7 cm, greenish buds, clear yellow flowers with an apex of yolk-yellow petals, collected in plateau forest, common, found in tropical South America.

Palynological description: monad, isopolar, large size ($P = 83.4 \pm 2.46 \mu\text{m}$; $E = 64.0 \pm 1.76 \mu\text{m}$), $P/E = 1.30$, subprolate, amb circular (Plate 6, figure 1), tricolporate. Colpi are short ($34.3 \times 4.5 \mu\text{m}$), with an ornamented membrane, with a costa, endoaperture endocingulate ($13.4 \mu\text{m}$) (Plate 6, figures 2–4), with a fastigium, exine thin ($1 \mu\text{m}$), sexine densely perforated.

Tabernaemontana macrocalyx Mull. Arg. (Plate 6, figures 5–7)

Characteristics: treelet, DBH = 5 cm, 2.5 m in height, cream buds and cream flowers with internally yellow petals, fragrant flowers, collected in lowland forest, rare, widely distributed in the Amazon.

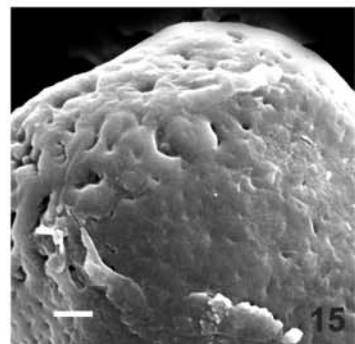
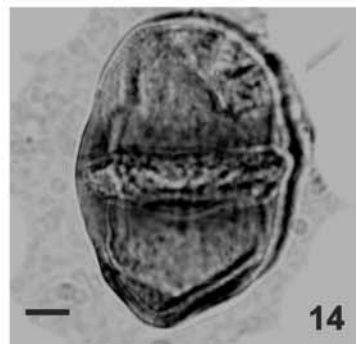
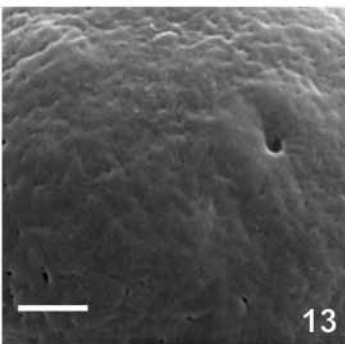
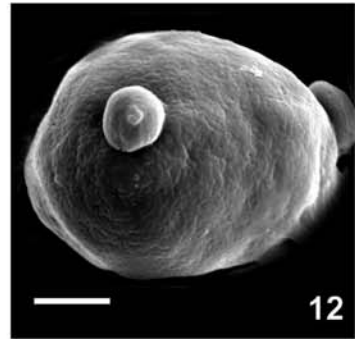
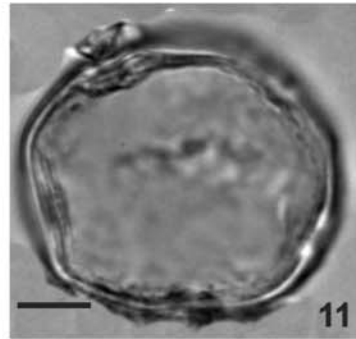
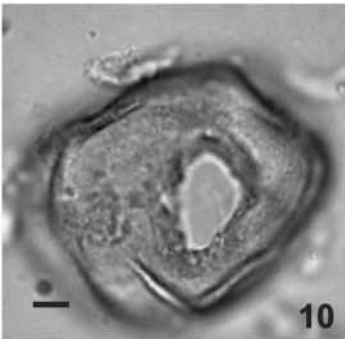
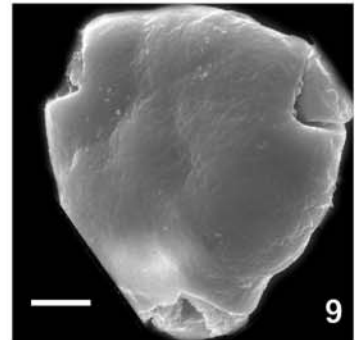
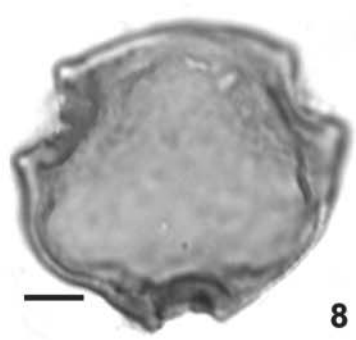
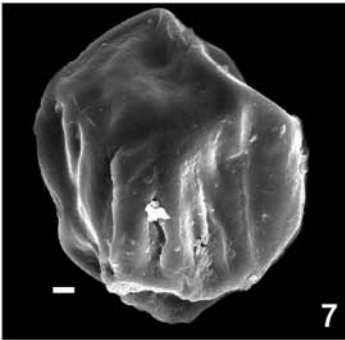
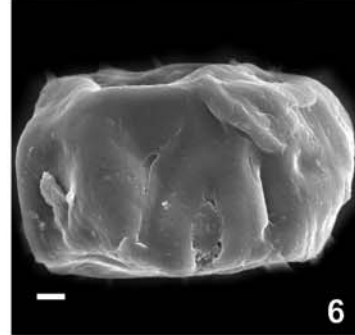
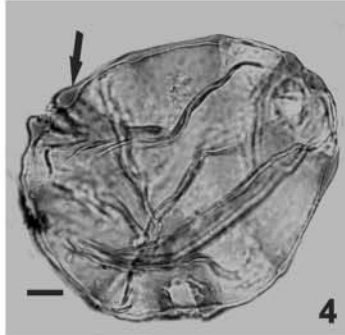
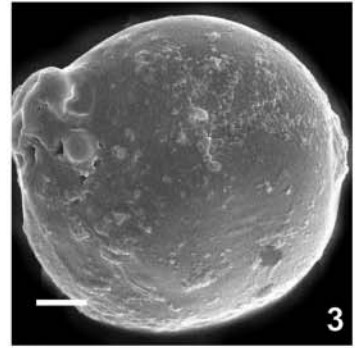
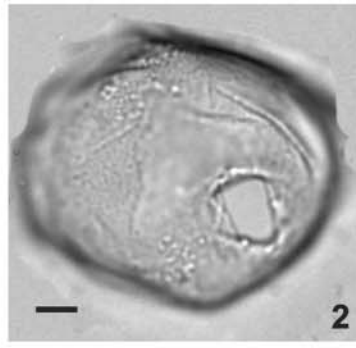
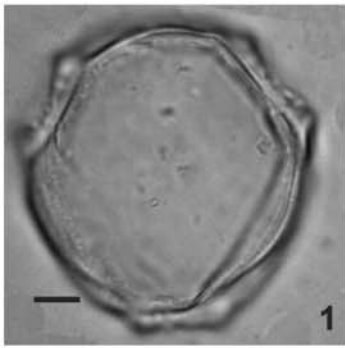
Palynological description: monad, isopolar, very large size ($P = 91.0 \pm 1.64 \mu\text{m}$; $E = 46.0 \pm 0.88 \mu\text{m}$), $P/E = 1.98$, prolate, amb circular, tricolporate. Colpi are short ($30.3 \times 1.8 \mu\text{m}$), with an ornamented membrane, with a costa, endoaperture endocingulate ($9.4 \mu\text{m}$) (Plate 6, figure 6), exine thick ($3.2 \mu\text{m}$), sexine (ca. $2 \mu\text{m}$) thicker than nexine (ca. $1.2 \mu\text{m}$), sexine scabrate, with conspicuous perforations at the poles (Plate 6, figure 7).

