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## The metareticulate pollen morphology of *Alternanthera* Forssk. (Gomphrenoideae, Amaranthaceae) and its taxonomic implications

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### Abstract

*Alternanthera* (Amaranthaceae) is a diverse genus largely restricted to the American Tropics that belongs to the alternantheroid clade containing C<sub>4</sub> and C<sub>3</sub>–C<sub>4</sub> intermediate species. This research focuses on the study of pollen characters by studying 13 species, representatives of the two major clades and subclades of *Alternanthera*. General palynological comparisons were conducted with light microscopy (LM), scanning electron microscopy (SEM) and with confocal laser scanning microscopy (CLSM) for exine ultrastructure. Twenty-five characters were measured and described for *Alternanthera* and among these, 14 pollen characters were used to discriminate pollen groups using cluster analysis and canonical analysis of principal coordinates (CAP). Pollen form and ornamentation, pores number, spines length, number of ektexinous bodies and nanospines on the ektexinous bodies on pore membranes, arrangement of nanopores and spines on structural elements, and metareticula form were taxonomically important and therefore used to construct the first palynological key to the alternantheroid clade species. Our study indicates that the seemingly subtle morphological variation of pollen is useful for recognising three main pollen types within *Alternanthera*. The much needed palynological terminology for describing the mesoporum in the metareticulate pollen of Amaranthaceae is provided.

**Keywords:** *alternantheroids, mesoporia, pollen morphology, scanning electron microscopy, confocal laser scanning microscopy*

*Alternanthera* includes 80–200 species (Mears 1977; Robertson 1981; Eliasson 1987, 1990; Townsend 1993; Siqueira 2004; Sánchez-del Pino et al. 2012) and is the third or fourth most diverse genus in the family Amaranthaceae. The genus is Neotropical (Townsend 1993) with the greatest centres of diversity in South America (Mears 1977), India, Pakistan and China (Chin & Lim 2011) in order of relevance. Thirteen indigenous species (nine endemics) are distributed in the Galápagos Islands (Eliasson 1988, 2004) and 14 species (seven natives) in Australia (APNI 2010+). Recently, *c.* four new species and six infraspecific taxa from Argentina and Paraguay have been reported (Pedersen 1997, 2000). *Alternanthera* includes ornamentals (Robertson 1981; Eliasson 1987), and some species are considered invasive weeds in different parts of the world

(Robertson 1981; Eliasson 1987; Iamónico & Sánchez-del Pino 2012).

Most *Alternanthera* species are annual or perennial herbs, rarely shrubs or trees (Robertson 1981). The genus is characterised by its short spike inflorescences; flowers with stamens united into a cup; globose or subglobose stigmas (Townsend 1993); and triangular, ligulate and lacinate pseudostaminodia (Eliasson 1988). Species of the monophyletic *Alternanthera* are easily recognised by their globose or subglobose stigmas (Sánchez-del Pino et al. 2012).

Important contributions to the description and classification of the morphologically conserved (i.e. stenopalynous) pollen types within Amaranthaceae *s. str.* have been published in the last 65 years (Nowicke 1975; Skvarla & Nowicke 1976; Nowicke & Skvarla 1977, 1979; Erdtman 1986; Eliasson 1988;

Borsch 1998; Borsch & Barthlott 1998; Müller & Borsch 2005b). Erdtman (1986), following Schinz's (1934) pollen classification, recognised two basic pollen types within the Amaranthaceae: the *Amaranthus*-type, characterising the Amaranthoideae, and the *Gomphrena*-type, typical of the Gomphrenoideae. This last pollen type is distinguished by the presence of a reticulum that is not present in the *Amaranthus*-type. The *Gomphrena*-type pollen is, according to Erdtman (1986, p. 41), differentiated by 'muroid ridges separated by luminoid, aperturiferous (or aperturoidiferous) depressions' and differentiated from the *Amaranthus*-type pollen in having pores at the bottom of these depressions (Zandonella & Lecocq 1977).

Vishnu-Mittre (1963) distinguished nine pollen types based on the size and distribution of supra-rectal processes or sculpture on the tectum for the Indian Amaranthaceae species. Later, Zandonella and Lecocq (1977), based on pollen form, pore number and tectum sculpture, further subdivided Erdtman's (1952) Amaranthaceae pollen type classes into five subtypes. More recent palynological contributions for the Amaranthaceae by Eliasson (1988), Borsch and Barthlott (1998) and Borsch (1998) have shown the usefulness of pollen characters in the taxonomy of the family. In a proposal for a revised and updated terminology to describe the pollen types in Amaranthaceae, Borsch and Barthlott (1998) introduced the concepts of metareticulate pollen, mesoporia, conjunction points and structural elements to describe the ornamentation of the amaranthoid pollen and changed the whole understanding of pollen morphology in the family.

The metareticulum (singular of metareticula) is a type of reticulum-like ornamentation composed by vaulted mesoporia, a structure homologous to the meshes of the 'reticulate pollen' (Borsch & Barthlott 1998, p. 68). The mesoporia refers to the tectated areas surrounding two sunken pores. This type of pollen sculpture and aperture organisation is synapomorphic for the core gomphrenoid (Kadereit et al. 2003; Müller & Borsch 2005a, 2005b) and the gomphrenoids+alternantheroids clade (Sánchez-del Pino et al. 2009).

Borsch (1998) used pollen shape, mesoporia form (flat or vaulted), tectum sculpture and variants of ektexinous bodies (e.g. body types, shape, density and number) distributed on the pore membranes to recognise 17 well-defined pollen types in Amaranthaceae. Among them, the *Pfaffia*-type pollen diagnostic of *Alternanthera* and other genera is defined as '... dodecahedral or spheroidal, metareticulate, tectate, punctate, mostly with a few, unevenly distributed perforations, microspines distally  $\pm$  reg-

ularly arranged in a line, pores of type P' (Borsch 1998, pp. 130, 134).

Chin and Lim (2011), most recently summarised in a table the 20 species of *Alternanthera* from the New World, India, Pakistan and China with respective references published for palynological data since 1962. These authors also studied the palynological variation of three widespread species present in Peninsular Malaysia. Their findings supported that pollen of *Alternanthera* is similar to *Pfaffia* pollen as suggested by Borsch (1998) and indicated that number and shape of apertures (pores) and distribution of microspines on the sexine were important pollen characters for differentiating *Pfaffia* from *Alternanthera*. The exine ultrastructure in *Alternanthera* is organised as in all *Centrospermae* and consists of four layers, the tectum, columellae, foot layer and a single continuous layer below the foot layer or endexine (Skvarla & Nowicke 1976; Eliasson 1988).

Pollen morphology in Amaranthaceae has been informative at different taxonomic levels (Borsch 1998). Its variation has supported the potential resurrection of genera (e.g. *Hebanthe*: Borsch & Pedersen 1997), recognition of clades (e.g. subfamily Gomphrenoideae: Sánchez-del Pino et al. 2009) and species and infraspecific categories (e.g. *Tidestromia*: Sánchez-del Pino 2001; Sánchez-del Pino et al. 2002; Sánchez-del Pino & Flores Olvera 2006).

Recent palynological sources focused on Neotropical *Alternanthera* consists of only microphotographs as those from endemic species in the Galápagos Islands (Jaramillo et al. 2014) or palynological descriptions for few species in Mexican palynofloras like that of Martínez and Matuda (1979), Quiroz-García et al. (1994, *Alternanthera* cf. *Alternanthera pycnantha* Standl.) and Palacios-Chávez et al. (1991, *A. ramosissima* [Mart.] Chodat et Hassl.). Although these works have been relevant to define basic pollen characters in the genus (Erdtman 1986; Eliasson 1988; Borsch 1998), studies about patterns of pollen evolution within *Alternanthera* in a phylogenetic context are yet to be developed (Sánchez-del Pino & Fuentes-Soriano, own observation, March 2015).

In this work, we assess the morphological variation of pollen within *Alternanthera* with the main objectives of (i) testing the utility of pollen variation for the circumscription of species of *Alternanthera*, (ii) describing, analysing and providing detailed pollen descriptions in *Alternanthera* species and (iii) revising and updating palynological terms used to describe the mesoporia in pollen grains of *Alternanthera*. The complementary approach developed here integrates light microscopy (LM), scanning electron microscopy (SEM) and confocal laser scanning micro-

scopy (CLSM), multivariate ordination techniques to facilitate the evaluation of potential pollen types in *Alternanthera*.

## Material and methods

### *Plant material*

A total of 13 *Alternanthera* species were selected for study to represent all subclades of the *Alternanthera* clade resolved in a recent morphological- and molecular-based phylogenetic tree (Sánchez del-Pino et al. 2012).

Pollen samples for *Alternanthera* were obtained from material collected and fixed in FAA (formaldehyde-acetic acid-alcohol; Ruzin 1999); voucher herbarium specimens were prepared for deposit at the Centro de Investigación Científica de Yucatán (CICY) herbarium. Additional samples were obtained from F, GB, MEXU, NY and S herbaria collections (acronyms following Thiers 2011).

### *Scanning electron microscopy (SEM)*

Pollen grains were acetolysed using the technique of Erdtman (1960) with modified acid proportions and reaction times as needed to remove the pollen kit and clean the pollen grain exine of organic material. Material analysed with SEM was dehydrated in an ethanol series (50%, 70%, 90%, 100%) for ten minutes for each step and dried in a semi-automatic critical point dryer (SAMDRI 795). The dry material was mounted on aluminium stubs and coated with gold using a sputter coater (Denton vacuum, model DESK-II; COLD SPUTTER/ETCH UNIT). SEM samples were observed and photographed in a JEOL JSM6360LV SEM at CICY.

### *Light microscopy (LM)*

Acetolysed pollen grains were embedded in glycerol jelly on slides sealed with nail polish, described and measured using a NIKON LM (model ECLIPSE E200) at 100× magnification with oil immersion. Images were captured using an INFINITY1-3C digital camera (Lumenera).

For most of the species, except for *Alternanthera littoralis* var. *maritima*, 28 or 30 pollen grains pooled from two or three biological replicates (e.g. anthers from flowers from a single plant or anthers from flowers from different plants) were measured using the INFINITY ANALYZE software version 4.6.0 (2008). For *A. littoralis* var. *maritima*, 21 pollen grains were measured. Columellae number and diameter (in structural elements and conjunction points), number of rows of columellae per structural

element, and mesopodium width were measured for nine pollen grains pooled from three or two biological replicates using SEM and CLSM images.

### *Confocal laser scanning microscopy (CLSM)*

Palynological studies based on LM and complemented with SEM observations are standard to analyse general pollen morphology. Traditionally, the ultrastructure of the exine has been analysed with expensive and labour intensive transmission electron microscopy (TEM). CLSM has emerged as a cost-efficient alternative to analyse internal ultrastructure of the exine and permits optical sectioning of a single pollen (Castro et al. 2010; Sivaguru et al. 2012; Holt & Bennett 2014). CLSM observations in the present study allowed us to study and characterise the ultrastructure of exine layers such as the ectexine, collumelae, nexine in the lumen, exine in aperture regions, conjunction points and structural elements (structures described later).

The *Alternanthera* pollen samples previously prepared for LM were also observed with an OLYMPUS FluoView™ FV1000 confocal microscope, configured as follows: Lens UPLFLN 40× O (oil, NA: 1.3), scan speed: 2 µs/pixel, an excitation laser at 405 nm to 5% (Al-morin complex has a wavelength of excitation and emission of 420 and 515 nm, respectively). Images were collected at the 1.0 µm Z position, and approximately 30 sections of 512 × 512 pixels per image were obtained; all sections were projected into a single image using FV10-ASW software version 3.01b.

A sequential scanning in two phases was performed to avoid background interference. We used a 40× O UPLFLN lens (oil, NA: 1.3), scan rate of 10 µs/pixel, a 405 nm excitation laser for morin and DAPI, and a 543 nm excitation laser for FM4-64. Morin, DAPI and FM4-64 have excitation and emission wavelengths of 420, 515 nm; 358, 461 nm; and 515, 670 nm, respectively. All images have a resolution of 512 × 512 pixels, and Z-stacking for each fluorophore was projected into a single image. The images for the three fluorophores were merged using FV10-ASW software 3.01b.

### *Pollen terminology*

Descriptions of general pollen morphology in this study followed the terminology of Punt et al. (1994), Borsch and Barthlott (1998) and Borsch (1998). Pollen-specific characters of Amaranthaceae were used according to several other studies (Skvarla & Nowicke 1976; Zandonella & Lecocq 1977; Eliasson 1988; Borsch 1998; Sánchez-del Pino 2001; Sánchez-del Pino et al. 2002). We provide a diagram

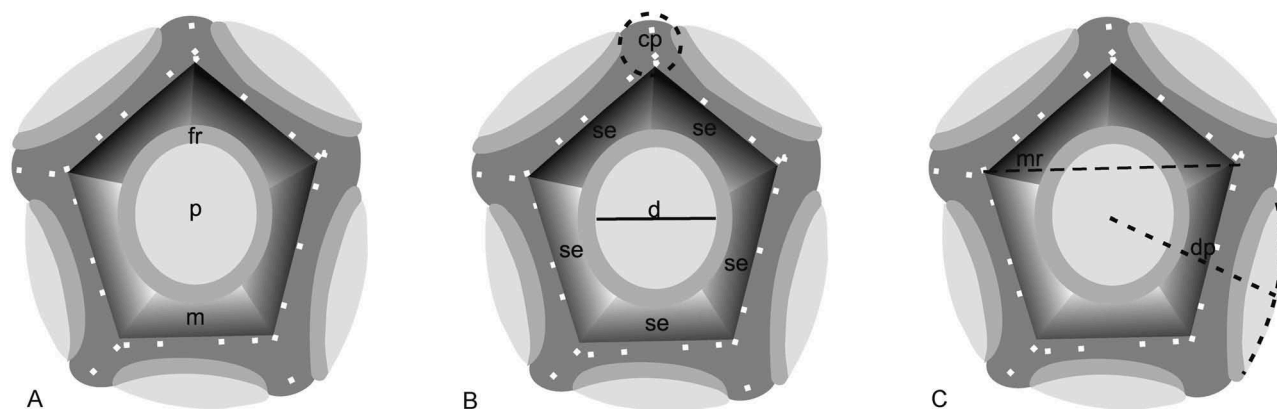


Figure 1. Pollen diagram showing parts of the metareticulum. **A.** Mesoporiolum (m), flat ring (fr), poral membrane (p). **B.** Conjunction points (cp), structural elements (se) and pore membrane diameter (d). **C.** Metareticulum diameter (mr) and distance between two pores (dp).

that shows the structures and morphological landmarks of the pollen grains that were observed and measured here (Figure 1).

