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Pollen morphology of some Cuban *Guettarda* species (Rubiaceae: Guettardeae)

LÁZARA SOTOLONGO MOLINA, MAIRA FERNÁNDEZ ZEQUEIRA and PEDRO HERRERA OLIVER

Sotolongo Molina, L., Fernández Zequeira, M. & Herrera Oliver, P. 2002. Pollen morphology of some Cuban *Guettarda* species (Rubiaceae: Guettardeae). – Grana 41: 142–148.

Pollen grains of 19 taxa of the genus *Guettarda* (Rubiaceae: Guettardeae) were studied using light microscopy, scanning electron microscopy (9 species), and fractured pollen grains (7 species). Most of the species have suboblate to oblate spheroidal, small to medium-sized grains with reticulate, microreticulate, perforate or foveolate exine. There is a great variability in the morphology of ectoapertures and endoapertures. Three pollen types are established, mainly based on differences in ectoapertures and sexine ornamentation.

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The genus *Guettarda* L. (Cuban vernacular name: “cuero”) has over 85 species distributed in all tropical regions but mostly neotropical and notably abundant in the Antilles (Alain 1964). There are 34 species in Cuba, 85% of which are endemic (Borhidi et al. 1977). Being an extremely variable and heterogeneous group from a morphological standpoint, a palynologic survey becomes necessary for clarifying both taxonomic position and relations.

Using optic microscopy, pollen morphology of one species not found in Cuba has been described by Mathew & Philip (1983) who characterized *Guettarda speciosa* as 4/5 zonoporate, aspidate, with foveolate tectum. Roubik & Moreno (1991) described pollen of *Guettarda foliacea* as inaperturate, reticulate, whereas Jung-Mendaçolli & Melhem (1994) refer the pollen of *Guettarda viburnoides* as oblate spheroidal, 2/3/4-porate, aspidate, with reticulate tectum.

Palacios-Chavéz et al. (1991) made a morphologic description of pollen of two species, which are quite common in Cuba, *Guettarda combsii* and *Guettarda elliptica* finding differences between them with reference to the aperture and ornamentation of exine.

This is the first paper where the pollen morphology of Cuban *Guettarda* species is described.

MATERIAL AND METHODS

The study was based on 19 species of the genus *Guettarda*. Pollen was obtained from the Herbarium (HAC) of the Institute of Ecology and Systematics.

For light microscopy (LM), the material was acetolyzed (Erdtman 1954, 1969; Nilsson & Praglowski 1992) and mounted in glycerine jelly. An Olympus BH-2 microscope was used for observation. 10 (?) pollen grains of each sample were measured.

For scanning electron microscopy (SEM), acetolyzed material was dehydrated in a series of ethanol, mounted on film and coated with gold palladium. Photomicrographs were obtained with a Jeol JSM 25 S-II electron microscope.

The material studied is deposited in the actuopalyntotheca of the

Institute of Ecology and Systematics (Havana, Cuba) and the Palynological Laboratory of the Swedish Museum of Natural History (Stockholm, Sweden).

The terminology is basically in accordance with Punt et al. (1994) and Vezey & Skvarla (1994).

RESULTS

General features

Pollen grains 3-colporate or 3-pororate, small to medium size. Suboblate or oblate spheroidal; polar outline (amb) circular, equatorial outline elliptical. Sexine microreticulate, reticulate, microperforate, perforate or foveolate.

Pollen types

Type I (Figs. 1–4)

Pollen grains 3-colporate, small to medium in size. P: 22–(26.5)–31 µm; E: 26–(31.0)–37 µm. Suboblate; polar outline (amb) circular (Fig. 1); equatorial outline elliptical.

Ectocolpus relatively long (*G. blodgettii*) to very short (*G. brevinodis*). Endoaperture is an almost circular pore, with costa endopori.

