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Pollen morphology and exine ultrastructure of selected species of *Waltheria* L. (Byttnerioideae-Malvaceae)

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ABSTRACT

The pollen morphology of five species: *Waltheria albicans* Turczaninow, *Waltheria brachypetala* Turczaninow, *Waltheria cinerascens* Auguste Saint-Hilaire, *Waltheria martiana* Bentham ex J.G. Saunders, and *Waltheria viscosissima* Auguste Saint-Hilaire were studied by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), focusing on apertural type and exine. Pollen grains are medium to large, isopolar, oblate spheroidal to prolate spheroidal, 3(-4)-5(-6)-zonocolporate, costate, and fastigiate. Exine ornamentation is microreticulate, homobrochate, suprareticulate (longistyled morph), and microechinate to echinate (brevistyled morph). Sexine thickness is equal to or greater than the nexine. In TEM, the sexine consists of four layers, having an inner sexine 1, and outer sexine 4. An ultrathin section of exine revealed a columellate-like intine on pollen grains of *W. cinerascens*.

1. Introduction

Waltheria Linnaeus is a tropical and subtropical genus of Malvaceae with approximately 60 species; 53 species are found in the Americas (Saunders, 2007), and 20 are endemic to Brazil (Saunders, 1993). Currently the genus, along with *Hermannia* L., *Melochia* L., and *Dicarpidium* F. Muell., comprises the tribe Hermannieae of the subfamily Byttnerioideae (Malvaceae), characterized by flowers with five stamens and staminodes which are absent or reduced (Bayer et al., 1999).

Morphologically, the genus is characterized by a unicarpellar gynoecium, lateral, simple style, and penicillate stigma (Esteves, 1986). According to Saunders (1993), approximately 40 species of *Waltheria* exhibit distyly. Ganders (1979) defined heterostyly as a heteromorphism that is genetically controlled, composed of two (distyly) or three (tristyly) morphs that differ in the length of the style and stamens. In Malvaceae, heterostyly is common in the genera *Melochia* and *Waltheria* (Byttnerioideae), which also exhibit pollen dimorphism. The pollen grains of the brevistyle morph are microechinate, while those of the longistyle morph are suprareticulate. Pollen grains of both morphs also differ in apertural number and pollen size (Köhler, 1973, 1976; Dorr and Barnett, 1989; Miranda and Andrade, 1989; Saunders, 1993; Saba et al., 2004; Saunders and Pozner, 2007).

The pollen morphology of the *Waltheria* species has been described previously, based on observations using light microscopy (LM) by Erdtman (1952), Chaudhuri (1969), Sharma (1970), Köhler (1971, 1976), Melhem et al. (1976), Moncada and Salas (1983), Miranda and Andrade (1989), Palacios-Chávez et al. (1990, 1991), Roubik and Moreno (1991), Saunders (1993), Saba and Santos (2000, 2003), Saunders and Pozner (2007), and using scanning electron microscopy (SEM) by El Ghazali (1993) and Saba et al. (2004). No observations in transmission electron microscopy (TEM) were previously published.

In the present study, the pollen morphology of five species of *Waltheria* (*Waltheria albicans* Turcz., *Waltheria brachypetala* Turcz., *Waltheria cinerascens* A. St.-Hil., *Waltheria martiana* Benth. ex J.G. Saunders, and *Waltheria viscosissima* A .St.-Hil.) was investigated using light microscopy (LM), scanning electron microscopy (SEM), and transmission electronic microscopy (TEM), with emphasis on the differences in pollen morphology from heterostyled morphs.

2. Materials and methods

Flower buds were collected from selected herbarium specimens (CEPEC, EAC, HRB, HUEFS, and SPF, abbreviations follow *Index Herbariorum*; Thiers, B., continuously updated). Prior to treatments, flower buds were examined for heterostyly and dehydrated in pure acetic acid. Pollen grains were treated by the acetolysis method (Erdtman, 1960). After acetolysis, each sample was split into two subsamples, for observations in LM and SEM.

For LM, the pollen grains were mounted on slides with glycerin jelly. The measurement of the main morphometric parameters (equatorial and polar diameters) was made, whenever possible, for 25 pollen grains, while the other parameters (diameter of the apertures and

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Table 1
Morphometric characters of the pollen grains of *Waltheria* L. species[a].