#### Statistical analyses

Fourteen quantitative morphological pollen characters (Tables I, II) were directly used in two complementary multivariate procedures: a constrained ordination canonical analysis of principal coordinates (CAP) and a cluster analysis. These techniques were used to investigate the grouping of taxa within the genus, on the basis of their overall palynological similarity. CAP and cluster analyses were implemented in PRIMER v6 and PERMANOVA add-on software (Clarke & Gorley 2006).

Morphological data from each simple variable were normalised to remove differences in scale among original variables and a similarity matrix based on Euclidean distances was constructed. Based on this distance matrix, the distances among centroids of sampling data were visualised using the CAP ordinations and considering the species as the predictor variables. The variables used for the CAP were correlated with the first two axes of correlation (0.9791 and 0.9705) of the first eight axis variables that contributed more than 40% of the variability in the original dissimilarity matrix. A plot of first cano-

nical axes was inferred to show patterns of differences among groups.

Further cluster analysis included a UPGMA using the Bray–Curtis coefficient to detect groups based on distance and the characters that influenced that grouping and a SIMPROF (similarity profile) test with 1000 permutations.

## Results

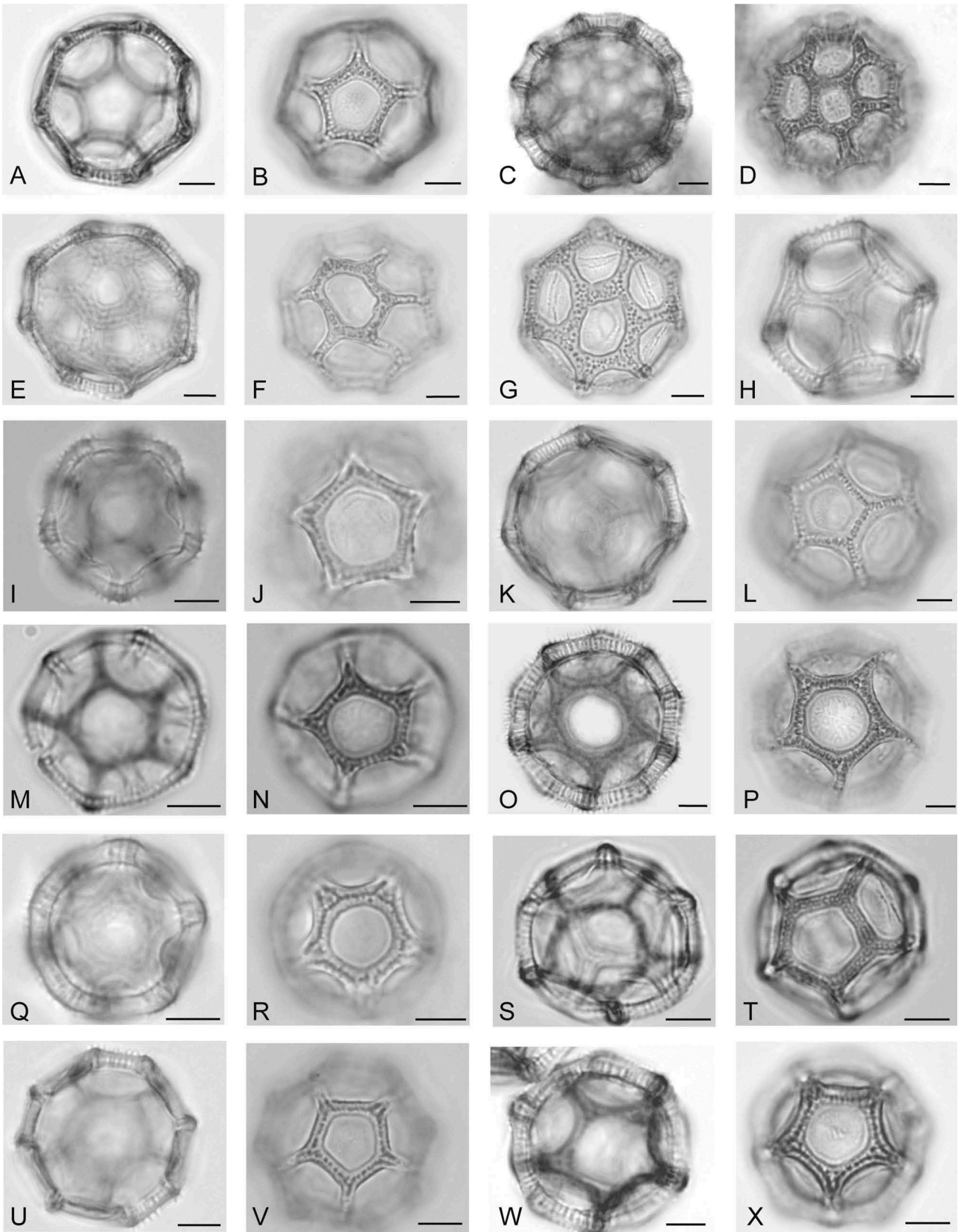
### General pollen description of *Alternanthera*

*Origin and shape.* — Monads, dodecahedric or circular with pollen 12.39–37.75  $\mu\text{m}$  in width (Table I).

*Pores (Figure 1A).* — Pollen pores in *Alternanthera* as in all Amaranthaceae include the pore membrane and a flat ring, which is the intectated area surrounding the pore membrane. Pores in *Alternanthera* are pantoporate in numbers of from 12 to 30 (Table IV). The distance between two pores (DBTP), here described as another source of aperture variation varied from 5.0  $\mu\text{m}$  to 14.26  $\mu\text{m}$  (as in *Alternanthera geniculata*; Table I).

*Pore membrane.* — Pore membrane diameter (PMD) of the studied species varied from 2.58  $\mu\text{m}$  to 10.97  $\mu\text{m}$  (Table I). The pore membrane was

Figure 2. Light microscopy of pollen grains of species of *Alternanthera*. **A.** Pollen grain of *A. caracasana*. **B.** Metareticula and pore membrane of *A. caracasana*. **C.** Pollen grain of *A. costaricensis*. **D.** Metareticula and pore membrane of *A. costaricensis*. **E.** Pollen grain of *A. echinocephala*. **F, G.** Metareticula and pore membrane of *A. echinocephala*. **H, I.** Pollen grain of *A. flava*. **J.** Metareticula and pore membrane of *A. flava*. **K.** Pollen grain of *A. flavescens*. **L.** Metareticula and pore membrane of *A. flavescens*. **M.** Pollen grain of *A. galapagensis*. **N.** Metareticula and pore membrane of *A. galapagensis*. **O.** Pollen grain of *A. geniculata*. **P.** Metareticula and pore membrane of *A. geniculata*. **Q.** Pollen grain of *A. lanceolata*. **R.** Metareticula and pore membrane of *A. lanceolata*. **S.** Pollen grain of *A. littoralis* var. *maritima*. **T.** Metareticula and pore membrane of *A. littoralis* var. *maritima*. **U.** Pollen grain of *A. macbridei*. **V.** Metareticula and pore membrane of *A. macbridei*. **W.** Pollen grain of *A. obovata*. **X.** Metareticula and pore membrane of *A. obovata*. Scale bars – 5  $\mu\text{m}$ .



covered with ektexinous bodies and nanospines. Ektexinous bodies varied in number (7–84), shape (rounded or elongated), and arrangement (towards the centre of the pore membrane [Figure 6E] or dispersed over the pore membrane [Figure 4E]; Table IV). Number of nanospines on the ektexinous bodies on the pore membranes group species with 10–16, 20–56 or species with 60–174 nanospines.

*Flat ring (Figure 1A).* — All pollen observed had a psilate flat ring (FR), a variably sized area that flanks the pore membrane, ranging from 0.20 (Figure 5M) to 0.64  $\mu\text{m}$  wide (Figure 5F; Table I).

*Pollen metareticulum.* — The metareticulum width (MW) varied in size from 4.76 to 16.66  $\mu\text{m}$  (Table I).

*Mesoporium (Figure 1A).* — In *Alternanthera*, the mesoporium (singular), an ‘area of a pollen grain surface delimited by lines between the apices of adjacent colpi or the margins of adjacent pores’ (Punt et al. 2007, p. 43), also named mesh by Vishnu-Mittre (1963) or luminoid depressions by Livingstone et al. (1973), is defined by 5–6 tectate regions called structural elements (SEs; Figure 1B) and conjunction points (CPs; Figure 1B). These structures define depending on the species a hexagonal (Figures 2F, 3A, 9H, K–L) or pentagonal metareticulum (Figures 2B, 4H, 7B). Mesoporium size ranged from 0.97 to 2.8  $\mu\text{m}$  width.

*Exine ultrastructure.* — Our CLSM and SEM images confirmed that basically all tectate regions have a complete exine wall comprising two main layers, the sexine (tectum+columellae) and nexine (including the footlayer, nexine, and intine, with the intine lost during acetolysis). The exine in *Alternanthera* consisted of an ektexine (sexine and nexine layers) that varied in thickness in different areas of the pollen and across species. The sexine comprises the tectum and rod-like structures that make the columellar layer. In general, the sexine was more variable than the nexine in thickness. Nexine layer in the structural elements (NSE) and in conjunction points

(NCP) were from 0.20 to 0.53  $\mu\text{m}$  and 0.49  $\mu\text{m}$  thickness, respectively (Table II).

*Tectum (Figure 8G).* — Pollen of *Alternanthera* is clearly tectate (Borsch 1998). The thinnest tectate areas were found on the structural elements (TSE) and varied in size (0.19–0.75  $\mu\text{m}$ ; Table II). The thickest tectate areas were found on the conjunction points (TCP) and varied from 0.42 to 1.45  $\mu\text{m}$  (Figures 7H, 9D, G, Table II).

*Tectum ornamentation.* — Pollen sculpture varied in the number and arrangement of pores or perforations. Pollen of *Alternanthera*, when punctated, have very small perforations (Skvarla & Nowicke 1976; Nowicke & Skvarla 1979) that vary in diameter from 40 to 400 nm width (Borsch 1998). Our observations suggest that perforations were arranged either as relatively ordered in a single row on each structural element (Figure 4L) or they were relatively forming two rows (Figures 4I, 5C). Most of the species observed in this study had nanopores relatively forming two rows (Table IV).

In addition to pores, pollen grains, with the exception of *Alternanthera serpyllifolia*, had spine-like sculptures (nano-, micro- or echinae). The smallest spines (SL) measured varied from 0.30 to 1.55  $\mu\text{m}$ . (Table I). The spines arrange in one or two rows per structural element (SRSE; Table IV)

*Sexine.* — Sexine and columellae layers of conjunction point regions (SCP, CCP) were thicker than in the structural elements (SSE). The sexine of the structural elements (SSE; 0.70  $\mu\text{m}$ ), sexine of the conjunction points (SCP; 1.03  $\mu\text{m}$ ), columellae of the structural elements (CSE; 0.40  $\mu\text{m}$ ) and columellae on the conjunction points (CCP; 0.52  $\mu\text{m}$ ) were from thinnest to thickest SSE and SCP (3.07  $\mu\text{m}$ , 3.71  $\mu\text{m}$ , respectively; Table II) and the CSE and CCP (2.3  $\mu\text{m}$ , 2.90  $\mu\text{m}$ , respectively; Table II).

The number of columellae in the structural elements varied among the species from 3–4 to 14 (Table IV). The columellae diameter ranged from 0.27 to 0.54  $\mu\text{m}$ , whereas the diameter of the colu-

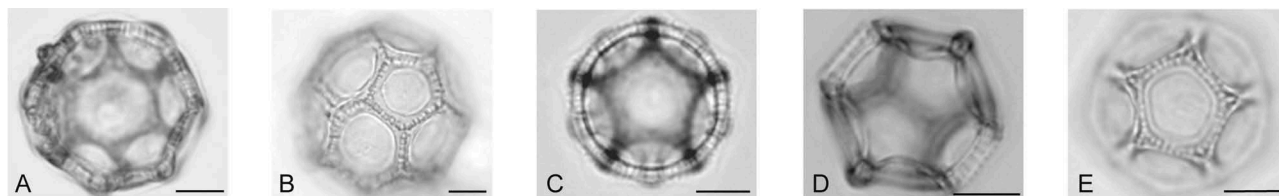


Figure 3. Light microscopy of pollen grains of species of *Alternanthera*. **A.** Pollen grain of *A. serpyllifolia*. **B.** Metareticula and pore membrane of *A. serpyllifolia*. **C, D.** Pollen grain of *A. tenella*. **E.** Metareticula and pore membrane of *A. tenella*. Scale bars – 5  $\mu\text{m}$ .