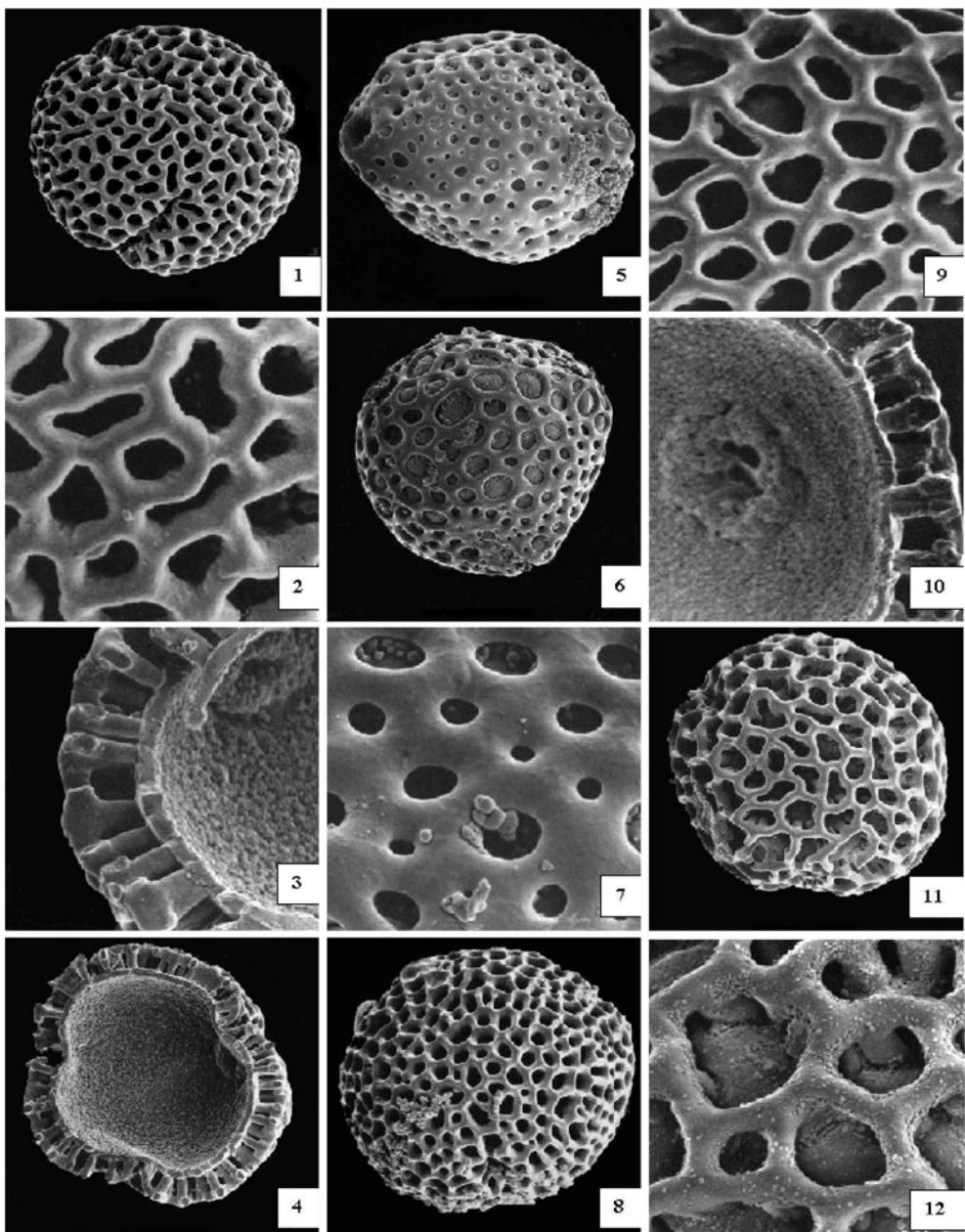
Exine 3.0–4.3 µm thick; sexine semitectate-reticulate, heterobrochate (Fig. 2); lumina 1.0–5.0 µm in diameter; muri 1.0–2.0 µm wide, simplicolumellate; nexine thickened around endoapertures.

Species examined: *G. blodgettii*, *G. brevinodis*.

G. blodgettii (Figs. 1–4). – Pollen grains 3-colporate, small to medium in size. P: 25–(26.7)–30 µm; E: 30–(32.1)–35 µm. Suboblate; polar outline circular; equatorial outline elliptical.

Ectocolpus relatively long (Fig. 1). Endoaperture is a pore almost circular in shape with costa endopori; granules present on endoporus (Fig. 3).