Tabernaemontana muricata Spruce ex Müll. Arg. (Plate 6, figures 8–10)

Characteristics: treelet, white flowers, collected in terra-firme forest, common, found around Manaus.

Palynological description: monad, isopolar, large size ($P = 57.6 \pm 1.08 \mu\text{m}$; $E = 44.4 \pm 0.91 \mu\text{m}$), $P/E = 1.30$, subprolate, amb circular, tricolporate. Colpi are short ($23.4 \times 2.8 \mu\text{m}$), with a costa, with an ornamented membrane (Plate 6, figure 9), endoaperture endocingulate ($10.3 \mu\text{m}$), with a costa (Plate 6, figure 8), exine thick ($2 \mu\text{m}$), sexine (ca. $1 \mu\text{m}$) as thick as nexine

Plate 4. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Mandevilla scabra*: figure 1, polar view, general view (LM); figure 2, equatorial view, general view (LM); figure 3, general view (SEM). *Odontadenia perrottetii*: figure 4, general view (LM); figure 5, general view (LM); figure 6, surface detail (SEM). *Odontadenia punctulosa*: figure 7, general view (LM); figures 8, 9, general view (SEM); figure 10, surface detail (SEM). *Odontadenia verrucosa*: figure 11, general view (LM); figure 12, aperture (LM); figures 13, 14, general view, aperture (SEM); figure 15, surface detail (SEM). Scale bars: 1–5, 7–9, 11–14 = $5 \mu\text{m}$; 6 = $2 \mu\text{m}$, 10, 15 = $1 \mu\text{m}$.



(ca. 1 μm), sexine scabrate with perforations at the poles (Plate 6, figure 10).

Tabernaemontana undulata Vahl (Plate 6, figures 11–15)

Characteristics: treelet, 3 m in height, DBH = 5 cm, pink-white calyx, corolla tube purple-whitish in its lower part, pinkish in its upper part, collected in slope forest, common, found in southern Central America and northern South America.

Palynological description: monad, isopolar, large size ($P = 61.5 \pm 1.10 \mu\text{m}$; $E = 41.6 \pm 0.62 \mu\text{m}$), $P = 1.48$, prolate, amb circular, tricolporate. Colpi are short ($26.4 \times 0.7 \mu\text{m}$), membrane ornamented (Plate 6, figure 11–13), with a costa (Plate 6, figure 12), endoaperture endocingulate ($4.6 \mu\text{m}$) (Plate 6, figure 12, 14), exine thick ($3 \mu\text{m}$), sexine (ca. $2 \mu\text{m}$) thicker than nexine (ca. $1 \mu\text{m}$), sexine scabrate with distinct perforations in the polar regions (Plate 6, figure 15).

3.1. Pollen key for species separation

1. Apolar, porate pollen grains
 2. Sexine psilate or scabrate
 3. Sexine psilate.....*Odontadenia perrottetii*
 3. Sexine scabrate.....*Odontadenia verrucosa*
 2. Sexine rugulate
 4. Sexine with inconspicuous rugulae, pores ca. $20.5 \times 21 \mu\text{m}$*Mandevilla scabra*
 4. Sexine with conspicuous rugulae and sparse perforations, pores ca. $6.2 \mu\text{m}$*Odontadenia puncticulosa*
1. Isopolar pollen grains
 5. Porate pollen grains
 6. Diporate pollen grains.....*Macoubea sprucei*
 6. triporate – hexaporate pollen grains
 7. Sexine rugulate or scabrate
 8. Sexine rugulate-perforated.....*Forsteronia acouci*
 8. Sexine scabrate-perforated.....*Forsteronia gracilis*
 7. Sexine psilate to scabrate
 9. Sexine psilate to scabrate at the mesoporum.....*Parahancornia fasciculata*
 9. Sexine scabrate with perforations.....*Secondatia duckei*
 5. Colporate pollen grains
 10. Solely stephanocolporate pollen grains, colpi surrounded by two thick 'cords'.....*Geissospermum argenteum*
 10. Tricolporate or tetracolporate pollen grains
 11. Tricolporate – tetracolporate pollen grains.....*Lacmellea arborescens* and *L. gracilis*
 11. Solely tricolporate pollen grains
 12. Pollen grains with an endocingulate endoaperture
 13. Sexine densely perforated.....*Tabernaemontana flavicans*