Species/specimens	PD		ED		EDpv		P/E	Ecto	Endo	Sex	Nex
	$\bar{x} \pm S_{\bar{x}}$	R	$\bar{x} \pm S_{\bar{x}}$	R	$\bar{x} \pm S_{\bar{x}}$	R					
Waltheria albicans Turcz. (Plate I, 1-9)											
Saunders & Carvalho 3120 (CEPEC) (L)	35.1 ± 0.3	31.5–37.5	33.1 ± 0.3	30.0–36.0	33.3 ± 0.3	31.5–36.0	1.06	22.5 × 1.1	3.8	1.4	0.8
Saunders & Carvalho 3117 (CEPEC) (L)	33.9 ± 0.4	30.0–34.5	32.3 ± 0.4	28.5–34.5	34.3 ± 0.4	31.5–37.5	1.05	22.3 × 1.6	4.1	1.4	0.8
Fernandes & Nunes s.n. (EAC 8517) (B)	43.6*	39.0–48.0	48.9*	43.5–52.5	48.5*	45.0–51.0	0.89	3.5 × 3.8	–	1.4	1.2
Waltheria brachypetala Turcz. (Plate I, 10–12)											
Saunders & Carvalho 3116 (CEPEC) (L)	35.3 ± 0.3	31.5–39.0	35.2 ± 0.4	31.5–37.5	35.3 ± 0.3	31.5–37.5	1.00	21.2 × 2.5	4.2	1.2	0.6
Hatschbach & Kummrow 48064 (CEPEC) (L)	33.5 ± 0.4	30.0–37.5	35.3 ± 0.5	31.5–39.0	35.6*	30.0–39.0	0.95	18.8 × 2.4	2.8	1.2	0.6
Waltheria cinerascens A. St.-Hil. (Plate II, 13–17)											
Harley et al. 20540 (SPF) (L)	41.8 ± 0.3	39.0–45.0	42.1 ± 0.3	39.0–45.0	42.6*	40.5–43.5	0.99	25.6 × 3.6	4.5	2.0	0.6
Harley et al. 18570 (CEPEC) (L)	37.7*	36.0–40.5	37.0*	34.5–40.5	36.8*	36.0–39.0	1.01	17.2 × 1.6	3.2	1.8	1.0
Waltheria martiana Benth. ex J.G. Saunders (Plate III, 18–19)											
Harley 16683 (CEPEC) (L)	49.5*	46.5–54.0	47.8*	45.0–54.0	46.0*	43.5–48.0	1.03	31.7 × 2.3	5.3	1.0	1.0
Queiroz & Nascimento 3480 (HUEFS) (B)	58.1*	54.0–63.0	59.8*	57.0–66.0	60.0*	55.5–64.5	0.97	–	–	1.0	1.0
Waltheria viscosissima A. St.-Hil. (Plate III, 20–26)											
Hatschbach & Kummrow 48065 (CEPEC) (L)	40.1 ± 0.5	34.5–42.7	36.4 ± 0.5	31.5–39.0	35.4*	32.3–40.5	1.10	15.2 × 2.5	5.6	1.0	0.6
Mattos-Silva et al. 2738 (HUEFS) (L)	36.7*	33.0–42.0	35.6*	33.0–39.0	34.0*	31.5–36.0	1.03	16.0 × 1.0	4.0	1.4	1.0
Longa & Campos 3 (HRB) (B)	43.5 ± 0.6	36.0–46.5	48.2 ± 0.6	39.0–52.5	46.8 ± 0.6	37.5–51.0	0.90	–	3.5	1.9	0.6

[a] PD = polar diameter; ED = equatorial diameter; EDpv = equatorial diameter in polar view; Ecto = length × width of the ectoaperture; Endo = height of the endoaperture; Sex = sexine; Nex = nexine; $\bar{x}$ = arithmetic mean; **s** = medium standard; R = range variation; L = longistyle morph; B = brevistyle morph; *$n < 25$; measurements in μm and indices in absolute numbers.

exine thickness, sexine, and nexine) were measured in ten randomly selected pollen grains.

For SEM, acetolyzed pollen grains were rinsed in an ethanol series up to 100%, pipetted onto specimen stubs. The specimens were air dried, coated with gold, and photographed with a Zeiss LEO 1430 VP microscope (SEM Lab, Biological Sciences Department, Universidade Estadual de Feira de Santana).