Exine 3.0–4.3 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 1.0–5.0 µm in diameter, irregular



Figs. 1–4. Pollen of *G. blodgettii*: (1) oblique polar view, 3-colporate, reticulate pollen grain, $\times 1600$; (2) detail of reticulate exine, $\times 4800$; (3) Inside of pollen grain with granular nexine and endoporus with costa, $\times 4800$; (4) Cross-section of exine showing relatively long columellae, $\times 1650$. Pollen type I.

Figs. 5–7. Pollen of *G. ambigua*: (5) equatorial view, 3-pororate reticulate pollen grain, $\times 1350$; (6) polar view, showing smooth muri and numerous processes in the lumina, $\times 1400$; (7) detail of perforate-foveolate exine, $\times 6000$. Pollen type II.

Figs. 8–10. Pollen of *G. calcicola*: (8) polar view, 3-pororate, reticulate pollen grain, $\times 2000$; (9) Detail of reticulate exine, $\times 5600$; (10) Inside of pollen grain with granular nexine, endoporus with costa and cross section of pollen wall, $\times 5200$. Pollen type II.

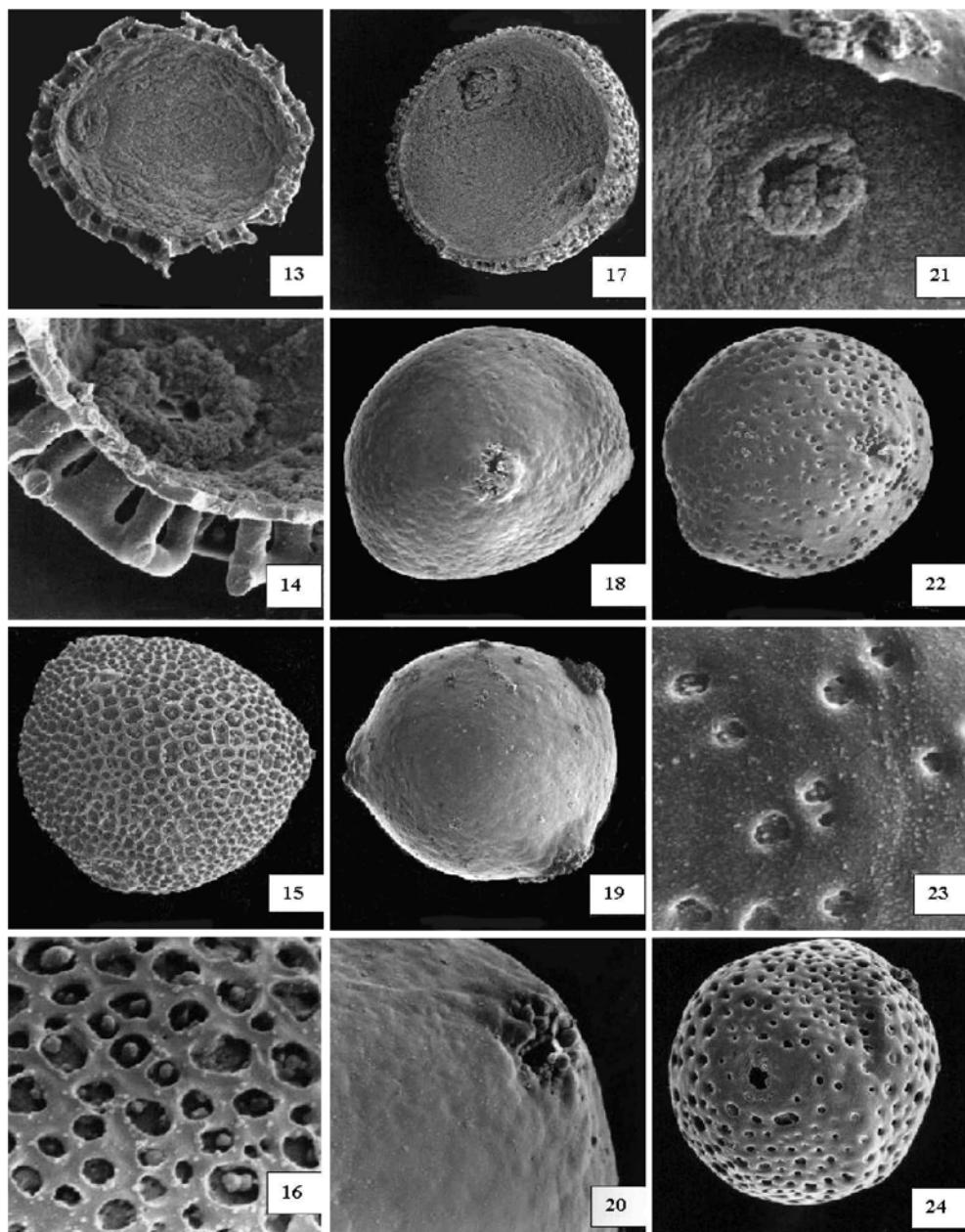
Figs. 11, 12. *G. elegans*: (11) polar view, 3-pororate, reticulate pollen grain, $\times 1350$; (12) detail of reticulate exine, muri beset with granules on the surface, $\times 4200$; Pollen type II.