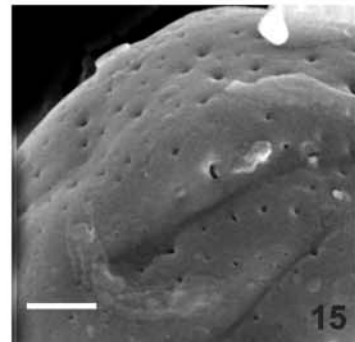
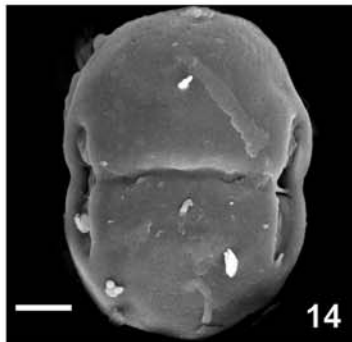
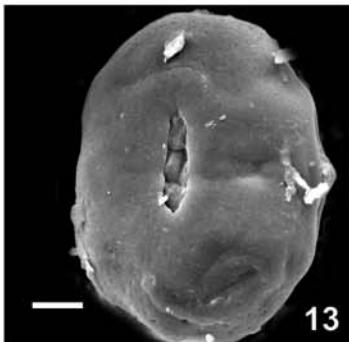
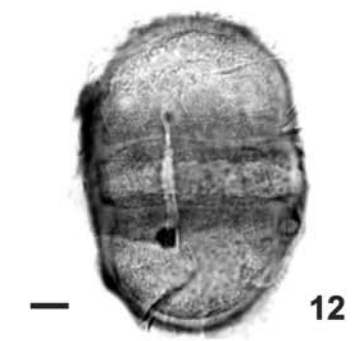
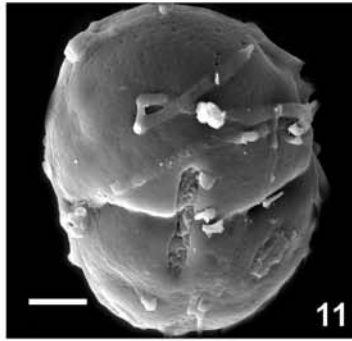
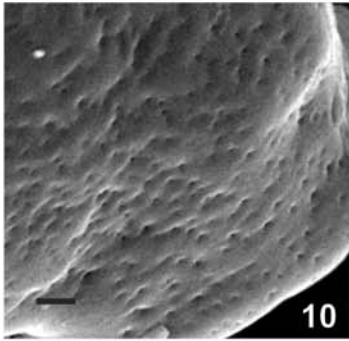
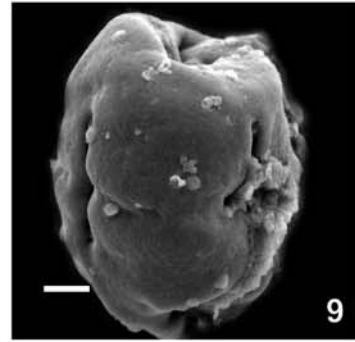
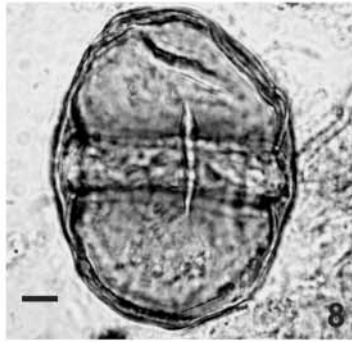
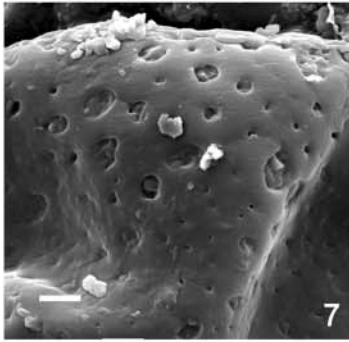
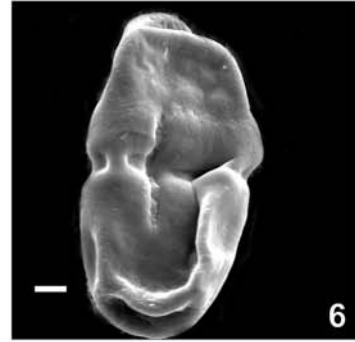
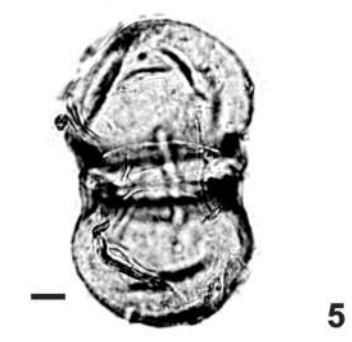
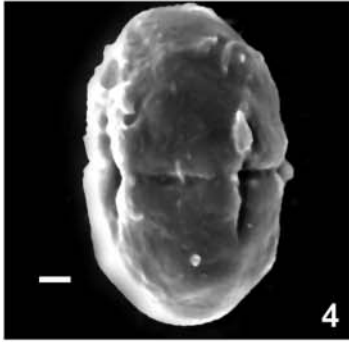
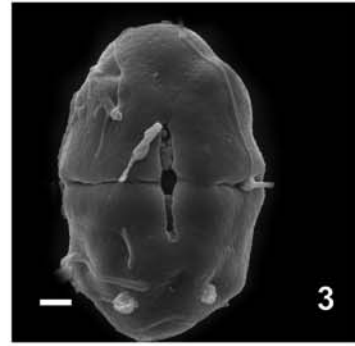
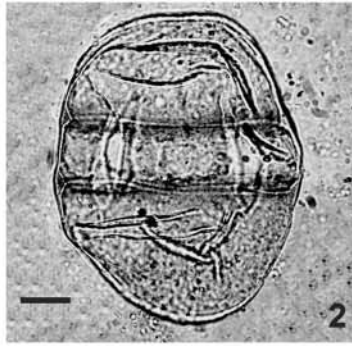
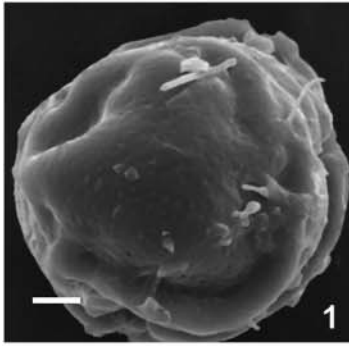
13. Sexine scabrate with perforations at the poles, or scabrate in the mesocolpium and rugulate at the poles
14. Sexine scabrate in the mesocolpium and rugulate at the poles.....*Tabernaemontana angulata*
14. Sexine scabrate with perforations at the poles.....*Tabernaemontana macrocalyx*, *Tabernaemontana muricata*, *Tabernaemontana undulata*
12. Pollen grains without endocingulate endoaperture
15. Sexine scabrate
 16. Endoaperture (ca. $3\text{--}6 \mu\text{m}$), with granulated surroundings.....*Ambelania acida*
 16. Endoaperture ($11.2 \times 6.2 \mu\text{m}$), without granulated surroundings.....*Couma guianensis*
15. Sexine perforated or psilate
 17. Sexine perforated, conspicuous perforations; in the medial mesocolpium region there is a depression with granulated ornamentation.....*Couma utilis*
 17. Sexine psilate
 18. In the mesocolpium region there is a depression with rugulate ornamentation, with perforations.....*Himatanthus bracteatus* var. *bracteatus* and *Himatanthus stenophyllus*
 18. Without a depression in the mesocolpium
 19. Colpi located in a depression formed by two wide and thick margins.....*Rauvolfia sprucei*
 19. Colpi not located in a depression
 20. Colpi with a distinct fastigium and granules in the surroundings of the aperture.....*Rhigospira quadrangularis*
 20. Colpi without a fastigium and without granules in the surroundings of the aperture.....*Himatanthus sucuuba*