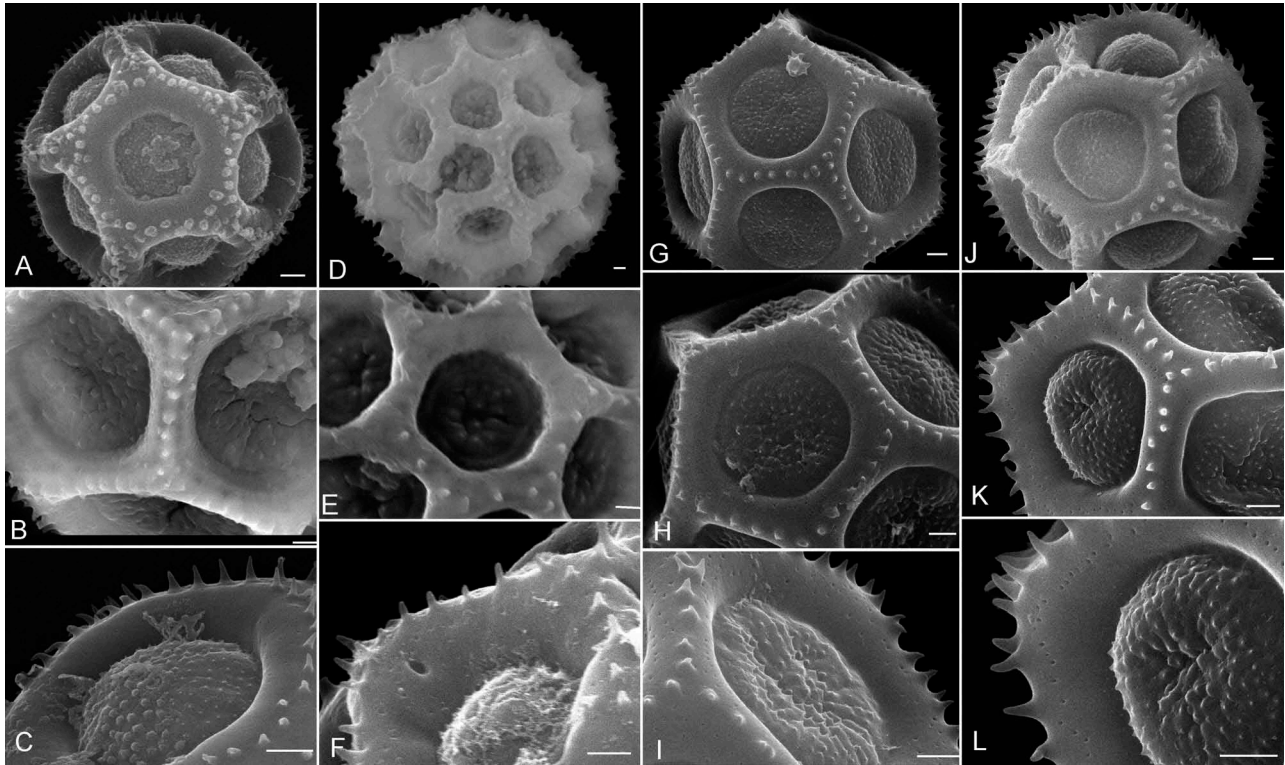


Figure 4. Scanning electron microscopy of pollen grains of species of *Alternanthera*. **A**. Pollen grain of *A. caracasana*. **B, C**. Metareticula and pore membrane of *A. caracasana*. **D**. Pollen grain of *A. costaricensis*. **E, F**. Metareticula and pore membrane of *A. costaricensis*. **G**. Pollen grain of *A. echinocephala*. **H, I**. Metareticula and pore membrane of *A. echinocephala*. **J**. Pollen grain of *A. flava*. **K, L**. Metareticula and pore membrane of *A. flava*. Scale bars – 1  $\mu\text{m}$ .

mellae on the conjunction points was 0.39 to 0.70  $\mu\text{m}$ .

The palynological characters such as pollen form, pollen ornamentation, metareticulum form, pore number, spines length and arrangement on structural elements, arrangement of nanopores on structural elements and number of nanopores on the ektexinous bodies, and number of ektexinous bodies on the pore membrane were useful in recognising *Alternanthera* species and constructing the first palynological key for the alternantheroid clade and *Alternanthera*.

*Palynological key to identify species of Alternanthera in the New World*

- 1a Pollen tectum punctate and psilate.....  
.....*Alternanthera serpyllifolia*
- 1b Pollen tectum punctate and spinulate (nano-, micro-, echinate) ..... 2
- 2a Pollen dodecahedral (with 12–14 pores) and/or spheroidal (with more than 16 pores) ..... 3
- 2b Pollen dodecahedral (with 12–14 pores) ..... 4
- 3a Pollen spheroidal with 24–30 pores .....  
..... *Alternanthera costaricensis*

- 3b Pollen dodecahedral (with 12 pores) and spheroidal (with 20–24 pores) .....  
..... *Alternanthera echinocephala*
- 4a Mean spine length  $1.08 \pm 0.27 \mu\text{m}$  .....  
..... *Alternanthera geniculata*
- 4b Mean spine length from  $0.41 \pm 0.06$  to  $0.72 \pm 0.16 \mu\text{m}$  ..... 5
- 5a Nanopores on individual structural element relatively ordered in a row ..... 6
- 5b Nanopores on individual structural elements relatively forming two rows ..... 9
- 6a Spines in a single row on each structural element ..... *Alternanthera galapagensis*
- 6b Spines in one or two rows on each structural element ..... 7
- 7a Ektexinous bodies per poral membrane 28–33 ..... 8
- 7b Ektexinous bodies per poral membrane 37–84 .....  
..... *Alternanthera flava*
- 8a Pollen with 12 pores; nanopores 6–13 per structural element *Alternanthera littoralis* var. *maritima*
- 8b Pollen with 12–14 pores; nanopores 11–25 per structural element ..... *Alternanthera tenella*
- 9a Spines 14–20 on tectum per structural element .....  
..... *Alternanthera obovata*



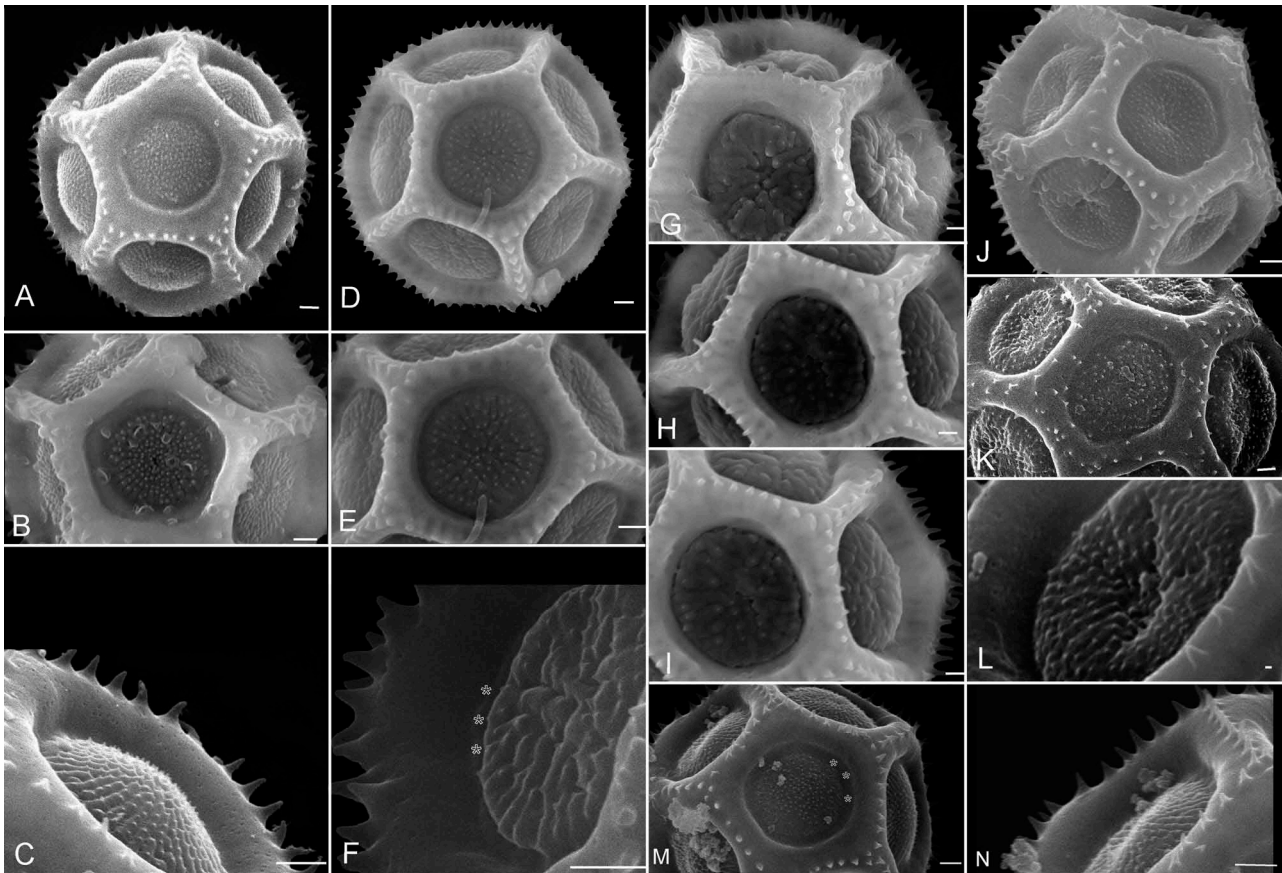


Figure 5. Scanning electron microscopy of pollen grains of species of *Alternanthera*. **A**. Pollen grain of *A. flavescens*. **B, C**. Metareticula and pore membrane of *A. flavescens*. **D**. Pollen grain of *A. galapagensis*. **E, F**. Metareticula and pore membrane of *A. galapagensis*. **G**. Pollen grain of *A. geniculata*. **H, I**. Metareticula and pore membrane of *A. geniculata*. **J**. Pollen grain of *A. lanceolata*. **K, L**. Metareticula and pore membrane of *A. lanceolata*. **M**. Pollen grain of *A. macbridei*. **N**. Metareticula and pore membrane of *A. macbridei*. Flat ring indicated by asterisks. Scale bars – 1  $\mu\text{m}$ .

- 9b Spines 6–10 on tectum per structural element ..... 10
- 10a Spines in a single row on each structural element ..... *Alternanthera flavescens*
- 10b Spines in one or two rows on each structural element ..... 11
- 11a Metareticulum pentagonal and hexagonal ..... 12
- 11b Metareticulum pentagonal... *Althermaria lanceolata*
- 12a Nanospines 7–8 per structural element ..... *Alternanthera macbridei*
- 12b Nanospines 8–10 per structural element ..... *Alternanthera caracasana*

*Pollen descriptions of species of Alternanthera*

*Alternanthera caracasana* (Figures 2A, 2B, 4A–C, 7A–D). — Pollen dodecahedric, 20.02–(24.69  $\pm$  2.54)–29.64  $\mu\text{m}$  in diameter. Pores 12–14, distance between two pores 6.82–(8.76  $\pm$  0.99)–10.61  $\mu\text{m}$ ; pore membrane 3.76–(5.67  $\pm$  1.06)–9.06  $\mu\text{m}$  in diameter, ectexinous bodies 15–40, nanospines about 70–130. Flat ring

0.32–(0.42  $\pm$  0.07)–0.55  $\mu\text{m}$  wide. Metareticulum pentagonal or hexagonal, 9.08–(10.90  $\pm$  1.47)–13.72  $\mu\text{m}$  wide; structural elements and conjunction points 5–6. Mesoporiium 1.61–1.81  $\mu\text{m}$  wide. Sculpture usually punctate-, nano- and microspinulate; 15–20 nanopores per structural element, relatively forming two rows; 8–10 nanospines-microspines per structural element, 0.33–(0.42  $\pm$  0.07)–0.61  $\mu\text{m}$  long, in one or two rows on each structural element. Tectum on structural elements 0.39–(0.48  $\pm$  0.06)–0.61  $\mu\text{m}$  high, tectum on conjunction points 0.79–(0.99  $\pm$  0.13)–1.22  $\mu\text{m}$  high; columellae 6–9, arranged in one or two rows on structural elements, each 0.55–(0.82  $\pm$  0.15)–1.24  $\mu\text{m}$  high, 0.34–0.43  $\mu\text{m}$  wide, columellae on the conjunction points 0.70–(0.98  $\pm$  0.19)–1.37  $\mu\text{m}$  high, 0.46–0.53  $\mu\text{m}$  wide; nexine of structural elements 0.30–(0.34  $\pm$  0.04)–0.44  $\mu\text{m}$  thick, nexine of conjunction points 0.30–(0.37  $\pm$  0.04)–0.46  $\mu\text{m}$  thick; sexine of structural elements 1.02–(1.30  $\pm$  0.14)–1.64  $\mu\text{m}$  thick, sexine of conjunction points 1.18–(1.80  $\pm$  0.36)–2.43  $\mu\text{m}$  thick.