in shape; muri 1.0–2.0 μm , simplicolumellate, with smooth upper surface; inner nexine granular (Figs. 3, 4), thickened around endoapertures.

G. brevinodis. — Pollen grains 3-colporate, small to medium in size. P: 22–(24.6)–26 μm ; E 27–(28.6)–30 μm . Sub-oblance; polar outline circular; equatorial outline elliptical.

Ectocolpus is very short. Endoaperture is an almost circular pore, with costa endopori.

Exine 3.0–4.0 μm thick; sexine semitectate-reticulate, heterobrochate; lumina 1.4–2.9 μm in diameter, irregular in shape; muri 1.0 μm , simplicolumellate, columella diameter <1.0 μm ; nexine thickened around endoapertures.



Figs. 13, 14. *G. elegans*: (13) inside of pollen grain showing granular nexine, $\times 1450$; (14) cross section of exine showing endoporus with costa and long columellae, $\times 4800$. Pollen type II.

Figs. 15–17. *G. crassipes*: (15) polar view, 3-pororate pollen grain, microreticulate ornamentation, $\times 1450$; (16) detail of perforate-foveolate exine, lumina with numerous processes and muri with granules, $\times 4800$; (17) Inside of pollen grain showing granular nexine and endoporus with costa, $\times 1400$. Pollen type II.

Figs. 18–21. *G. calyptata*: (18) equatorial view 3-pororate pollen grains, with a few perforations, $\times 1700$; (19) polar view, $\times 1400$; (20) detail of exine and ectoaperture, $\times 4200$; (21) Inside of pollen grain with granular nexine and endoporus with costa, $\times 3200$. Pollen type III.

Figs. 22, 23. *G. ferruginea*: (22) equatorial view, 3-pororate, perforate pollen grains, $\times 1200$; (23) Detail of exine with perforations, $\times 4800$. Pollen type III.

Fig. 24. *G. monocarpa*. Equatorial view, 3-pororate, perforate pollen grain, $\times 1100$. Pollen type III.

Type II (Figs. 5–17)

Pollen grains 3-pororate, small to medium in size. P: 21–(30.3)–41 μm ; E: 24–(34.1)–46 μm . Suboblate to oblate-spheroidal; polar outline (amb) circular (Figs. 6, 8, 11 & 15); equatorial outline elliptical.

Ectoporus diameter 1.9–5.4 μm , circular in shape. Endoaperture is a circular pore.

Exine 1.0–4.3 μm thick; sexine semitectate-reticulate or microreticulate, heterobrochate (Figs. 6, 9, 12 & 16); lumina 1.3–2.3 μm in diameter, irregular in shape; muri simplicolumellate; nexine thickened around endoapertures.

Included species: *G. ambigua*, *G. calcicola*, *G. camagueyensis*, *G. coxiana*, *G. crassipes*, *G. elegans*, *G. elegans*, *G. lindeniana*, *G. nervosa*, *G. rigida* & *G. sciaphila*.

G. ambigua (Figs. 5–7). – Pollen grains 3-pororate; of medium size. P: 30–(35.2)–40 µm; E: 34–(39.1)–46 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus 2.1–2.7 µm in diameter, almost circular in shape. Endoaperture is a circular pore, with costa endopori.