4. Discussion and conclusion

In general, there was a great degree of morphological variation among the pollen grains, which were apolar or isopolar, diporate to stephano (col)porate, with ornamentation ranging from psilate, scabrate to rugulate.

Endress & Bruyns (2000) proposed the tribal classification within the subfamilies of Apocynaceae. Pollen grains of Rauvolfioideae (tribes Alstonieae, Vinceae, Willughbeieae, Tabernaemontaneae, Melodineae, Hunterieae, Plumerieae, Carisseae and Alyxieae) are usually colporate, while those of Apocynoideae (tribes Wrightieae, Malouetieae, Apocynae, Mesechiteae and Echiteae) are nearly always porate. Psilate-perforate is the most common ornamentation type (Middleton 2007). In the species

Plate 5. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Parahancornia fasciculata*: figure 1, polar view, general view (LM); figure 2, equatorial view, aperture (LM); figure 3, general view (SEM). *Rauvolfia sprucei*: figure 4, polar view, general view (LM), arrow indicates the costa; figure 5, equatorial view, general view (LM); figures 6, 7, general view (SEM). *Rhigospira quadrangularis*: figure 8, polar view, general view (LM); figure 9, general view (SEM); figure 10, equatorial view, aperture (LM). *Secondatia duckei*: figure 11, polar view, general view (LM); figure 12, equatorial view, general view (SEM); figure 13, surface detail (SEM). *Tabernaemontana angulata*: figure 14, equatorial view, general view (LM); figure 15, surface detail (SEM). Scale bars: 1–3, 6–12, 14 = 5 μm ; 4, 5 = 10 μm ; 13, 15 = 2 μm .



found in the RFAD, the great majority presented the same characters as the type of aperture and ornamentation in the subfamilies Rauvolfioideae: *Geissospermum argenteum* (colporate, scabrate), *Lacmellea arborescens* (colporate, scabrate with perforations), *L. gracilis* (colporate, scabrate with sparse perforations), *Tabernaemontana angulata*, *T. macrocalyx*, *T. muricata* and *T. undulata* (colporate, scabrate perforate), *T. flavicans* (colporate, perforate); and Apocynoideae: genera *Odontadenia* and *Secundaria* (porate) and *Mandevilla scabra* (pantoporate). According to phylogenetic studies of Endress & Bruyns (2000), Rauvolfioideae represents a basal group within the subfamilies Apocynaceae s. l. Particularities such as pollen grains usually porate and inaperturate, in most of Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae, as opposed to the brevicolporate condition in the most Rauvolfioideae, represent derived characters (Sennblad & Bremer 2002). In the RFAD, monad pollen grains of Rauvolfioideae and Apocynoideae are found. Characters such as tetrads and polynyas of the other subfamilies correspond to derived structures within Apocynaceae s. l. According to Middleton (2007), this condition correlates with the increase of floral morphological complexity within Apocynaceae s. str.; tetrads occur in only six out of the 162 genera.

According to the classification of the Apocynaceae s. l. by Endress & Bruyns (2000), the genus *Rauvolfia* is inserted into the tribe Vinceae, subfamily Rauvolfioideae. The morphology of *Rauvolfia sprucei* described in this study is not significantly different from that described by Barth & Luz (2008) with respect to polarity (isopolar), size (large; $P = 98.9$; $E = 106.8 \mu\text{m}$), amb (subcircular), number and type of apertures (tricolporate), as well as exine (psilate). However, the shape (oblate-spheroidal) and the presence of a thick margo between the colpi described in the present study were different from earlier descriptions, which reported suboblate grains lacking a significant margin between the colpi. Two species of the Colombian Caribbean have only one characteristic in common with *R. sprucei* of the RFAD, namely, a psilate sexine; however, they differ in various characteristics, such as the prolate spheroidal shape, triporate and amb circular in *R. littoralis* and subprolate shape, tetraporate – stephanoporate and amb circular in *R. tetraphylla* (García-M. et al. 2011).

The grain of *Odontadenia puncticulosa*, described by Roubik & Moreno (1991), is apolar and tetraperiporate; *O. macrantha* is apolar and diporate – triporate; while the grain of *O. puncticulosa* of RFAD is apolar and periporate (6–8 pores). In *Odontadenia geminata* (Colombian Amazon), described by Rangel Ch (2008), the grain is triporate, tetraporate – stephanoporate or periporate (5–7 pores), amb circular, suboblate, apolar or isopolar. The same species described by Carreira & Barth (2003) grains are triporate, amb circular and isopolar. The two species *O. perrottetii* and *O. verrucosa* in this study also provide similar descriptions to previous results, isopolar grains and triporate or

tetraporate for the first species and apolar and periporate (6–8 pores) for the second species. The *Odontadenia* genus studied by Marques & Melhem (1966) also presents variability in the number of pores. *Odontadenia*, belonging to the tribe Apocynaceae, subfamily Apocynoideae, from Malaysia, has monad grains, small- to medium-sized or medium-sized to large, mostly stephanoporate (3–4 pores), sometimes stephanoporate (2–5 pores), oblate to prolate. The ornamentation is psilate to scabrate, sparsely to densely perforate or imperforate (*Anodendron*, *Beaumontia*, *Elytropus*, *Odontadenia*, *Urceola*) (Middleton 2007). In the RFAD the genus *Odontadenia* has grains similar to those of Malesia, in the presence of monad grains, large, ornamentation scabrate (*O. perrottetii* and *O. verrucosa*) and pantoporate (*O. puncticulosa* and *O. verrucosa*).