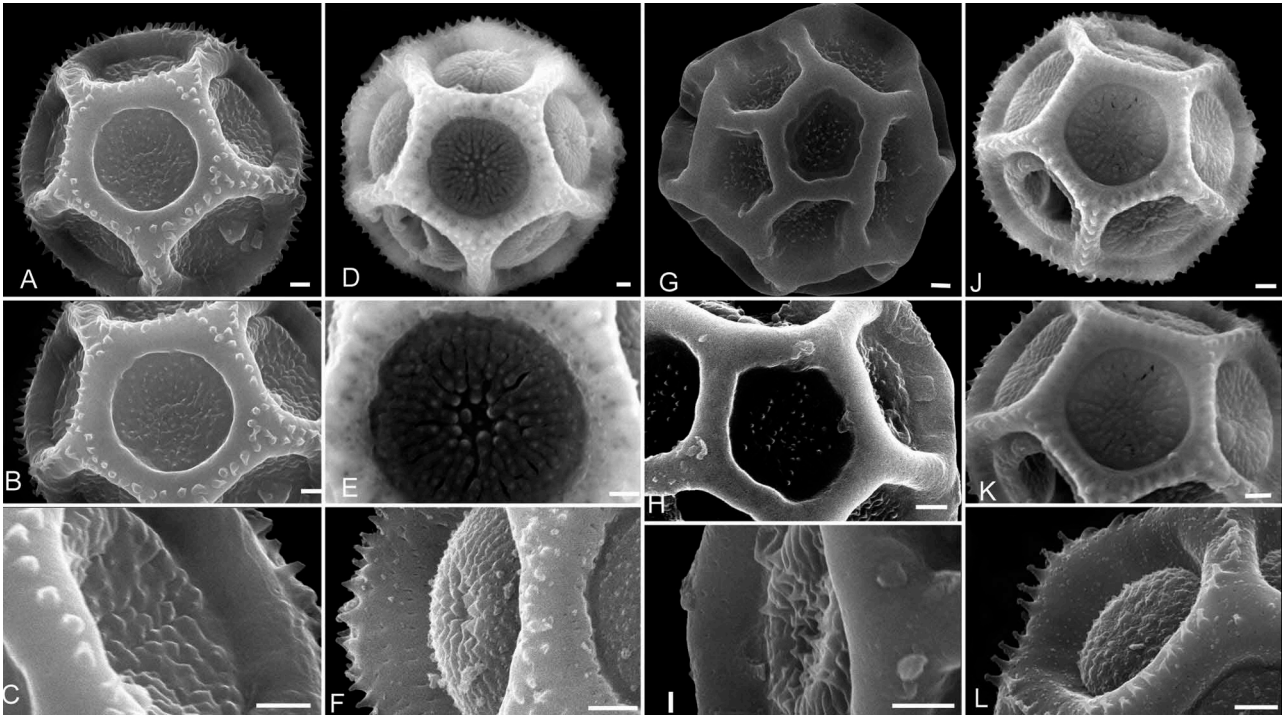


Figure 6. Scanning electron microscopy of pollen grains of species of *Alternanthera*. **A**. Pollen grain of *A. littoralis* var. *maritima*. **B**, **C**. Metareticula and pore membrane of *A. littoralis* var. *maritima*. **D**. Pollen grain of *A. obovata*. **E**, **F**. Metareticula and pore membrane of *A. obovata*. **G**. Pollen grain of *A. serpyllifolia*. **H**, **I**. Metareticula and pore membrane of *A. serpyllifolia*. **J**. Pollen grain of *A. tenella*. **K**, **L**. Metareticula and pore membrane of *A. tenella*. Scale bars – 1  $\mu\text{m}$ .

*Alternanthera costaricensis* (Figures 2C–D, 4D–F, 7E–H). — Pollen spheroidal, 23.94–(30.98  $\pm$  4.01)–37.75  $\mu\text{m}$  in diameter. Pores 24–30, distance between two pores 6.0–(7.65  $\pm$  0.95)–9.11  $\mu\text{m}$ ; pore membrane 4.48–(5.87  $\pm$  0.65)–7.12  $\mu\text{m}$  in diameter, ektexinous bodies 7–10, nanospines about 10–16. Flat ring 0.30–(0.34  $\pm$  0.04)–0.42  $\mu\text{m}$  wide. Metareticulum pentagonal or hexagonal, 6.28–(8.54  $\pm$  1.04)–10.24  $\mu\text{m}$  wide; structural elements and conjunction points 5–6. Mesopodium 1.36–1.77  $\mu\text{m}$  wide. Sculpture punctate, nano- and microspinulate; 8–15 nanopores per structural element, relatively forming two rows; 4–7 nanospines, microspines and equinulas 0.47–(0.72  $\pm$  0.16)–1.03  $\mu\text{m}$  long, in one row per structural element. Tectum on structural elements, 0.30–(0.46  $\pm$  0.10)–0.68  $\mu\text{m}$  high, tectum on conjunction points 0.54–(0.76  $\pm$  0.12)–0.95  $\mu\text{m}$  high; columellae 3–4, arranged in one row on structural elements, each 1.10–(1.52  $\pm$  0.30)–2.25  $\mu\text{m}$  high, 0.45–0.54  $\mu\text{m}$  wide, columellae on the conjunction points 1.54–(2.02  $\pm$  0.34)–2.87  $\mu\text{m}$  high, 0.53–0.63  $\mu\text{m}$  wide; nexine of structural elements 0.29–(0.36  $\pm$  0.04)–0.44  $\mu\text{m}$  thick, nexine of conjunction points 0.30–(0.36  $\pm$  0.03)–0.42  $\mu\text{m}$  thick; sexine of structural elements 1.52–(2.21  $\pm$  0.44)–3.07  $\mu\text{m}$  thick, sexine of conjunction points 1.99–(2.81  $\pm$  0.43)–3.71  $\mu\text{m}$  thick.

*Alternanthera echinocephala* (Figures 2E–G, 4G–I, 7I–L). — Pollen spheroidal and dodecahedral, 17.51–(25.08  $\pm$  5.52)–36.26  $\mu\text{m}$  in diameter. Pores both 12 and 20–24, distance between two pores 5.50–(7.71  $\pm$  1.34)–10.31  $\mu\text{m}$ ; pore membrane 3.23–(5.47  $\pm$  1.02)–8.12  $\mu\text{m}$  in diameter, ektexinous bodies 36–54, nanospines 68–148. Flat ring 0.20–(0.28  $\pm$  0.03)–0.36  $\mu\text{m}$  wide. Metareticulum pentagonal or hexagonal, 4.76–(8.35  $\pm$  1.31)–10.22  $\mu\text{m}$  wide; structural elements and conjunction points 5–6. Mesopodium 1.19–1.97  $\mu\text{m}$  wide. Sculpture punctate, nano- and microspinulate; 14–30 nanopores per structural element, relatively forming two rows; 6–7 nanospines and microspines per structural element, 0.33–(0.53  $\pm$  0.12)–0.73  $\mu\text{m}$  long, in one row on each structural element. Tectum on structural elements 0.19–(0.34  $\pm$  0.07)–0.48  $\mu\text{m}$  high, tectum on conjunction points 0.50–(0.76  $\pm$  0.14)–1.0  $\mu\text{m}$  high; columellae 6–12, arranged in one or two rows on structural elements, each 0.50–(0.85  $\pm$  0.22)–1.33  $\mu\text{m}$  high, 0.31–0.35  $\mu\text{m}$  wide, columellae on the conjunction points 0.80–(1.12  $\pm$  0.20)–1.53  $\mu\text{m}$  high, 0.39–0.41  $\mu\text{m}$  wide; nexine of structural elements 0.22–(0.31  $\pm$  0.04)–0.40  $\mu\text{m}$  thick, nexine of conjunction points 0.21–(0.30  $\pm$  0.05)–0.42  $\mu\text{m}$  thick; sexine of structural elements 0.74–(1.27  $\pm$  0.31)–1.87  $\mu\text{m}$  thick, sexine of conjunction points 1.17–(1.88  $\pm$  0.32)–2.43  $\mu\text{m}$  thick.

*Alternanthera flava* (Figures 2H–J, 4J–L, 7M–P). — Pollen dodecahedral, 18.16–(22.34 ± 1.96)–24.71 µm in diameter. Pores 12, distance between two pores 5.92–(8.35 ± 1.28)–10.44 µm; pore membrane 4.73–(6.81 ± 1.03)–8.94 µm in diameter, ektextinous bodies 37–84, nanopines about 80–112. Flat ring 0.20–(0.29 ± 0.05)–0.39 µm wide. Metareticulum pentagonal, 6.03–(9.33 ± 1.52)–11.71 µm wide; structural elements and conjunction points 5. Mesopodium 1.68–2.1 µm wide. Sculpture punctate, nano- and microspinulate; 15–22 nanopores per structural element, relatively ordered in a row; 7–8 nanopines and microspines per structural element, 0.44–(0.62 ± 0.11)–0.79 µm long, in one or two rows on each structural element. Tectum on structural elements 0.20–(0.37 ± 0.10)–0.68 µm high, tectum on conjunction points 0.50–(0.82 ± 0.13)–1.07 µm high; columellae 6–12, arranged in one or two rows on structural elements, each 0.53–(0.80 ± 0.13)–1.07 µm high, 0.33–0.37 µm wide, columellae on the conjunction points 0.70–(1.03 ± 0.16)–1.38 µm high, 0.39–0.45 µm wide; nexine of structural elements 0.20–(0.32 ± 0.05)–0.40 µm thick, nexine of conjunction points 0.20–(0.28 ± 0.04)–0.37 µm thick; sexine of structural elements 0.82–(1.33 ± 0.27)–1.92 µm thick, sexine of conjunction points 1.13–(1.93 ± 0.34)–2.49 µm thick.

*Alternanthera flavescens* (Figures 2K–L, 5A–C, 7Q–T). — Pollen dodecahedral, 19.31–(23.80 ± 1.72)–27.78 µm in diameter. Pores 12, distance between two pores 7.38–(9.0 ± 0.68)–10.97 µm; pore membrane 5.56–(7.06 ± 0.77)–8.48 µm in diameter, ektextinous bodies 46–64, nanopines 146–174. Flat ring 0.23–(0.32 ± 0.05)–0.40 µm wide. Metareticulum pentagonal, 7.00–(9.77 ± 1.88)–13.27 µm wide; structural elements and conjunction points 5. Mesopodium 1.92–2.10 µm wide. Sculpture punctate and microspinulate; 14–30 nanopores per structural element, relatively forming two rows; 6–8 microspines per structural element, 0.50–(0.70 ± 0.12)–0.99 µm long, in one row per structural element. Tectum on structural elements 0.25–(0.42 ± 0.11)–0.66 µm high, tectum on conjunction points 0.7–(0.92 ± 0.11)–1.09 µm high; columellae 7–13, arranged in one or two rows on structural elements, each 0.70–(1.08 ± 0.17)–1.36 µm high, 0.35–0.38 µm wide, columellae on the conjunction points 0.97–(1.29 ± 0.18)–1.62 µm high, 0.41–0.51 µm wide; nexine of structural elements 0.22–(0.30 ± 0.04)–0.39 µm thick, nexine of conjunction points 0.24–(0.33 ± 0.04)–0.40 µm thick; sexine of structural elements 1.29–(1.64 ± 0.28)–2.41 µm thick, sexine of conjunction points 1.52–(2.22 ± 0.32)–2.87 µm thick.

*Alternanthera galapagensis* (Figures 2M–N, 5D–F, 8A–D). — Pollen dodecahedral, 12.89–(18.19 ± 2.73)–23.42 µm in diameter. Pores 12, distance between two pores 4.75–(7.45 ± 1.16)–10.31 µm; pore membrane 3.25–(4.90 ± 0.84)–6.16 µm in diameter, ektextinous bodies 27–42, nanopines 60–93. Flat ring 0.33–(0.48 ± 0.08)–0.64 µm wide. Metareticulum pentagonal, 6.43–(10.89 ± 1.61)–13.22 µm wide; structural elements and conjunction points 5. Mesopodium 1.26–1.83 µm wide. Sculpture punctate, nano- and microspinulate; 6–13 nanopores per structural element, relatively ordered in a row; 7–9 nanopines-microspines per structural element, 0.43–(0.62 ± 0.10)–0.80 µm long, in one row on each structural element. Tectum on structural elements 0.36–(0.49 ± 0.07)–0.67 µm high, tectum on conjunction points 0.61–(0.82 ± 0.10)–0.99 µm high; columella 7–8, arranged in one row on structural elements, each 0.50–(0.71 ± 0.12)–0.94 µm high, 0.4–0.5 µm wide, columellae on the conjunction points 0.71–(1.03 ± 0.16)–1.38 µm high, 0.54–0.59 µm wide; nexine of structural elements 0.33–(0.41 ± 0.05)–0.49 µm thick, nexine of conjunction points 0.32–(0.40 ± 0.04)–0.47 µm thick; sexine of structural elements 1.02–(1.21 ± 0.13)–1.46 µm thick, sexine of conjunction points 1.07–(1.75 ± 0.25)–2.28 µm thick.

*Alternanthera geniculata* (Figures 2O–P, 5G–I, 8E–H). — Pollen dodecahedral, 22.01–(29.77 ± 3.22)–35.56 µm in diameter. Pores 12, distance between two pores 7.69–(10.57 ± 1.40)–14.26 µm; pore membrane 6.42–(8.86 ± 1.09)–10.97 µm in diameter, ektextinous bodies 20–22, nanopines 27–29. Flat ring 0.23–(0.34 ± 0.05)–0.45 µm wide. Metareticulum pentagonal, 10.76–(14.77 ± 1.63)–16.66 µm wide; structural elements and conjunction points 5. Mesopodium 2.21–2.80 µm wide. Sculpture punctate, micro- and equinulate; 10–25 nanopores per structural element, relatively forming two rows; 4–9 microspines and equinulas per structural element, 0.58–(1.08 ± 0.27)–1.55 µm long, in a row on each structural element. Tectum on structural elements 0.35–(0.53 ± 0.10)–0.74 µm high, tectum on conjunction points 0.67–(1.03 ± 0.17)–1.45 µm high; columellae 7–14, arranged in one or two rows on structural elements, each 1.10–(1.74 ± 0.32)–2.30 µm high, 0.45–0.51 wide, columellae on the conjunction points 1.15–(2.15 ± 0.41)–2.90 µm high, 0.41–0.51 µm wide; nexine of structural elements 0.25–(0.32 ± 0.05)–0.50 µm thick, nexine of conjunction points 0.22–(0.32 ± 0.04)–0.41 µm thick; sexine of structural elements 1.55–(2.24 ± 0.33)–2.95 µm thick, sexine of conjunction points 1.67–(2.59 ± 0.42)–3.64 µm thick.

*Alternanthera lanceolata* (Figures 2Q–R, 5J–L, 8I–L). — Pollen dodecahedral, 14.63–(17.39 ± 1.50)–20.53 µm in diameter. Pores 12, distance between two pores 5.07–(6.23 ± 0.63)–7.43 µm; pore membrane 3.36–(4.43 ± 0.57)–5.69 µm in diameter, ektexinous bodies 29–30, nanospines 140–164. Flat ring 0.21–(0.30 ± 0.05)–0.40 µm wide. Metareticulum pentagonal, 5.81–(8.15 ± 1.07)–10.71 µm wide; structural elements and conjunction points 5. Mesopodium 1.6–1.8 µm wide. Sculpture punctate, nano- and microspinulate; 14–19 nanopores per structural element, relatively forming two rows; 6–10 nanospines and microspines per structural element, 0.30–(0.48 ± 0.08)–0.62 µm long, in one or two rows on each structural element. Tectum on structural elements 0.22–(0.36 ± 0.06)–0.53 µm high, tectum on the conjunction points 0.44–(0.60 ± 0.12)–0.92 µm high; columellae 7, arranged in a row on structural elements, each 0.45–(0.66 ± 0.09)–0.83 µm high, 0.31–0.43 µm wide, columellae on the conjunction points 0.63–(0.90 ± 0.15)–1.26 µm high, 0.43–0.54 µm wide; nexine of structural elements 0.21 (0.29 ± 0.05)–0.38 µm thick, nexine of conjunction points 0.22–(0.31 ± 0.04)–0.39 µm thick; sexine of structural elements 0.85–(1.18 ± 0.19)–1.62 µm thick, sexine of conjunction points 1.04–(1.42 ± 0.21)–1.79 µm thick.