Exine 2.3–2.7 thick; sexine semitectate-reticulate, heterobrochate; lumina 0.3–2.3 µm in diameter, circular-elliptical in shape (Fig. 7), the bottom of lumina has numerous processes (Fig. 6); muri 1.0–2.0 µm, simplicolumellate, with smooth upper surface; nexine thickened around endoapertures.

G. calcicola (Figs. 8–10). – Pollen grains 3-pororate; small to medium in size. P: 24–(25.4)–27 µm; E: 27–(30.1)–32 µm. Suboblate; polar outline circular; equatorial outline elliptical.

Ectoporus diameter 1.9 µm, circular in shape. Endoaperture is a circular pore; costa endopori; granules present on endoporus (Fig. 10).

Exine 2.1–2.6 µm thick; sexine semitectate-reticulate, heterobrochate; endexine granular (Fig. 10); lumina 0.5–2.0 µm in diameter, irregular in shape; muri 0.7 µm, simplicolumellate, with smooth upper surface (Fig. 9); nexine thickened around endoapertures.

G. camagueyensis. – Pollen grains 3-pororate, medium size. P: 32–(34.7)–41 µm; E: 37–(39.8)–44 µm. Suboblate; polar outline circular; equatorial outline elliptical.

Ectoporus diameter 2.5–2.7 µm, circular in shape. Endoaperture is a circular pore.

Exine <1.0 µm thick; sexine semitectate-microreticulate, heterobrochate; lumina 0.3–0.4 µm in diameter, irregular in shape; muri simplicolumellate; nexine thickened around endoapertures.

G. coxiana. – Pollen grains 3-pororate, small to medium in size. P: 23–(24.7)–26 µm; E: 27–(30.1)–32 µm. Suboblate; polar outline circular; equatorial outline elliptical.

Ectoporus circular in shape. Endoaperture is a circular pore, with costa endopori.

Exine 3.0–3.2 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 1.2–1.7 µm in diameter; muri 2.0 µm, simplicolumellate; nexine thickened around endoapertures.

G. crassipes (Figs. 15–17). – Pollen grains 3-pororate, medium size. P: 32–(36.1)–40 µm; E: 37–(39.6)–44 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus is a circular pore; costa endopori; granules present on endoporus (Fig. 17).

Exine 1.0–2.0 µm thick; sexine semitectate-microreticulate, heterobrochate; lumina 0.3–1.3 µm in diameter, irregular in shape; the bottom of lumina with numerous processes (Figs. 15, 16); muri simplicolumellate, with granules on surface (Fig. 16); nexine granular (Fig. 17), thickened around endoapertures.

G. elegans (Figs. 11–14). – Pollen grains 3-pororate; medium size. P: 31–(34.7)–39 µm; E: 36–(38.6)–41 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus circular in shape. Endoaperture is a circular pore; costa endopori; granules present on endoporus (Fig. 14).

Exine 3.0–4.3 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 1.0–5.0 µm in diameter; muri 1.0–2.0 µm, simplicolumellate, with granules on surface (Fig. 12); granular inner nexine (Fig. 13), thickened around endoapertures.

G. lindeniana. – Pollen grains 3-pororate; medium size. P: 29–(32.2)–37 µm; E: 32–(34.1)–39 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus circular in shape. Endoaperture is a circular pore; costa endopori.

Exine 3.0–4.0 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 2.7–3.8 µm in diameter, with numerous processes; muri 1.3–1.6 µm, simplicolumellate; nexine thickened around endoapertures.

G. nervosa. – Pollen grains 3-pororate; small to medium in size. P: 21–(24.1)–26 µm; E: 24–(28.0)–30 µm. Suboblate; polar outline circular; equatorial outline elliptical.

Ectoporus circular in shape. Endoaperture is a circular pore; costa endopori.

Exine 2.0–2.9 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 0.5–1.2 µm in diameter; muri 1.3 µm, simplicolumellate, columellae diameter 1.0 µm; nexine thickened around endoapertures.

G. rigida. – Pollen grains 3-pororate; small to medium in size. P: 24–(24.7)–26 µm; E: 26–(27.4)–30 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical. Ectoporus circular in shape. Endoaperture is a circular pore; costa endopori.