For TEM, the most commonly widespread species were selected. In this case, a longistyled morph of *W. cinerascens* was used. Closed, mature anthers were fixed for 48 h in glutaraldehyde (2.5%) solution in 0.1 M sodium phosphate, pH 7.4. The material was post-fixed in osmium tetroxide (OsO_4) dissolved in 1% buffered solution, dehydrated in ascending acetonic series, and then infiltrated in Epon resin. The ultrathin sections were obtained using an ultramicrotome, placed on copper grids and contrasted with uranyl acetate and 7% aqueous lead citrate. The samples were then analyzed on a Zeiss M 109 (Platform for Electron Microscopy, Gonçalo Moniz Research Center—FIOCRUZ).

The pollen terminology follows Punt et al. (2007).

3. Results and discussion

In this study, brevistyled and longistyled specimens were investigated of *Waltheria albicans*, *W. martiniana*, and *W. viscosissima*, and only longistyled specimens of *W. brachypetala* and *W. cinerascens*, according to the vouchers available in herbaria. The analyses of pollen developed in this study have confirmed the data found in the literature about pollen dimorphism between heterostyle species *Waltheria*.

All *Waltheria* species generally have medium to large-sized pollen grains (Table 1), oblate spheroidal to prolate spheroidal, amb subcircular to polygonal. Pollen grains are angulaperturate, with 3(-4)-5(-6) apertures (colporus). Exine is microreticulate, suprareticulate in longistyled morph, and microechinate to echinate in brevistyled morph (Plates I–III, Table 2). Sexine has the same thickness as that of the nexine or it is greater. In TEM, the sexine consists of four layers (sexines 1, 2, 3, 4), and intine exhibits a columellate-like structure.

Pollen diameters of the brevistyled morphs are comparatively larger compared to specimens of the longistyled morphs (Table 1), confirming previous observations for heterostyled species (Köhler, 1973, 1976; Miranda and Andrade, 1989; Saunders, 1993; Saba et al., 2004; Saunders and Pozner, 2007).

3.1. Aperture

Waltheria exhibits a colporate apertural system. In the pollen of longistyled morphs, the ectoapertures are elongated with tapered ends (Plate I, 3; Plate III, 18), while in the brevistyled morphs, the ectoapertures are considerably shorter, narrower, and slightly larger than the endoaperture (Plate I, 9; Plate III, 25; Table 1). The endoapertures can be both lalongate to circular. The lalongate form has upper and lower parallel margins, with indistinct equatorial ends.

Apertural heteromorphism was observed in all species studied (within the same sample). However, variation is greater in the pollen of brevistyled morphs, which have more apertures (4–6) compared to those of the longistyled morph (3) of the same species (Table 2). Pollen grains with three and five apertures are more predominant. In pollen grains with more than three apertures, the apertures are arranged in a sinuous line in equatorial view (Plate I, 6).

In some pollen grains, there is a separation of the layers of the exine in the apertural region (Plate I, 1), forming a fastigium. There are reports of the presence of annuli in pollen apertures of brevistyled morphs of *W. viscosissima* (Miranda and Andrade, 1989) and *W. belizensis* (Saunders and Pozner, 2007). There is certainly a terminological confusion since an annulus is a pollen feature restricted to a porus (Punt et al., 2007). The thickening corresponds to the costa (Plate I, 6 and 12, Plate II, 15)

Plate I. Pollen grains of *Waltheria* L. (see on page 206)

1–4 *W. albicans* Turcz. (longistylous): 1—Polar view (optical section). 2—LO analysis. 3—General view (SEM). 4—Surface (SEM).
5–9 *W. albicans* Turcz. (brevistylous): 5—Polar view (optical section). 6—Equatorial view (optical section). 7—LO analysis. 8—Surface (SEM). 9—Aperture detail (SEM).
10–12 *W. brachypetala* Turcz. (longistylous): 10—Polar view (optical section). 11—LO analysis. 12—Equatorial view (optical section).

Plate II. Pollen grains of *Waltheria* L. (see on page 207)

13-17 *W. cinerascens* A. St.-Hil. (longistylous): 13—Polar view (optical section). 14—LO analysis. 15—Equatorial view (optical section). 16—Radial section across apertural region (TEM). 17—Radial section across mesocolpium (TEM). (In = intine; S1 = sexine 1; S2 = sexine 2; S3 = sexine 3; S4 = sexine 4).