*Alternanthera littoralis* var. *maritima* (Figures 2S–T, 6A–C, 9A–D). — Pollen dodecahedral, 16.22–(21.79 ± 3.26)–28.08 µm in diameter. Pores 12, distance between two pores 5.72–(8.16 ± 1.36)–10.56 µm; pore membrane 4.07–(5.91 ± 1.27)–7.54 µm in diameter, ektexinous bodies 28–29, nanospines 66–85. Flat ring 0.30–(0.41 ± 0.05)–0.48 µm wide. Metareticulum pentagonal or hexagonal, 7.56–(10.74 ± 1.88)–13.62 µm wide; structural elements and conjunction points 5. Mesopodium 1.43–1.96 µm wide. Sculpture punctate, nano- and microspinulate; around 6–13 nanopores per structural element, relatively ordered in a row; 8–10 nanospines and microspines per structural element, 0.33–(0.50 ± 0.09)–0.70 µm long, in one or two rows on each structural element. Tectum on structural elements 0.38–(0.48 ± 0.06)–0.57 µm high, tectum on conjunction points 0.60–(0.80 ± 0.13)–1.08 µm high; columellae 6–9, arranged in one or two rows on structural elements, each 0.51–(0.79 ± 0.18)–1.29 µm high, 0.34–0.43 µm wide, columellae on the conjunction points 0.89 (1.10 ± 0.17)–1.50 µm high, 0.46–0.53 µm wide; nexine of structural elements 0.30–(0.37 ± 0.04)–0.45 µm thick, nexine of conjunction points 0.30–(0.38 ± 0.04)–0.44 µm thick; sexine of structural elements 0.88–(1.25 ± 0.23)–1.94 µm thick, sexine of conjunction points 1.52–(1.90 ± 0.27)–2.57 µm thick.

Table 1. Pollen characters of 13 species of *Alternanthera* measured using light microscopy.

Taxon	D min (mean ± var)		DBTP min (mean ± var)		PMD min (mean ± var)		FR min (mean ± var)		MW min (mean ± var)		SL min (mean ± var)							
	max	(µm)	max	(µm)	max	(µm)	max	(µm)	max	(µm)	max	(µm)						
<i>A. caracasana</i>	20.02	(24.69 ± 2.54)	29.64	6.82	(8.76 ± 0.99)	10.61	3.76	(5.67 ± 1.06)	9.06	0.32	(0.42 ± 0.07)	0.55	9.08	(10.90 ± 1.47)	13.72	0.33	(0.42 ± 0.07)	0.61
<i>A. costaricensis</i>	23.94	(30.98 ± 4.01)	37.75	6.0	(7.65 ± 0.95)	9.11	4.48	(5.87 ± 0.65)	7.12	0.30	(0.34 ± 0.04)	0.42	6.28	(8.54 ± 1.04)	10.24	0.47	(0.72 ± 0.16)	1.03
<i>A. echinocephala</i>	17.51	(25.08 ± 5.52)	36.26	5.50	(7.71 ± 1.34)	10.31	3.23	(5.47 ± 1.02)	8.12	0.20	(0.28 ± 0.03)	0.36	4.76	(8.35 ± 1.31)	10.22	0.33	(0.53 ± 0.12)	0.73
<i>A. flava</i>	18.16	(22.34 ± 1.96)	24.71	5.92	(8.35 ± 1.28)	10.44	4.73	(6.81 ± 1.03)	8.94	0.20	(0.29 ± 0.05)	0.39	6.03	(9.33 ± 1.52)	11.71	0.44	(0.62 ± 0.11)	0.79
<i>A. flavescens</i>	19.31	(23.80 ± 1.72)	27.78	7.38	(9.0 ± 0.68)	10.97	5.56	(7.06 ± 0.77)	8.48	0.23	(0.32 ± 0.05)	0.40	7.00	(9.77 ± 1.88)	13.27	0.50	(0.70 ± 0.12)	0.99
<i>A. galapagensis</i>	12.89	(18.19 ± 2.73)	23.42	4.75	(7.45 ± 1.16)	10.31	3.25	(4.90 ± 0.84)	6.16	0.33	(0.48 ± 0.08)	0.64	6.43	(10.89 ± 1.61)	13.22	0.43	(0.62 ± 0.10)	0.80
<i>A. geniculata</i>	22.01	(29.77 ± 3.22)	35.56	7.69	(10.57 ± 1.40)	14.26	6.42	(8.86 ± 1.09)	10.97	0.23	(0.34 ± 0.05)	0.45	10.76	(14.77 ± 1.63)	16.66	0.58	(1.08 ± 0.27)	1.55
<i>A. lanceolata</i>	14.63	(17.39 ± 1.50)	20.53	5.07	(6.23 ± 0.63)	7.43	3.36	(4.43 ± 0.57)	5.69	0.21	(0.30 ± 0.05)	0.40	5.81	(8.15 ± 1.07)	10.71	0.30	(0.48 ± 0.08)	0.62
<i>A. maeburdei</i>	14.55	(22.00 ± 2.87)	27.76	5.54	(7.74 ± 1.35)	10.93	3.84	(5.43 ± 1.04)	7.32	0.20	(0.31 ± 0.06)	0.40	5.20	(8.15 ± 1.60)	11.60	0.36	(0.56 ± 0.11)	0.80
<i>A. littoralis</i> var. <i>maritima</i>	16.22	(21.79 ± 3.26)	28.08	5.72	(8.16 ± 1.36)	10.56	4.07	(5.91 ± 1.27)	7.54	0.30	(0.41 ± 0.05)	0.48	7.56	(10.74 ± 1.88)	13.62	0.33	(0.50 ± 0.09)	0.70
<i>A. obovata</i>	17.20	(21.75 ± 2.29)	26.50	5.0	(8.32 ± 1.59)	10.88	3.0	(5.95 ± 1.58)	8.94	0.20	(0.36 ± 0.08)	0.50	6.96	(11.50 ± 2.46)	15.65	0.33	(0.47 ± 0.08)	0.59
<i>A. serpyllifolia</i>	18.43	(21.90 ± 2.91)	30.87	6.42	(8.50 ± 1.42)	12.28	4.17	(5.71 ± 1.23)	9.47	0.20	(0.31 ± 0.04)	0.40	8.07	(9.65 ± 1.36)	12.87	0		
<i>A. tenella</i>	12.39	(17.65 ± 2.19)	21.23	5.10	(7.19 ± 0.92)	8.68	2.58	(4.75 ± 0.98)	6.43	0.28	(0.45 ± 0.07)	0.62	7.23	(9.67 ± 1.06)	11.23	0.30	(0.41 ± 0.06)	0.50

Note: D, diameter; DBTP, distance between two pores; FR, flat ring; MW, metareticulum width; PMD, pore membrane diameter; SL, spine length.

Table II. Pollen characters of 13 species of *Alternanthera* measured using light microscopy.

Taxon	TSE min (mean ± var)		TCP min (mean ± var)		CSE min (mean ± var)		CCP min (mean ± var)		NSE min (mean ± var)		NCP min (mean ± var)		SSE min (mean ± var)		SCP min (mean ± var)	
	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)	max (µm)
<i>A. canacasana</i>	0.39 (0.48 ± 0.06)	0.61	0.79 (0.99 ± 0.13)	1.22	0.55 (0.82 ± 0.15)	1.24	0.70 (0.98 ± 0.19)	1.37	0.30 (0.34 ± 0.04)	0.44	0.30 (0.37 ± 0.04)	0.46	1.02 (1.30 ± 0.14)	1.64	1.18 (1.80 ± 0.36)	2.43
<i>A. costaricensis</i>	0.30 (0.46 ± 0.10)	0.68	0.54 (0.76 ± 0.12)	0.95	1.10 (1.52 ± 0.30)	2.25	1.54 (2.02 ± 0.34)	2.87	0.29 (0.36 ± 0.04)	0.44	0.30 (0.36 ± 0.03)	0.42	1.52 (2.21 ± 0.44)	3.07	1.99 (2.81 ± 0.43)	3.71
<i>A. echinocephala</i>	0.19 (0.34 ± 0.07)	0.48	0.50 (0.76 ± 0.14)	1.0	0.50 (0.85 ± 0.22)	1.33	0.80 (1.12 ± 0.20)	1.53	0.22 (0.31 ± 0.04)	0.40	0.21 (0.30 ± 0.05)	0.42	0.74 (1.27 ± 0.31)	1.87	1.17 (1.88 ± 0.32)	2.43
<i>A. flava</i>	0.20 (0.37 ± 0.10)	0.68	0.50 (0.82 ± 0.13)	1.07	0.53 (0.80 ± 0.13)	1.07	0.70 (1.03 ± 0.16)	1.38	0.20 (0.32 ± 0.05)	0.40	0.20 (0.28 ± 0.04)	0.37	0.82 (1.33 ± 0.27)	1.92	1.13 (1.93 ± 0.34)	2.49
<i>A. flavescens</i>	0.25 (0.42 ± 0.11)	0.66	0.70 (0.92 ± 0.11)	1.09	0.70 (1.08 ± 0.17)	1.36	0.97 (1.29 ± 0.18)	1.62	0.22 (0.30 ± 0.04)	0.39	0.24 (0.33 ± 0.04)	0.40	1.29 (1.64 ± 0.28)	2.41	1.52 (2.22 ± 0.32)	2.87
<i>A. galapagensis</i>	0.36 (0.49 ± 0.07)	0.67	0.61 (0.82 ± 0.10)	0.99	0.50 (0.71 ± 0.12)	0.94	0.71 (1.03 ± 0.16)	1.38	0.33 (0.41 ± 0.05)	0.49	0.32 (0.40 ± 0.04)	0.47	1.02 (1.21 ± 0.13)	1.46	1.07 (1.75 ± 0.25)	2.88
<i>A. geniculata</i>	0.35 (0.53 ± 0.10)	0.74	0.67 (1.03 ± 0.17)	1.45	1.10 (1.74 ± 0.32)	2.30	1.15 (2.15 ± 0.41)	2.90	0.25 (0.32 ± 0.05)	0.50	0.22 (0.32 ± 0.04)	0.41	1.55 (2.24 ± 0.33)	2.95	1.67 (2.59 ± 0.42)	3.64
<i>A. lanceolata</i>	0.22 (0.36 ± 0.06)	0.53	0.44 (0.60 ± 0.12)	0.92	0.45 (0.66 ± 0.09)	0.83	0.63 (0.90 ± 0.15)	1.26	0.21 (0.29 ± 0.05)	0.38	0.22 (0.31 ± 0.04)	0.39	0.85 (1.18 ± 0.19)	1.62	1.04 (1.42 ± 0.21)	1.79
<i>A. macbridei</i>	0.20 (0.36 ± 0.11)	0.57	0.47 (0.72 ± 0.16)	0.97	0.40 (0.68 ± 0.18)	1.20	0.52 (0.86 ± 0.21)	1.34	0.23 (0.29 ± 0.04)	0.38	0.25 (0.31 ± 0.03)	0.36	0.70 (1.17 ± 0.28)	1.73	1.03 (1.52 ± 0.33)	2.20
<i>A. litorealis</i> var. <i>maritima</i>	0.38 (0.48 ± 0.06)	0.57	0.60 (0.80 ± 0.13)	1.08	0.51 (0.79 ± 0.18)	1.29	0.89 (1.10 ± 0.17)	1.50	0.30 (0.37 ± 0.04)	0.45	0.30 (0.38 ± 0.04)	0.44	0.88 (1.25 ± 0.23)	1.94	1.52 (1.90 ± 0.27)	2.57
<i>A. obovata</i>	0.32 (0.48 ± 0.08)	0.72	0.53 (0.89 ± 0.19)	1.24	0.65 (0.95 ± 0.17)	1.33	0.72 (1.07 ± 0.25)	1.90	0.24 (0.36 ± 0.07)	0.52	0.28 (0.39 ± 0.05)	0.49	1.15 (1.47 ± 0.23)	2.01	1.37 (1.92 ± 0.37)	2.85
<i>A. serpyllifolia</i>	0.33 (0.47 ± 0.08)	0.75	0.42 (0.85 ± 0.15)	1.15	0.58 (0.98 ± 0.26)	1.51	0.62 (0.87 ± 0.19)	1.39	0.23 (0.31 ± 0.04)	0.45	0.23 (0.31 ± 0.04)	0.45	1.03 (1.47 ± 0.28)	1.95	1.12 (1.82 ± 0.36)	2.51
<i>A. tenella</i>	0.34 (0.43 ± 0.06)	0.57	0.55 (0.79 ± 0.11)	0.97	0.45 (0.71 ± 0.12)	0.94	0.68 (0.97 ± 0.18)	1.52	0.28 (0.38 ± 0.06)	0.53	0.29 (0.40 ± 0.06)	0.49	0.94 (1.18 ± 0.15)	1.57	1.18 (1.62 ± 0.23)	2.0

Note: CCP, columellae on conjunction points; CSE, columellae on structural elements; NCP, nexine of conjunction points; NSE, nexine of structural elements; SCP, sexine of conjunction points; SSE, sexine of structural elements; TCP, tectum on conjunction points; TSE, tectum on structural elements.