Exine <2.0 µm thick; sexine semitectate-reticulum, heterobrochate; lumina 0.8–1.7 µm in diameter; muri 1.2 µm, simplicolumellate, columellae diameter <1.0 µm; nexine thickened around endoapertures.

G. sciaphila. – Pollen grains 3-pororate; medium size. P: 26–(28.7)–32 µm; E: 29–(32.0)–35.0 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus circular in shape. Endoaperture is a circular pore; costa endopori.

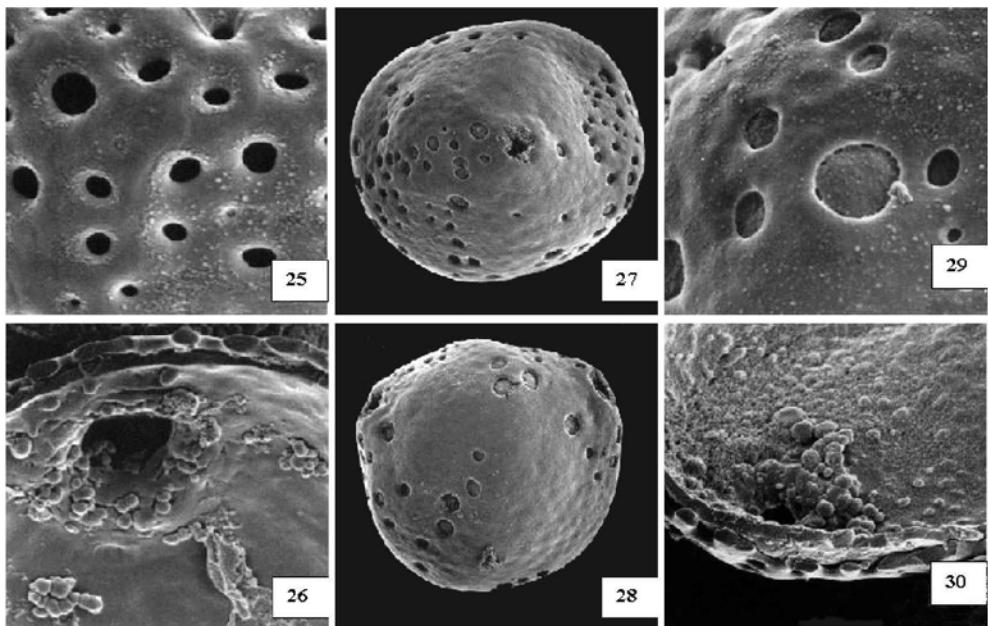
Exine <2.0 µm thick; sexine semitectate-reticulate, heterobrochate; lumina 0.8–1.5 µm in diameter, circular, oval, quadrangular; muri 0.6–1.2 µm, simplicolumellate, columellae diameter 1.0 µm; nexine thickened around endoapertures.

Type III (Figs. 18–30)

Pollen grains 3-pororate; small to medium in size. P: 22–(29.6)–36 µm; E: 27–(39.7)–43 µm. Suboblate or oblate-spheroidal; polar outline (amb) circular (Figs. 19 & 28); equatorial outline elliptical (Figs. 18, 22, 24 & 27).

Ectoporus is a pore circular in shape. Endoporus circular in shape; costa endopori.

Exine 1.0–2.0 µm thick; sexine tectate-psilate or perforate to foveolate; nexine thickened around endoapertures.



Figs. 25, 26. *G. monocarpa*: (25) Detail of perforate-foveolate exine, $\times 6000$; (26) Inside of pollen grains with clava-like processes, note the smooth inner nexine surface and absence of columellae layer, $\times 4500$. Pollen type III.

Figs. 27–30. *G. combsii* (27) Equatorial view, 3-pororate, foveolate pollen grains, $\times 1300$; (28) Polar view, 3-pororate pollen grains, apoporum, 1200; (29) Detail of perforate-foveolate exine, $\times 5200$; (30) Inside of pollen grains with granular nexine and endoaperture, note the absence of columellae, $\times 3000$. Pollen type III.

Included species: *G. calyprata*, *G. cueroensis*, *G. combsii*, *G. densiflora*, *G. ferruginea*, *G. monocarpa*, *G. valenzuelana*.