For *Geissospermum argenteum*, belonging to the subfamily Rauvolfioideae, tribe Alstonieae, Nilsson et al. (1993) described the pollen grains as tricolporate, tectate and spheroidal to subprolate, whereas pollen grains from the same species collected in the Adolpho Ducke Forest Reserve were stephanocolporate, prolate-spheroidal and had a distinct margin forming ‘cords’ around the colpus. Middleton (2007) described *Geissospermum* and *Aspidosperma* with more or less prominent extracolpal ridges. Some species of *Aspidosperma* Juss. have been synonymised with *Geissospermum*, and analyses of the pollen grains from species of these two genera have revealed many similarities. Moreira et al. (2004) performed a palynological analysis of *Aspidosperma parvifolium* A. DC., and Barth & Luz (2008) examined four species of this genus, and their results are similar to those reported for *Geissospermum argenteum* in this study.

The pollen of *Lacmellea* was described by Colinvaux et al. (1999) and it is peroblate, with longate pores and amb circular, which differs from the species *Lacmellea arborescens* and *L. gracilis* from RFAD, which have prolate spheroidal and prolate grains, respectively, of amb varying circular or triangular and endoaperture circular. Rangel Ch (2008) described the grains of *L. arborescens* of Brazilian Amazonia (Rondônia) with different variations in this species, such as tricolporate, tetraporate – stephanocolporate, endoaperture longate, amb circular to convex triangular or quadrangular straight and peroblate. Middleton (2007) described the pollen species belonging to this subfamily Rauvolfioideae (tribe Willughbeieae) of Malaysia as monads, oblate spheroidal to three- or four-aperturate, sometimes porate (*Lacmellea*). The psilate-scabrate ornamentation in *Lacmellea* from Malaysia finds some correspondence to the genus of RFAD, which is scabrate with perforations. The morphological characterisation of *Tabernaemontana* pollen has subsidised phylogenetic studies and allowed the species classification of the New and Old World in the tribe Tabernaemontaneae.

Tabernaemontana belongs to the subfamily Rauvolfioideae (tribe Tabernaemontaneae) (Middleton 2007). Tabernaemontaneae

Plate 6. Light micrographs (LM) and scanning electron micrographs (SEM) of Apocynaceae. *Tabernaemontana flavicans*: figure 1, polar view, general view (SEM); figure 2, equatorial view, aperture (LM); figure 3–4, general view (SEM). *Tabernaemontana macrocalyx*: figure 5, equatorial view, aperture (LM); figure 6, general view (SEM); figure 7, surface detail (SEM). *Tabernaemontana muricata*: figure 8, equatorial view, aperture (LM); figure 9, general view (SEM), figure 10, surface detail (SEM). *Tabernaemontana undulata*: figure 11, polar view, general view (SEM); figure 12, aperture (LM); figure 13, general view, aperture (SEM); figure 14, general view, mesocolpi (SEM); figure 15, surface detail (SEM). Scale bars: 1, 3–6, 8, 9, 11–14 = 5 μm ; 2 = 10 μm ; 7, 10 = 1 μm ; 15 = 2 μm .

is considered monophyletic by the occurrence of distinctly lalongate endoapertures that might be fused to form an endocingulum, which occurs in species of the Old World. The clade *Tabernaemontana* of the Old World is supported by the occurrence of densely perforate pollen with long colpi (Van der Weide & Van der Ham 2012). *Tabernaemontana* is the largest genus in *Tabernaemontaneae* and contains both Old and New World species (Leeuwenberg 1991, 1994a, 1994b).

In *Tabernaemontana*, pollen grain size varied from large in most species to very large in *T. macrocalyx*. Zonorate grains characterise the *Tabernaemontanoideae* (Erdtman 1966). The equatorial zone was quite wide and continuous and consisted of endocingulum costate in all species from RFAD, as described by Van Campo et al. (1979) for *T. flavicans*.

The last mentioned authors characterised *T. flavicans* pollen grains as tetracolporate (solely tricolporate in the present study), similar to the present study. Moreira et al. (2004) described *T. flavicans* pollen grains as triporate – tetraporate, with a very large polar area, circular amb and endocingulate aperture; this latter characteristic has also been observed for all *Tabernaemontana* species described in the RFAD. The presence of a costate endocingulum was observed in all pollen grains from this genus. Characteristics such as a circular amb and an extremely short colpi, as described by these authors for *T. flavicans*, were also consistent with the pollen samples from the RFAD. *T. undulata* grains were brevicolporate, costate, amb circular and possessed an endocingulum, an exine with a thickness of 3 µm and a sexine with a thickness of 2 µm. Likewise, Nilsson (1990) described the same species with these characteristics, except with an exine of 2 µm. Van Campo et al. (1979) also observed tricolporate pollen grains in *T. undulata*. Species with common characteristics such as *T. submollis*, occurring in the Serra dos Carajás (Pará Amazon), are isopolar but are tetracolporate (Carreira & Barth 2003). *T. cimosa* and *T. arborea* are also tricolporate (Garcia-M et al. 2011; Roubik & Moreno 1991). In the same way, *T. cerea* has a prolate grain and short colpi, differing from the species of the RFAD in the presence of lalongate pores and aperture (tetraporate, stephanoaperturate) (Leal et al. 2011). Middleton (2007) characterised the *Tabernaemontana* pollen grains as medium sized, prolate; with short colpi (brevicolporate) and lalongate endopores, which are fused into an endocingulum (mostly with equatorial costae), similar to *Tabernaemontana* of RFAD in the following aspects: prolate or subprolate grains, short colpi and endocingulate endoapertures, differing in the size from large to very large.