*Alternanthera macbridei* (Figures 2U–V, 5M–N, 8M–P). — Pollen dodecahedral, 14.55–(22.00 ± 2.87)–27.76 µm in diameter. Pores 12, distance between two pores 5.54–(7.74 ± 1.35)–10.93 µm; pore membrane 3.84–(5.43 ± 1.04)–7.32 µm in diameter, ektexinous bodies 40, nanospines 77–128. Flat ring 0.20–(0.31 ± 0.06)–0.40 µm wide. Metareticulum pentagonal or hexagonal, 5.20–(8.15 ± 1.60)–11.60 µm wide; structural elements and conjunction points 5–6. Mesopodium 1.46–1.65 µm wide. Sculpture punctate, nano- and microspinulate; around 15–20 nanopores per structural element, relatively forming two rows; 7–8 nanospines and microspines per structural element, 0.36–(0.56 ± 0.11)–0.80 µm long, in one or two rows on each structural element. Tectum on structural elements 0.20–(0.36 ± 0.11)–0.57 µm high, tectum on conjunction points 0.47–(0.72 ± 0.16)–0.97 µm high; columellae 6–9, arranged in one or two rows on structural elements, each 0.40–(0.68 ± 0.18)–1.20 µm high, 0.33–0.37 µm wide, columellae on the conjunction points 0.52–(0.86 ± 0.21)–1.34 µm high, 0.43–0.53 µm wide; nexine of structural elements 0.23–(0.29 ± 0.04)–0.38 µm thick, nexine of conjunction points 0.25–(0.31 ± 0.03)–0.36 µm thick; sexine of structural elements 0.70–(1.17 ± 0.28)–1.73 µm thick, sexine of conjunction points 1.03–(1.52 ± 0.33)–2.20 µm thick.

*Alternanthera obovata* (Figures 2W–X, 6D–F, 9E–G). — Pollen dodecahedral, 17.20–(21.75 ± 2.29)–26.50 µm in diameter. Pores 12, distance between two pores 5.00–(8.32 ± 1.59)–10.88 µm; pore membrane 3.00–(5.95 ± 1.58)–8.94 µm in diameter, ektexinous bodies 26–36, nanospines 88–151. Flat ring 0.20–(0.36 ± 0.08)–0.50 µm wide. Metareticulum pentagonal, 6.96–(11.50 ± 2.46)–15.65 µm wide; structural elements and conjunction points 5. Mesopodium 1.3–1.99 µm wide. Sculpture punctate, nano- and microspinulate; 22–43 nanopores per structural element, relatively forming two rows; 14–20 nanospines and microspines per structural element, 0.33–(0.47 ± 0.08)–0.59 µm long, in one or two rows on each structural element. Tectum on structural elements 0.32–(0.48 ± 0.08)–0.72 µm high, tectum on conjunction points 0.53–(0.89 ± 0.19)–1.24 µm high; columellae 6–9, arranged in one or two rows in the structural elements, each 0.65–(0.95 ± 0.17)–1.33 µm high, 0.36–0.57 µm wide, columellae on the conjunction points 0.72–(1.07 ± 0.25)–1.90 µm high, 0.45–0.57 µm wide; nexine of structural elements 0.24–(0.36 ± 0.07)–0.52 µm thick, nexine of conjunction points 0.28–(0.39 ± 0.05)–0.49 µm thick; sexine of structural elements 1.15–(1.47 ± 0.23)–2.01 µm thick, sexine of conjunction points 1.37–(1.92 ± 0.37)–2.85 µm thick.

*Alternanthera serpyllifolia* (Figures 3A–B, 6G–I, 9H–J). — Pollen dodecahedral and spheroidal, 18.43–(21.90 ± 2.91)–30.87 µm in diameter. Pores both 12 and 14–16, distance between two pores 6.42–(8.50 ± 1.42)–12.28 µm; pore membrane 4.17–(5.71 ± 1.23)–9.47 µm in diameter, ektexinous bodies 20–22, nanospines 32–59. Flat ring 0.20–(0.31 ± 0.04)–0.40 µm wide. Metareticulum pentagonal or hexagonal, 8.07–(9.65 ± 1.36)–12.87 µm in wide; structural elements and conjunction points 5–6. Mesopodium 1.31–2.0 µm wide. Sculpture punctate, tectum surface never has spines; 10–13 nanopores per structural element, relatively forming two rows. Tectum on structural elements 0.33–(0.47 ± 0.08)–0.75 µm high, tectum on conjunction points 0.42–(0.85 ± 0.15)–1.15 µm high; columellae 8–9, arranged in one or two rows on structural elements, each 0.58–(0.98 ± 0.26)–1.51 µm high, 0.38–0.41 µm wide, columellae on the conjunction points 0.62–(0.87 ± 0.19)–1.39 µm high, 0.48–0.55 µm wide; nexine of structural elements 0.23–(0.31 ± 0.04)–0.45 µm thick, nexine of conjunction points 0.23–(0.31 ± 0.04)–0.45 µm thick; sexine of structural elements 1.03–(1.47 ± 0.28)–1.95 µm thick, sexine of conjunction points 1.12–(1.82 ± 0.36)–2.51 µm thick.

*Alternanthera tenella* (Figures 3C–E, 6J–L, 9K–N). — Pollen dodecahedral, 12.39–(17.65 ± 2.19)–21.23 µm in diameter. Pores 12–14, distance between two pores 5.10–(7.19 ± 0.92)–8.68 µm; pore membrane 2.58–(4.75 ± 0.98)–6.43 µm in diameter, ektexinous bodies 26–33, nanospines 74–82. Flat ring 0.28–(0.45 ± 0.07)–0.62 µm wide. Metareticulum pentagonal or hexagonal, 7.23–(9.67 ± 1.06)–11.23 µm in wide; structural elements and conjunction points 5. Mesopodium 0.97–1.61 µm wide. Sculpture punctate and nanospinate; 11–25 nanopores per structural element, relatively ordered in a row; 8–16 nanospines per structural element, 0.30–(0.41 ± 0.06)–0.50 µm long, forming one or two rows on each structural element. Tectum on structural elements 0.34–(0.43 ± 0.06)–0.57 µm high, tectum on conjunction points 0.55–(0.79 ± 0.11)–0.97 µm high; columellae 7–12, arranged in one or two rows on structural elements, each 0.45–(0.71 ± 0.12)–0.94 µm high, 0.27–0.37 µm wide, columellae on the conjunction points 0.68–(0.97 ± 0.18)–1.52 µm high, 0.46–0.51 µm wide; nexine of structural elements 0.28–(0.38 ± 0.06)–0.53 µm thick, nexine of conjunction points 0.29–(0.40 ± 0.06)–0.49 µm thick; sexine of structural elements 0.94–(1.18 ± 0.15)–1.57 µm thick, sexine of conjunction points 1.18–(1.62 ± 0.23)–2.0 µm thick.

#### Statistical analyses

Twenty-five pollen characters, 22 quantitative and three qualitative, were analysed to study and describe pollen variation within *Alternanthera* (Tables I–IV). Among them were only 14 quantitative informative characters to be used for multivariate analyses (Tables I, II). Global similarities and dissimilarities of the pollen among species of *Alternanthera* and delimitation of the pollen types defined by the CAP and cluster analyses (based on 14 characters) suggested that ten characters were discriminative to identify differences among the species (Figures 10, 11, Table V). Characters were distributed mainly according to correlation cluster CAP2 ( $\delta = 94.19\%$ ). The discriminative characters (with values in parentheses) were pollen diameter ( $D$ , 0.46661); sexine thickness in conjunction points (SCP, 0.4324); sexine thickness in structural elements (SSE, 0.41372); columellae height at the conjunction points (CCP, 0.32758); columellae height at the structural elements (CSE, 0.31612); pore membrane diameter (PMD, 0.05061). Characters that were distributed mainly according to correlation cluster CAP1 ( $\delta = 97.91\%$ ) were spines length (SL, –0.85981), nexine of the conjunction points (NCP, 0.54919), flat ring (FR, 0.45756), and nexine of the structural elements (NSE, 0.41204) (Figure 10A, Table V). A cluster analysis was performed to identify discriminative groups among the species. Cluster analysis using a Euclidian distance of 4.5 independently recognised three major clusters of pollen types (Figures 10, 11). Cluster one included pollen specimens from *A. costaricensis*, cluster two comprised pollen samples from *A. geniculata* and cluster three consisted of pollen samples from 11 species. Cluster analyses depicted congruence with CAP analyses supporting recognition of three pollen types in *Alternanthera*.

Overlay of pollen characters such as pollen general shape, metareticulum form and size, pore number and size, distance between pores, flat ring size, pore membrane ornamentations, and sculpture of the exine are shown in the LM and SEM micrographs (Figures 2–6) and into UPGMA tree suggested that Pollen type I and II have the lowest number of ektexinous bodies on pore membranes (7–10 in pollen type I versus 15–84 in pollen type III). Based on statistical analyses and structure of the pollen exine in *Alternanthera* presented in this study (Figures 7–9), we constructed a key to pollen types for species of *Alternanthera* distributed in the New World and describe three pollen types defined by pollen diameter, pore membrane diameter, flat ring and exine ultrastructure and ornamentation (Figures 10, 11, Tables III, V).

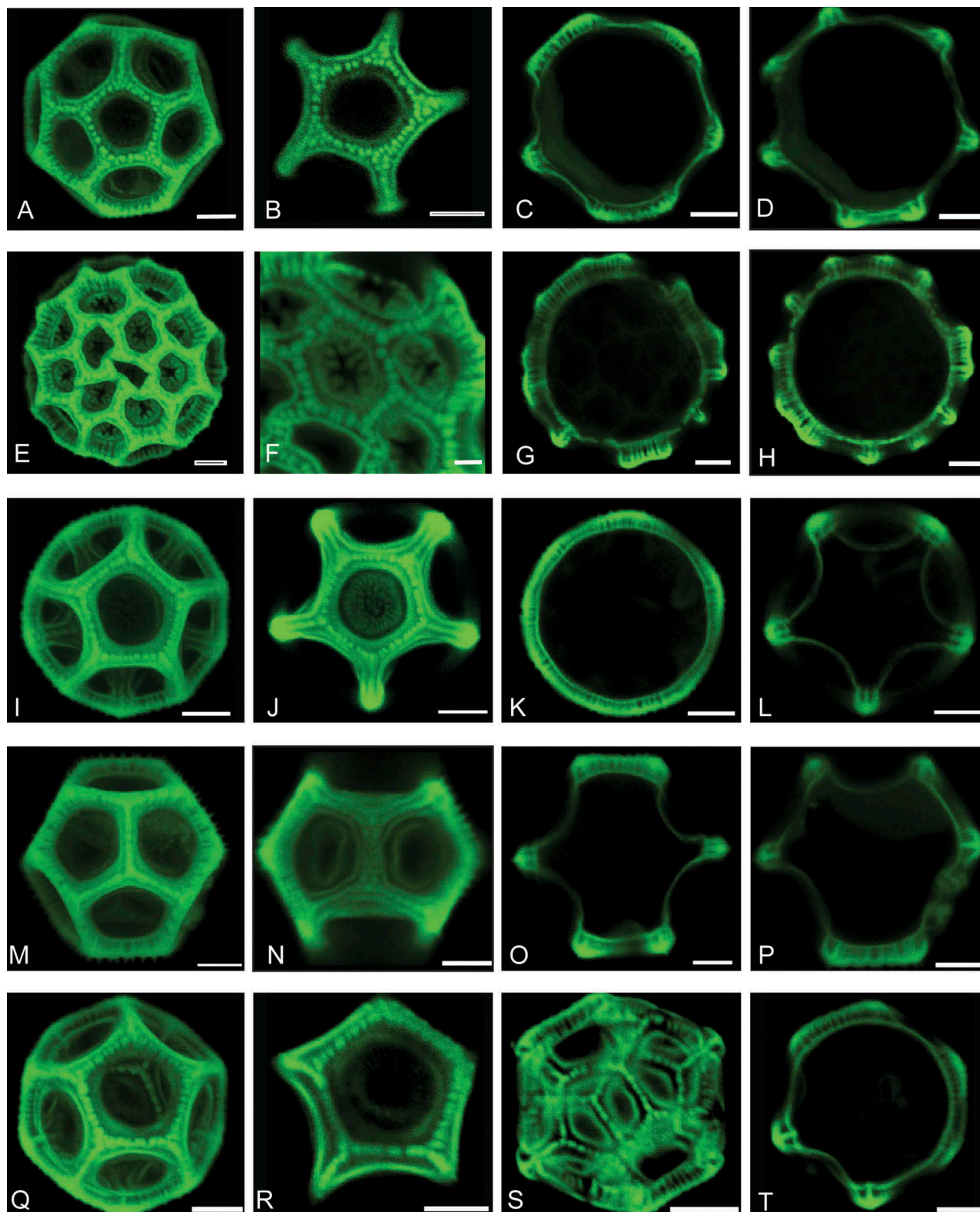


Figure 7. Confocal laser scanning microscopy of pollen grains of species of *Alternanthera*. **A–D**. Pollen grain of *A. caracasana*. **B**. Metareticula. **C**. Sexine and nexine of structural elements. **D**. Sexine and nexine of conjunction points (CPs). **E–H**. Pollen grain of *A. costaricensis*. **F**. Metareticula. **G**. Sexine and nexine in structural elements. **H**. Sexine and nexine in CPs. **I–L**. Pollen grain of *A. echinocephala*. **J**. Metareticula. **K**. Sexine and nexine of structural elements. **L**. Sexine and nexine of CPs. **M–P**. Pollen grain of *A. flava*. **N**. Metareticula. **O**. Sexine and nexine of structural elements. **P**. Sexine and nexine of CPs. **Q–T**. Pollen grain of *A. flavescens*. **R**. Metareticula. **S**. Sexine and nexine of structural elements. **T**. Sexine and nexine of CPs. Scale bars – 5  $\mu\text{m}$ .