G. calyprata (Figs. 18–21). – Pollen grains 3-pororate; medium size. P: 25–(29.0)–34 μm ; E: 30–(32.1)–36 μm . Oblate-spheroidal; polar outline circular (Fig. 19); equatorial outline elliptical (Fig. 18).

Ectoaperture is a pore, circular in shape, diameter 2.1–2.7 μm ; with clavae around ectoporus (Figs. 18–20). Endopori circular in shape; costa endopori; granules present on endoporus (Fig. 21).

Exine 1.3–1.5 μm thick; sexine tectate-microperforate, a few perforations on surface; perforations diameter 0.2–0.3 μm (Fig. 20); granular inner nexine (Fig. 21) thickened around endoapertures.

G. cueroensis. – Pollen grains 3-pororate; small to medium in size. P: 22–(25.8)–29 μm ; E: 29–(30.3)–33 μm . Suboblate; polar outline circular; equatorial outline elliptical. Ectoaperture is a pore circular in shape. Endopori circular in shape; with costa endopori.

Exine 0.7–0.9 μm thick, sexine tectate-perforate, perforations diameter 0.2–0.6 μm ; nexine thickened around endoapertures.

G. combsii (Figs. 27–30). – Pollen grains 3-pororate; medium size. P: 31–(33.0)–36 μm ; E: 35–(37.6)–40 μm . Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus diameter 2.0–2.5 μm , circular in shape (Fig. 27). Endoporus circular in shape, costa endopori, and clavae around apertures (Fig. 30).

Exine 1.0–1.5 μm thick; sexine tectate-foveolate; foveolae membrane diameter 0.6–3.8 μm , becoming fewer near the apoporum (Figs. 28, 29); granular inner nexine (Fig. 30) thickened around endoapertures.

G. densiflora. – Pollen grains 3-pororate; small or medium in size. P: 22–(25.9)–30 μm ; E: 27–(30.2)–34 μm . Suboblate; polar outline circular; equatorial outline elliptical.

Ectoaperture is a circular pore, with diameter <2.0 μm . Endopori circular in shape, with costa endopori.

Exine <1.0 μm thick; sexine tectate-perforate, perforations diameter 0.1–0.6 μm ; nexine thickened around endoapertures.

G. ferruginea (Figs. 22, 23). – Pollen grains 3-pororate; medium size. P: 28–(33.2)–34 μm ; E: 32–(34.8)–38 μm . Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoaperture is a circular pore, with clavae around ectoporus (Fig. 22). Endopori circular in shape, with costa endopori.

Exine 1.0–1.6 μm thick; sexine tectate-perforate, perforations diameter 0.1–0.8 μm (Fig. 23); nexine thickened around endoapertures.

G. monocarpa (Figs. 24–26). – Pollen grains 3-pororate; medium size. P: 30–(33.1)–35 μm ; E: 33–(37.1)–42 μm . Oblate-spheroidal; polar outline circular; equatorial outline elliptical.

Ectoporus is a pore, circular in shape. Endopori circular in shape, costa endopori with clavae (Fig. 26).

Exine 1.0–2.0 μm thick; sexine tectate-perforate, perforations

diameter 0.3–0.8 µm (Fig. 25); granular inner nexine (Fig. 26) thickened around endoapertures.

G. valenzuelana. – Pollen grains 3-pororate; medium size. P: 27–(31.0)–35 µm; E: 35–(35.3)–43 µm. Oblate-spheroidal; polar outline circular; equatorial outline elliptical. Ectoporus diameter 1.9–3.6 µm, circular in shape. Endoporus circular in shape, with costa endopori.

Exine 1.0–1.4 µm thick; sexine tectate-foveolate, foveolae diameter 0.3–3.0 µm; nexine thickened around endoapertures.

DISCUSSION AND CONCLUSIONS

There is considerable variation in the pollen morphology of the species investigated, which has been grouped here into three pollen types. The differences mainly include ectoaperture and sexine ornamentation.

Our results do not agree with Mathew & Philip (1983) who characterised the pollen of *Guettarda* as homogeneous.

Variations in exine thickness and columellae size were observed in all types. Large columellae were found in types I, II, except in *G. camagueyensis* and *G. crassipes* where a reduction in size was seen. In type III, there is a considerable reduction in exine thickness and columellae are completely absent.