Van der Weide & Van der Ham (2012) described the *Tabernaemontana* pollen with the following characteristics: small to large and suboblate to prolate, the endoapertures are lalongate to endocingulate, and the ornamentation is psilate, and perforate to imperforate. Prolate, endocingulate pollen occurs mainly in the New World species. The pollen from the five species of *Tabernaemontana* from RFAD varied from large to very large. (*T. macrocalyx*), from subprolate to prolate, endocingulate and ornamentation scabrate. Endocingulate pollen occur in 15 of 23 New World *Tabernaemontana* species. In species of the Old World long colpi occur in most Madagascan and Asian species and in two basal African species. The *T. muricata* pollen size is 73.1 µm (Van der Weide & Van der Ham 2012). In the

Tabernaemontana of RFAD the colpi are short and the size of the *T. muricata* is large (57.6 × 44.4 µm). The endocingulum can be seen as a synapomorphy of *Neotabernaemontana* (*Stemmadenia* and the New World *Tabernaemontana* species) and might be regarded as an advanced feature. *Bonafousia* (included in present study: *T. angulata*, *T. coriacea*, *T. cuspidata*, *T. disticha*, *T. flavicans*, *T. lorifera*, *T. muricata*, *T. penduliflora*, *T. rupicola*, *T. sananho*, *T. siphilitica*, *T. undulata*) and *Woytkowski* (included in *T. cuspidata*) have endocingulate pollen (class E), while *Stenosolen* (included: *T. heterophylla*) has distinctly lalongate endoapertures (class C/D). (Van der Weide & Van der Ham 2012). The same condition is found in species of *Tabernaemontana* of RFAD, which have the endocingulum of class E (Plates 5 and 6), according to the endoapertures shape classes of Van der Weide & Van der Ham (2012).

The grain of *Macoubea sprucei* from RFAD has scabrate exine, 1 µm, prolate spheroidal shape and diporate. It differs from the verrucate grain and exine of 2 µm of *Macoubea* described by Colinvaux et al. (1999), as well as of *M. guianensis* from Rio Negro (Amazon), with an oblate grain, with amb elliptical circular to triangular convex and dicolporate – tricolporate (Rangel Ch 2008). Moreover, *M. sprucei* from RFAD has the same characteristics of *M. guianensis* described by Herrera & Urrego (1996), prolate spheroidal grains and scabrate, but differing in the apertures (dicolporate). Phylogenetic studies conducted by Zaruchi (1988) included *Macoubea* genus and six other genera, emphasising *Ambelania* and *Rhigospira* that were dealt with in this study, in the tribe *Tabernaemontaneae*, forming an unstable nucleus. In other studies *Macoubea* is included in *Macoubeeae* and the other six genera in *Ambelanieae* (Pichon 1948a, 1948b; Leeuwenberg 1994a, b). On the other hand, molecular studies conducted by Simões et al. (2010) show *Macoubea* as sister to *Ambelanieae*.

The species of *Himatanthus bracteatus* var. *bracteatus*, *H. stenophyllus* and *H. sucuuba* (subfamília *Rauvolfioideae*; tribe *Plumerieae*) of RFAD have tricolporate grains, characteristic also found in this genus in the study by Marques & Melhem (1966) and for *H. articulatus* of the Colombian Amazon (Rangel Ch 2008). *H. articulatus* also has the same characteristics as in the shape (suboblate) and amb (triangular) of *H. bracteatus* var. *bracteatus* and *H. stenophyllus*. Both differ from *H. sucuuba* in the shape (oblate spheroidal) and amb (subtriangular). Middleton (2007) described the *Himatanthus* grain of Malaysia as monads, medium-sized, usually oblate spheroidal, rarely oblate or subprolate, tricolporate and ornamentation usually psilate-perforate or psilate-scabrate.

The pollen grains of *Ambelania acida*, as observed in this study, have a lalongate endoaperture (3–6 µm), a thin exine (1.4 µm) and a sexine with perforations. Variations were observed in this species, with respect to the presence of stephanocolporate grains. This species, as described by Nilsson (1990), has an exine of 3 µm, and a lalongate endoaperture measuring 4–8 µm, as well as densely perforated ornamentation. By means of phylogenetic studies Zarucchi (1988) included genus *Ambelania* and another six genera, highlighting *Macoubea* and *Rhigospira* that were dealt with in this study, in the tribe *Tabernaemontana*, forming an unstable nucleus; together with nine genera, emphasising *Tabernaemontana* (treated in this study) that are more or less stable.

The largest genus in the Apocynaceae is *Mandevilla* containing 150 species in America (Middleton 2007). Four *Mandevilla* species were analysed by Moreira et al. (2004), and our results for *M. scabra* are similar to theirs. It is also similar to grains described by Marques & Melhem (1966) as to the variability of the number of pores. *Mandevilla scabra* from RFAD has the apolar and pantoporate grain, similar characters to *M. subsagitata* and *M. villosa* grains, which are apolar and periporate (Roubik & Moreno 1991). Grains of *M. annulariifolia* (stephanoporate (4–5 pores)) and *M. hirsuta* (tetraporate) were described by Rangel Ch (2008) and Carreira & Barth (2003), respectively. Leal et al. (2011) described *M. scabra* (savannas of Guyana) as spherical and pantoporate and *M. bentharii*, spheroidal and stephanoporate (3–6 pores). Luz et al. (2007) also described *M. emarginata* and *M. pohliana* as periporate (5 pores) and periporate (4–7 pores), respectively. Pollen grains described by Middleton (2007), tribe Mesechites (Apocynoideae), were monads, small size (*Secondatia*) to very large (*Mandevilla*), oblate to subprolate, sometimes irregular (*Tintinnabularia*), triporate or tetraporate, rarely also stephanoporate – hexaporate (*Mandevilla*, *Mesechites*). With similar characters, *Mandevilla* of RFAD presents monads grains, very large size and periporate (4–6 pores).