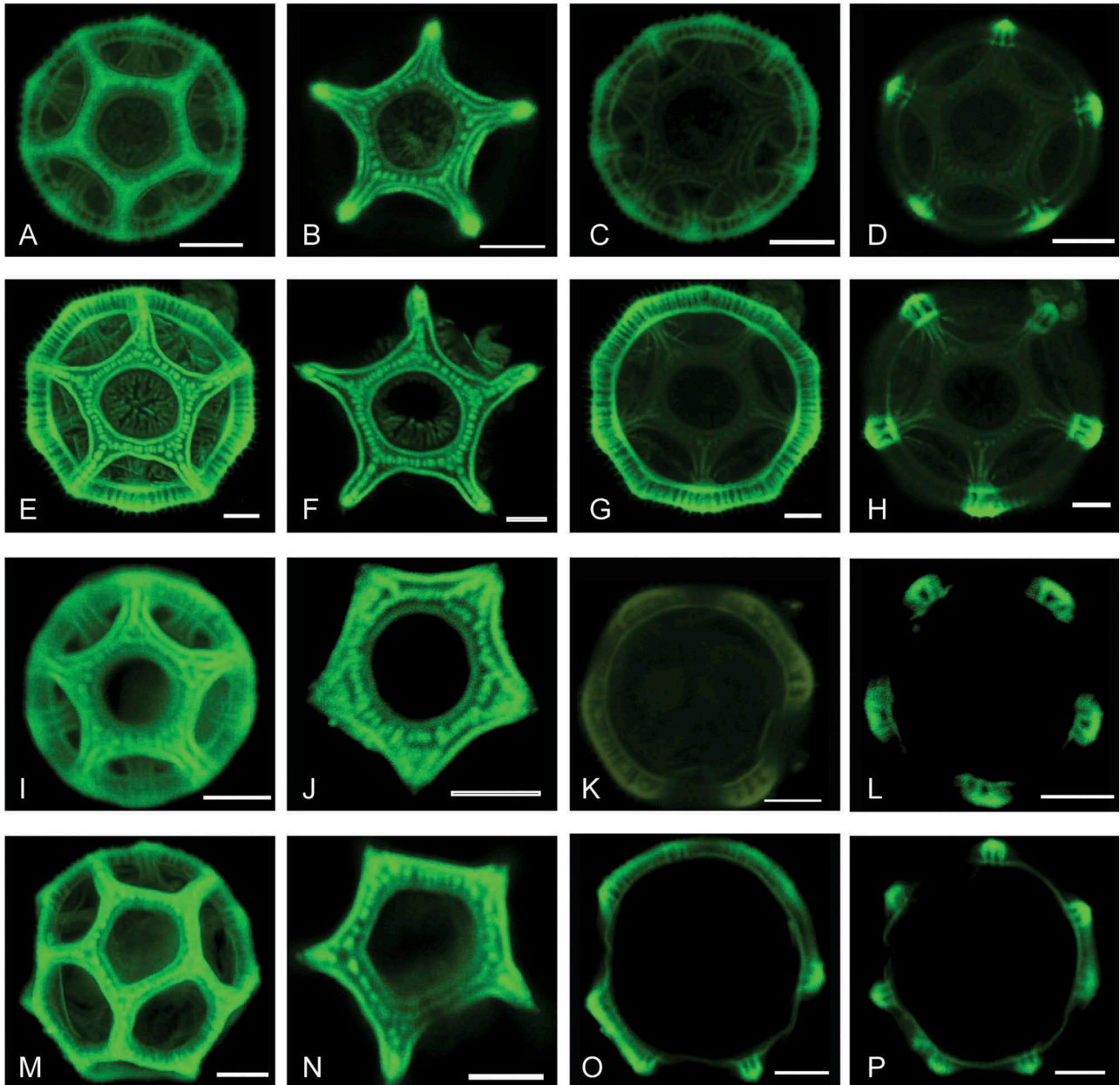


Figure 8. Confocal laser scanning microscopy of pollen grains of species of *Alternanthera*. **A–D.** Pollen grain of *A. galapagensis*. **B.** Metareticula. **C.** Sexine and nexine of structural elements. **D.** Sexine and nexine of conjunction points (CPs). **E–H.** Pollen grain of *A. geniculata*. **F.** Metareticula. **G.** Sexine and nexine of structural elements. **H.** Sexine and nexine of CPs. **I–L.** Pollen grain of *A. lanceolata*. **J.** Metareticula. **K.** Sexine and nexine of structural elements. **L.** Sexine and nexine of CPs. **M–P.** Pollen grain of *A. macbridei*. **N.** Metareticula. **O.** Sexine and nexine of structural elements. **P.** Sexine and nexine of CPs. Scale bars – 5  $\mu$ m.

*Key to pollen types in New World species of Alternanthera*

- 1a Pollen spheroidal with 24–30 pores, with *D* of 23.94–[30.98  $\pm$  4.01]–37.75  $\mu$ m and SCP of 1.99–[2.81  $\pm$  0.43]–3.71  $\mu$ m ..... Type I
- 1b Pollen dodecahedral and/or spheroidal with 12 or more pores, and *D* and SCP smaller than above ..... 2
- 2a Pollen dodecahedral with 12 pores, respectively, and with DBTP of 7.69–[10.57  $\pm$  1.40]–14.26  $\mu$ m and SL of 0.58–[1.08  $\pm$  0.27]–1.55  $\mu$ m, whereas the mean value for CSE measures 1.74  $\pm$  0.32  $\mu$ m, for CCP of 2.15  $\pm$  0.41  $\mu$ m and for SSE of 2.24  $\pm$  0.33  $\mu$ m ..... Type II
- 2b Pollen dodecahedral and spheroidal, with 12 pores or 14–24 pores, with DBTP of 4.75–



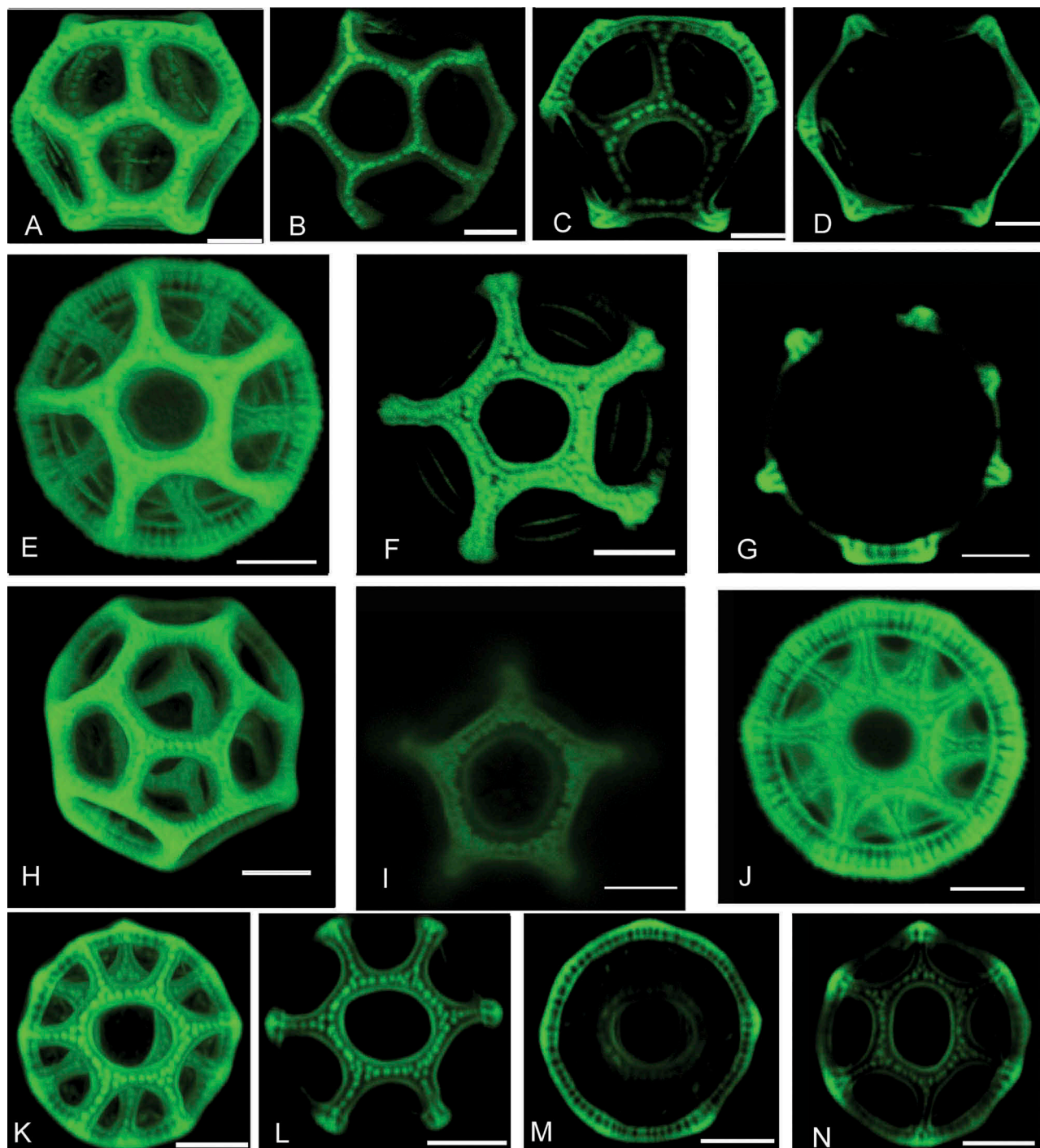


Figure 9. Confocal laser scanning microscopy of pollen grains of species of *Alternanthera*. **A–D**. Pollen grain of *A. littoralis* var. *maritima*. **B**. Metareticula. **C**. Sexine and nexine of structural elements. **D**. Sexine and nexine of conjunction points (CPs). **E–G**. Pollen grain of *A. obovata*. **F**. Metareticula. **G**. Sexine and nexine of structural elements. **H–J**. Pollen grain of *A. serpyllifolia*. **I**. Metareticula. **J**. Sexine and nexine of structural elements. **K–N**. Pollen grain of *A. tenella*. **L**. Metareticula. **M**. Sexine and nexine of structural elements. **N**. Sexine and nexine of CPs. Scale bars – 5  $\mu\text{m}$ .

( $6.23 \pm 0.63$ – $9.0 \pm 0.68$ )– $12.28 \mu\text{m}$  and SL of  $0.30$ –( $0.41 \pm 0.06$ – $0.70 \pm 0.12$ )– $0.99 \mu\text{m}$  to absent (in *Alternanthera serpyllifolia*), whereas the mean value for CSE measures

$0.68 \pm 0.18$ – $1.08 \pm 0.17 \mu\text{m}$ , for CCP of  $0.86 \pm 0.21$ – $1.29 \pm 0.18 \mu\text{m}$ , and for SSE of  $1.17 \pm 0.28$ – $1.64 \pm 0.28 \mu\text{m}$  ..... Type III

*Type I.* — Pollen spheroidal with 24–30 pores. Pollen type I has the largest mean and range dimensions among *Alternanthera* pollen for diameter ( $D$ ; 23.94–[30.98 ± 4.01]–37.75 µm) and sexine in conjunction points (SCP; 1.99–[2.81 ± 0.43]–3.71 µm). The largest mean dimensions in the genus ranged for nexine of the structural elements (NSE; 0.36 ± 0.04 µm) and nexine of the conjunction points (NCP; 0.36 ± 0.03 µm) (Figures 2C–D, 4D–F, 7E–H, 10A–B, 11, Tables III, IV). Number of ektexinous bodies 7–10. Species included: *A. costaricensis*.

*Type II.* — Pollen dodecahedral with 12 pores. Pollen type II has the largest mean and range dimensions among *Alternanthera* pollen for distance between two pores (DBTP; 7.69–[10.57 ± 1.40]–14.26 µm, pore membrane diameter (PMD; 6.42–[8.86 ± 1.09]–10.97 µm, metareticulum width (MW; 10.76–[14.77 ± 1.63]–16.66 µm), spines length (SL; 0.58–[1.08 ± 0.27]–1.55 µm), tectum width in structural elements (TSE; 0.35–[0.53 ± 0.10]–0.74 µm), tectum width in conjunction points (TCP; 0.67–[1.03 ± 0.17]–1.45 µm). The largest mean dimensions in the genus ranged for columellae height at the structural elements (CSE; 1.74 ± 0.32 µm), columellae height at the conjunction points (CCP; 2.15 ± 0.41 µm), and sexine thickness in structural elements (SSE; 2.24 ± 0.33 µm; Figures 2O, P, 5G–I, 8E–H, 10A, B, 11, Tables III, IV). Number of ektexinous bodies 20–22. Species included: *A. geniculata*.

*Type III.* — Pollen dimorphic based on pores number, mainly one dodecahedral with 12 pores and the other spheroidal with 14–24 pores. The species studied, has the smallest diameter ( $D$ ; 12.39–[17.39 ± 1.50–25.08 ± 5.52]–36.26 µm), spines length (SL; 0.30–[0.41 ± 0.06–0.70 ± 0.12]–0.99 µm) to absent (in *Alternanthera serpyllifolia*), columellae height at the structural elements (CSE; 0.40–[0.68 ± 0.18–1.08 ± 0.17]–1.51 µm), columellae height at the conjunction points (CCP; 0.52–[0.86 ± 0.21–1.29 ± 0.18]–1.90 µm), sexine thickness in structural elements (SSE; 0.70–[1.17 ± 0.28–1.64 ± 0.28]–2.41 µm), and sexine in conjunction points (SCP; 1.04–[1.42 ± 0.21–1.93 ± 0.34]–2.87 µm). In addition, DBTP measures 4.75–[6.23 ± 0.63–9.0 ± 0.68]–12.28 µm (Figures 2–9, 10A, B, 11, Tables III, IV). Number of ektexinous bodies 15–84. Species included: *A. caracasana*, *A. echinocephala*, *A. flava*, *A. flavescens*, *A. galapagensis*, *A. lanceolata*, *A. macbridei*, *A. littoralis* var. *maritima*, *A. obovata*, *A. serpyllifolia*, *A. tenella*.