Concerning evolutionary trends in apertures, type I (3-colporate) is regarded as less advanced than types II and III (3-pororate). However, in tectum ornamentation type I (semitectate-reticulate) is the most advanced since types II and III not only may have semitectate-reticulate ornamentation but also may have tectate-perforate and tectate-foveolate tecta. Therefore, a transformation and evolution of pollen grain characteristics is present in all species under study though in most cases the ornamented tecta remains unchanged, suggesting that evolution has taken place without any change in pollinators (entomophilous), a hypothesis that needs further testing.

Our results agree with those of some other researchers (Van Campo 1976, Mathew & Philip 1983, and Pire & Cabral 1996), who have proposed this kind of evolutionary pattern for Rubiaceae, in general.

Advanced traits were evident in inflorescences of some species such as small flowers, few-flowered, axillare inflorescences and reduction in number and size of floral parts while others are less advanced in having many-flowered inflorescences and large, glabrous flowers. However, advanced and less advanced species according to pollen type can have both types of inflorescences. Environmental conditions seem to have a definite influence on inflorescence transformation; on the other hand, the genus *Guettarda* has many endemic species, which include occurrence on serpentine on barren and limestone rock dwellers, adapted to hydric-stressed habitats though some thriving on more mesophytic habitats such as humid forests.

Regarding that the Cuban Guettardae are only 34 species out of more than 85 species distributed in all tropical regions and that our study comprised just 20 Cuban species (58,8%), we strongly recommend research to be continued in a larger scale so as to reach more ample conclusions on this genus.

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SPECIMENS EXAMINED

- Guettarda ambigua* Chapm. CUBA: Cienfuegos. Sabana Antón Recio, Fecha. 22/06/1932. Col. Hno. León 15599. LS.
- G. blodgettii* Shultz. ex Chapm. CUBA: Santiago de Cuba. Manigua costera Aeropuerto Santiago de Cuba. Fecha 09–10/04/1954. Col. López Figueiras 1206. SV.
- G. brevinodis* Urb. (endemic). CUBA: Pinar del Río. Camino El Ancón, Viñales. Fecha 19/05/1955. Col. Hno Alain 4267. LS.
- G. calcicola* Britt. (endemic). CUBA: Pinar del Río. Mogote de la Bandera, Viñales. Fecha 29/03/1953. Col. Hno. Alain 6887. LS.
- G. calyprata* A. Rich. (endemic). CUBA: Pinar del Río. Ensenada de La Bandera, Viñales. Fecha 30/03/1953. Col. Hno. Alain 2888. LS.
- G. camagueyensis* Britt. (endemic). CUBA: Camaguey. Sabanas serpentinas de Camaguey. Fecha 04/1966. Col. M. Yero 782. SV.
- G. combsii* Urb CUBA: Manigua cerca de La Habana. Fecha 07/1919. Col. Hnos. León y Edmundo 8803. LS.
- G. coxiana* Britt. (endemic). CUBA: Oriente. Playa Barracos. Santiago de Cuba, Fecha 18/04/1954. Col. López Figueiras 1344. SV.
- G. crassipes* Britt. (endemic). CUBA: Oriente. Pinares de Moa., Fecha 27/03/1942. Col. Hnos. León y Clemente. 20761. LS.
- G. cueroensis* Britt. (endemic). CUBA: Oriente. Camino del Morro, Santiago de Cuba. Fecha. 11/19/43. Col. Hno. Clemente. 3144. LS.
- G. densiflora* Urb. (endemic). CUBA: Oriente. Loma Mensura, Sierra de Nipe. Fecha. 21/04/1960. Col. Hno. Alain, Acuña y Ramos 7988. SV.
- G. elegans* Urb. (endemic). CUBA: Oriente. Pico Turquino. Fecha 07/1922. Col. Hno. León 10814. LS.
- G. ferruginea* Wr. ex Griseb. (endemic). CUBA: Oriente. Lengua de Pájaro, Nicaro, Mayarí. Fecha. 03/1943. Col. Van Herman. 24633. SV.
- G. lindeniana* A. Rich. CUBA: Oriente. Baracoa. Fecha. 07/1936. Col. C. Bucher 10407. SV.
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