The pollen grain of *Rhigospira quadrangulares* from RFAD is characterised by amb subtriangular, tricolporate and exine psilate, short colpi (ca. 10–14 μm), circular endoaperture, clear fastidium (Plate 5, figures 8, 9) and granules present around the apertures (Plate 5, figure 10). That same species, occurring in the savannas of Guyana, was described by Rangel Ch (2008), as tricolporate and with elliptical lalongate endoapertures and also studied by Leal et al. (2011), with samples of Colombia, as triporate, stephanoporate, and with circular pores. By means of phylogenetic studies *Rhigospira* and six more genera (*Ambelania*, *Macoubea*, *Molongum*, *Mucoa*, *Neocouma* and *Spongiosperma*) are still found unstably within the tribe Tabernaemontaneae (Rauvolfioideae), together with a more or less stable nucleus, containing nine genera (*Callichilia*, *Calocrater*, *Carvalhoa*, *Crioceras*, *Schizozygia*, *Stemmadenia*, *Tabernaemontana*, *Tabernanthe* and *Voacanga*) (Zarucchi 1988).

Parahancornia fasciculata from RFAD has prolate spheroidal shape, amb subcircular, triporate, circular pores and thin exine; while the same species from Santarém (Pará Amazon), differs in shape (suboblate), the amb (triangular convex to straight triangular) and the aperture (tricolporate) (Rangel Ch 2008). In common, these species share only the presence of thin exine.

Couma macrocarpa of the Colombian Amazon presents tricolporate, tetracolporate or stephanocolporate grains, endoaperture lalongate elliptical, amb triangular convex, spheroidal oblate (Rangel Ch 2008). It differs from the species of RFAD as to the opening (tricolporate), the shape (suboblate) and amb (subcircular) (*C. guianensis*); amb circular and circular endoaperture (*C. utilis*). *C. macrocarpa* described by Herrera & Urrego (1996) is also tricolporate.

For *Forsteronia acouci*, Nilsson et al. (1993) reported that the pollen grains are triporate – tetraporate, oblate to spheroid, and of irregular size. By contrast, the pollen grains of *F. acouci* described in the present study were tetraporate – hexaporate

and oblate spheroidal. This difference may be a common variation in this species. As in this study, the grain described of the Colombian Amazonia also presents circular pores, shape spheroidal oblate and amb circular, although with apertures varying of triporate, tetraporate, pentaporate or stephanoporate and circular pore to slightly lalongate (Rangel Ch 2008). The genus *Forsteronia*, belonging to the Apocynoideae subfamily, was in the tribe Apocynaceae; however, it was transferred to Mesechiteae (Endress et al. 2007).

The genus *Secondatia* was studied by Endress et al. (2007), who transferred it to the tribe Malouetieae, subfamily Apocynoideae. Middleton (2007) described the species of the tribe Mesechiteae, subfamily Apocynoideae and, among other characters, highlighted monad grains, sometimes small (18–33 μm ; *Secondatia*), oblate to subprolate ($P/E = 0.67–1.26$), triporate or tetraporate and ornamentation psilate-perforate, sometimes slightly scabrate (*Allomarkgrafia*, *Mandevilla*). These characteristics can be shared by *Secondatia duckei* da RFAD, as monads, small size (24.8 \times 20.6 μm), subprolate ($P/E = 1.20$), tetraporate – stephanoporate and ornamentation scabrate.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- APG. The Angiospermae Phylogeny Group III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical journal of the Linnean Society* 161:105–121.
- Barth OM, Luz CFP. 2008. Morfologia polínica das espécies arbóreas de Apocynaceae do Estado de Santa Catarina, Brasil. *Hoehnea* 35:577–582.
- Carreira LMM, Barth OM. 2003. Atlas de pólen da vegetação de Canga da Serra de Carajás (Pará, Brasil). Belém: Museu Paraense Emílio Goeldi.
- Colinvaux PA, De Oliveira PE, Moreno JE. 1999. *Amazon Pollen Manual and Atlas*. New York: Harwood Academic Press.
- Endress ME, Bruyns PV. 2000. A revised classification of Apocynaceae s. l. *Botanical Review* 66:1–56.
- Endress ME, Stevens WD. 2001. The renaissance of the Apocynaceae s.l. Recent advances in systematic phylogeny and evolution: introduction. *Ann Mo Bot Gard.* 88:517–522.
- Endress ME, Liede-Schumann S, Meve U. 2007. Advances in Apocynaceae: the enlightenment, an introduction. *Annals of the Missouri Botanical Garden* 94:259–267.
- Erdtman, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. Stockholm: Almqvist & Wiksell.
- Erdtman G. 1960. The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* 54:561–564.
- Erdtman G. 1966. Pollen morphology and plant taxonomy. Angiosperms (An Introduction to Palynology, I). New York and London: Hafner Publishing Company.
- Furness CA. 2007. Why does some pollen lack apertures? A review of inaperturate pollen in eudicots. *Botanical Journal of the Linnean Society* 155:29–48.
- Hopkins M JG. 2005. Flora da Reserva Ducke, Amazonas, Brasil. *Rodriguésia* 56:9–25.
- García-M Y, Rangel-Ch JO, Fernández D. 2011. Flora palinológica de la vegetación acuática, de pântano y de la llanura aluvial de los humedales de los Departamentos de Córdoba y Cesar (Caribe colombiano). *Caldasia* 33:573–618.
- Herrera LF, Urrego LE. 1996. Atlas de pólen de plantas útiles y cultivadas de la Amazonia colombiana. Colombia: Fundación Tropenbos.
- Hoorn C. 1993. Marine incursions and the influence of Andean tectonics on the Miocene depositional history of northwestern Amazonia: results of a palynostratigraphic study. *Palaeogeography Palaeoclimatology Palaeoecology* 105:267–309.
- Ionta GM, Judd WS. 2007. Phylogenetic relationships in Periplocoideae (Apocynaceae s. l.) and insights into the origin of pollinia. *Annals of the Missouri Botanical Garden* 94:360–375.
- Köppen W. 1948. *Climatología: con un estudio de los climas de la tierra*. México: Fondo Cultural Económico.
- Leal A, Berrio JC, Raimúndez E, Bilbao B. 2011. A pollen atlas of premontane woody and herbaceous communities from the upland savannas of Guayana, Venezuela. *Palynology* 35:226–266.
- Leeuwenberg AJM. 1991. A revision of *Tabernaemontana*: The Old World species. Kew: Royal Botanic Gardens Press.
- Leeuwenberg AJM. 1994a. A revision of *Tabernaemontana*: The New World species. Kew: Royal Botanic Gardens Press.
- Leeuwenberg AJM. 1994b. Taxa of the Apocynaceae above the genus level. Series of revisions of Apocynaceae. XXXVIII. *Agricultural University Wageningen Papers* 94:45–60.
- Luz CFP, Albanese FJ, Corrêa AMS. 2007. Flora polínica da Reserva do Parque Estadual das Fontes do Ipiranga (São Paulo, Brasil). *Hoehnea* 34:415–424.
- Marques M, Melhem TS. 1966. Pollen grains of plant of the cerrado - XI. Apocynaceae. *Anais da Academia Brasileira de Ciências* 2:371–378.
- Middleton DJ. 2007. Apocynaceae (subfamilies Rauvolfioideae and Apocynoideae). *Flora Malesiana. Series I, Volume 18, iv + 1–474*. National Herbarium Nederland, Universiteit Leiden branch, pp. 1–452.
- Moreira FF, Mendonça CBF, Pereira JF, Gonçalves-Esteves V. 2004. Palinotaxonomia de espécies de Apocynaceae ocorrentes na Restinga de Carapebus, Carapebus, Rio de Janeiro, Brasil. *Acta Botanica Brasilica* 18:711–721.
- Muller J. 1981. Fossil pollen records of extant angiosperms. *The Botanical Review* 47:1–142.
- Nilsson S. 1990. Taxonomic and evolutionary significance of pollen morphology in the Apocynaceae. *Plant Systematics and Evolution* 5:91–102.
- Nilsson S, Endress ME, Grafstrom E. 1993. On the relationship of the Apocynaceae and Periploceae. *Grana* 2:3–21.
- Pichon M. 1948a. Classification des Apocynacées: I. Carissées et Ambelaniées. *Mémoires du Muséum National d'Histoire Naturelle Serie B Botanique* 25:111–181.
- Pichon, M. 1948b. Classification des Apocynacées: IX. Rauvolfiées, Alstoniées, Allamandées et Tabernaemontanoidées. *Mémoires du Muséum National d'Histoire Naturelle Serie B Botanique* 27:152–251.
- Pires JM, Prance GT. 1985. The vegetation types of the Brazilian Amazon. In Prance GT, Lovejoy TE, editor. *Amazonia: key environments series*. Oxford: Pergamon Press; p. 108–145.
- Punt W, Hoen P, Blackmore S, Nilsson S, Le Thomas A. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143:1–81.

- Rangel Ch JO. 2008. Vegetación, Palinología y Paleocología de la Amazonia colombiana. Colômbia Diversidad Biotica VII. Bogotá DC: Universidad Nacional de Colombia.
- Rapini A. 2004. Apocynaceae: flowering plants of the neotropics. In: Smth N, Mori S, Henderson A, Stevenson D, Heald S, editors. *The New York botanical garden: Princeton University Press*; 594p.
- Rapini, A. 2012. Taxonomy "under construction": advances in the systematic of Apocynaceae, with emphasis on the Brazilian Asclepiadoideae. *Rodriguesia* 63:75–88.
- Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohmann LG, Assunção PAOL, Pereira EC, Silva CF, Mesquita MR, Procópio LC. 1999. Flora da Reserva Ducke: guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central. Amazonas: Instituto Nacional de Pesquisas da Amazônia/DFID.
- Roubik DW, Moreno JEP. 1991. Pollen and spores of Barro Colorado Island. Monographs in Systematic Botany. Panamá: Missouri Botanical Garden.
- Sennblad B, Bremer B. 2002. Classification of Apocynaceae s. l. according to a new approach combining Linnaean and phylogenetic taxonomy. *Systematic Biology* 51. 389–409.
- Silva-Caminha SAF, Jaramillo CA, Absy ML. 2010. Neogene palynology of the Solimões Basin, Brazilian Amazonia. *Palaeontographica Abteilung B* 283:1–67.
- Simões AO, Endress ME, Conti E. 2010. Systematics and character evolution of Tabernaemontaneae (Apocynaceae, Rauvolfioideae) based on molecular and morphological evidence. *Taxon* 59:772–790.
- Simões AO, Livshultz T, Conti E, Endress ME. 2007. Phylogeny and systematics of the Rauvolfioideae (Apocynaceae) based on molecular and morphological evidence. *Annals of the Missouri Botanical Garden* 94:268–297.
- Souza VC, Lorenzi H. 2005. Botânica sistemática: Guia ilustrado para identificação das famílias de Angiospermas da flora brasileira, baseado em APG II. Nova Odessa: Instituto Plantarum.
- Van Campo M, Nilsson S, Leeuwenberg AJM. 1979. Palynotaxonomic studies en Tabernaemontana L. sensu lato (Apocynaceae). *Grana* 18:5–14.
- Van Der Ham R, Zimmermann Y-M, Nilsson S, Igersheim A. 2010. Pollen morphology and phylogeny of the Alyxieae (Apocynaceae). *Grana* 40:169–191.
- Van Der Weide JC & Van der Ham RWJM. 2012. Pollen morphology and phylogeny of the tribe Tabernaemontaneae (Apocynaceae, subfamily Rauvolfioideae). *Taxon* 61:131–145.
- Verhoeven RL, Venter HJT. 1998. Pollinium structure in Periplocoideae Apocynaceae). *Grana* 37:1–14.
- Wyatt R, Lipow SR. 2007. A new explanation for the evolution of pollinia and loss of carpel fusion in Asclepias and the Apocynaceae s. l. *Annals of the Missouri Botanical Garden* 94:474–484.
- Zarucchi JL. 1988. Series of revisions of Apocynaceae XXIV. A revision of the tribe Ambelanieae (Apocynaceae-Plumerioideae). *Agricultural University Wageningen Papers* 87:1–106.