## Discussion

### *Pollen morphological characters of Amaranthaceae with special emphasis in Alternanthera*

Pollen morphology of *Alternanthera* has been described in various ways depending on the author (Fægri & Iversen 1964; Erdtman 1986; Moore et al. 1991); pollen terminology has been historically variable even to describe the same pollen structure. In recent years, Borsch (1998) and Borsch and Barthlott (1998) have provided foundations for modern interpretations of pollen variation in *Alternanthera* (e.g. Chin & Lim 2011). A discussion of palynological terms and their detailed graphical and textual description is offered later as an effort for standardising the use of the terminology for pollen in the genus and other Amaranthaceae.

SEM studies of pollen morphology provide valuable three-dimensional (3D) evidence for understanding variation in pollen with reticulate-like exine sculpture (Punt et al. 1994). Such studies have been especially important for interpreting exine sculpture of Amaranthaceae. Although pollen ornamentation within Amaranthaceae has been widely described as reticulate, this extensively debated concept has been misapplied (Zandonella & Lecocq 1977; Cuadrado 1988, 1989; Eliasson 1988; Townsend 1993; Borsch 1998). For example, Riollot and Bonnefille (1976, p. 74) reported pollen of *Gomphrena celosioides* Mart. as reticulate, and reflected on the uniqueness of the pollen ornamentation mentioning that ‘[in] each mesh [of the reticula] there was a pore in the center’. To settle the matter and based on detailed morphological pollen study, Borsch and Barthlott (1998) concluded that the pollen sculpture/pore complex is not homologous to the typical reticulum and thus proposed the term metareticulate (versus reticulum) as a high-order reticulum composed of mesoporia.

A careful and exhaustive study of each component of the mesoporium in *Alternanthera* here provides the most complete palynological description of metareticulate pollen so far available for the Gomphrenoidae.

### *Terminology for the description of mesoporia variation within Alternanthera*

*Mesoporium.* — Exine in the structural elements is simplicolumellate, with very thick columellae under the point of union of structural elements or conjunction points (CPs; Figure 8D, H, L, P; Borsch 1998; Borsch & Barthlott 1998). We confirm that pollen of *Alternanthera* is of the *Pfaffia*-type (Borsch 1998) with pores covered with a pore membrane, narrow

Table III. Measures of pollen-types based on characters indicated in Tables I and II

Characters	Pollen type I	Pollen type II	Pollen type III
D min (mean ± var) max (µm)	23.94 (30.98 ± 4.01) 37.75	22.01 (29.77 ± 3.22) 35.56	12.39 (17.39 ± 1.50–25.08 ± 5.52) 36.26
DBTP min (mean ± var) max (µm)	6.0 (7.65 ± 0.95) 9.11	7.69 (10.57 ± 1.40) 14.26	4.75(6.23 ± 0.63–9.0 ± 0.68) 12.28
PMD min (mean ± var) max (µm)	4.48 (5.87 ± 0.65) 7.12	6.42 (8.86 ± 1.09) 10.97	2.58 (4.43 ± 0.57–7.06 ± 0.77) 9.47
FR min (mean ± var) max (µm)	0.30 (0.34 ± 0.04) 0.42	0.23 (0.34 ± 0.05) 0.45	0.20 (0.28 ± 0.03–0.48 ± 0.08) 0.64
MW min (mean ± var) max (µm)	6.28 (8.54 ± 1.04) 10.24	10.76 (14.77 ± 1.63) 16.66	4.76 (8.15 ± 1.07–11.50 ± 2.46) 15.65
SL min (mean ± var) max (µm)	0.47 (0.72 ± 0.16) 1.03	0.58 (1.08 ± 0.27) 1.55	0.30 (0.41 ± 0.06–0.70 ± 0.12) 0.99
TSE min (mean ± var) max (µm)	0.30 (0.46 ± 0.10) 0.68	0.35 (0.53 ± 0.10) 0.74	0.19 (0.34 ± 0.07–0.49 ± 0.07) 0.75
TCP min (mean ± var) max (µm)	0.54 (0.76 ± 0.12) 0.95	0.67 (1.03 ± 0.17) 1.45	0.42 (0.60 ± 0.12–0.99 ± 0.13) 1.24
CSE min (mean ± var) max (µm)	1.10 (1.52 ± 0.30) 2.25	1.10 (1.74 ± 0.32) 2.30	0.40 (0.68 ± 0.18–1.08 ± 0.17) 1.51
CCP min (mean ± var) max (µm)	1.54 (2.02 ± 0.34) 2.87	1.15 (2.15 ± 0.41) 2.90	0.52 (0.86 ± 0.21–1.29 ± 0.18) 1.90
NSE min (mean ± var) max (µm)	0.29 (0.36 ± 0.04) 0.44	0.25 (0.32 ± 0.05) 0.50	0.20(0.29 ± 0.04–0.41 ± 0.05) 0.53
NCP min (mean ± var) max (µm)	0.30 (0.36 ± 0.03) 0.42	0.22 (0.32 ± 0.04) 0.41	0.20 (0.28 ± 0.04–0.40 ± 0.06) 0.49
SSE min (mean ± var) max (µm)	1.52 (2.21 ± 0.44) 3.07	1.55 (2.24 ± 0.33) 2.95	0.70 (1.17 ± 0.28–1.64 ± 0.28) 2.41
SCP min (mean ± var) max (µm)	1.99 (2.81 ± 0.43) 3.71	1.67 (2.59 ± 0.42) 3.64	1.04 (1.42 ± 0.21–1.93 ± 0.34) 2.87

Note: D, diameter; DBTP, distance between two pores; FR, flat ring; MW, metareticulum width; PMD, pore membrane diameter; SL, spine length; CCP, columellae on conjunction points; CSE, columellae on structural elements; NCP, nexine of conjunction points; NSE, nexine of structural elements; SCP, sexine of conjunction points; SSE, sexine of structural elements; TCP, tectum on conjunction points; TSE, tectum on structural elements.

and vaulted mesoporia and a flat-ring at the base of the simple columellate SEs. Our observations suggested that mesoporia can be either one or two rows and simple columellate only on CPs.

*Pores.* — According to Borsch (1998), the stenopalyinous pollen of *Alternanthera* has 12 or 14 pores in the majority of species, except for *A. costaricensis* with 25–30 pores. However, Borsch's (1998) report for the genus was based on only seven species; with our expanded sampling, we found that the pore number was even more variable in other species such as *A. echinocephala* (20–24 pores) and *A. serpyllifolia* (16–24 pores). Chin and Lim (2011) reported similar changes in pore number in two Asian species (*A. paronychioides* with 18 pores and *A. philoxeroides* with 20–24 pores). At this point, we cannot confirm the extent of the variation of this character for *Alternanthera* because the genus has 80–100 diverse species (Eliasson 1987, 1990).

*Pore membrane.* — This structure has been described as a unit-membrane-like layer (Skvarla & Nowicke 1976), as a verrucose pseudo-operculum covering the pores (Martínez-Hernández et al. 1993) or as a smooth poral membrane (Palacios-Chávez et al. 1991) in different representative species of the family. Amaranthaceae specialists (Vishnu-Mittre 1963; Nowicke & Skvarla 1979; Borsch 1998; Borsch & Barthlott 1998; Müller & Borsch 2005b) have also characterised the structure as poral membrane to be covered by ektexinous bodies in Amaranthaceae. Our analyses of the *Alternanthera* exine conducted in CLSM are appropriate to define this pore membrane-like structure as a

thinning of the exine lacking a tectum and columellae layers. Detailed TEM observations of different stages of pollen pore development are needed to reach a more conclusive understanding of the origin and ontogenetic nature of this pore membrane-like structure in the family.

Pore membrane ornamentation as defined by ektexinous bodies in Amaranthaceae is granulate (Livingstone et al. 1973; Sánchez-del Pino 2001), with angular ektexinous bodies (Skvarla & Nowicke 1976) or verrucose (Zandonella & Lecocq 1977). Based on SEM, Borsch (1998) described more than five ektexinous body shapes (tooth-shaped, roundish, polyhedral, triangular, quadrangular), variations in their number and arrangement and number of microspines on ektexinous bodies. Ektexinous bodies morphology and pore diameter are the basis for defining 11 types of Amaranthaceae pores. Pore type I, characteristic of *Alternanthera*, is described as having '... ektexinous bodies (12)20–60, closely adjoined, arranged in a mosaic-like pattern, ± rectangular, sinuous or elongate, 1.5–3.0 times as long as broad, the short sides pointing to the centre of the pore, ± equal in size, with 1–2(3–4) distinct microspines; pores (1.8–)3.0–6.0 µm in diameter, at the margin ± confluent into a solid, flat ring' (Borsch 1998, p. 130).

In the present study, we observed that the number of ektexinous bodies can be <12 and >60 (Table IV) and we agree with Chin and Lim (2011) that the number of ektexinous bodies in species such as *Alternanthera costaricensis* seems to be related to the size of the pores. Pollen types differentiate by this variation, too. The pore diameter can be bigger than 6.0 µm (Table I) and microspines on ektexinous bodies can

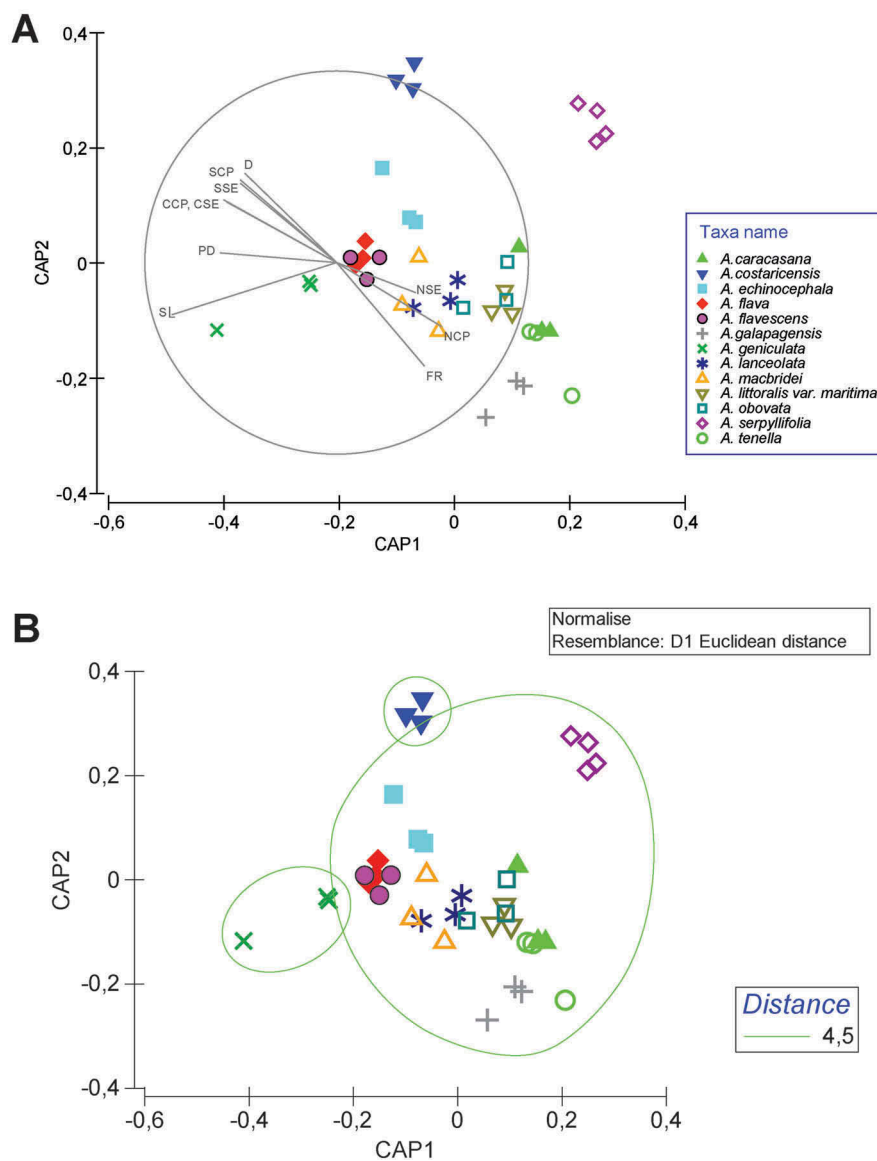


Figure 10. Canonical analysis of principal coordinates (CAP) and UPGMA tree from cluster analysis of pollen characters in *Alternanthera*. **A.** CAP ordination plots showing ten pollen characters as discriminative. **B.** CAP ordination plots showing three groups defined with Euclidean distance of 4.5.

be completely absent. Other than these variations, we concord with Borsch's (1998) description for ektexinous bodies (form and arrangement). An additional structure reported as part of the aperture is the flat ring that is described later.

*Flat ring.* — Different terms have been applied for this structure; it has been named torus (Sánchez-del Pino 2001 in *Tidestromia*); Punt et al. (1994) referred to it as an annulus or the area of the exine surrounding a pore that is sharply differentiated from the remainder of the exine, either in ornamentation or thickness. In the present work, we adopt Borsch's (1998) flat ring definition; it is the first, most complete and, in our opinion, the best description for differentiating the flanking area of the pollen pore membrane in Amaranthaceae. Based on canonical

studies and statistical values, this structure can be informative at species level and it is relevant to differentiate pollen types (Figure 10A).

*Tectum ornamentation.* — Microspines located on top of the tectum were classified, depending on size and shape (e.g. toothed conical or convex concave), by Borsch (1998) as small (*c.* 150 nm), medium (*c.* 400 nm) or long (*c.* 800 nm). However, the size of sculptures seen in the present sampling fall within the range of nanospines (<0.5  $\mu\text{m}$  in long), microspines (<1  $\mu\text{m}$  long) or echinae (>1  $\mu\text{m}$  in long) as defined by Punt et al. (1994) and we use these definitions to describe pollen sculpture of *Alternanthera*.

*Sexine.* — In the genus, sexine thickness of the SEs and the CPs differs considerably among species

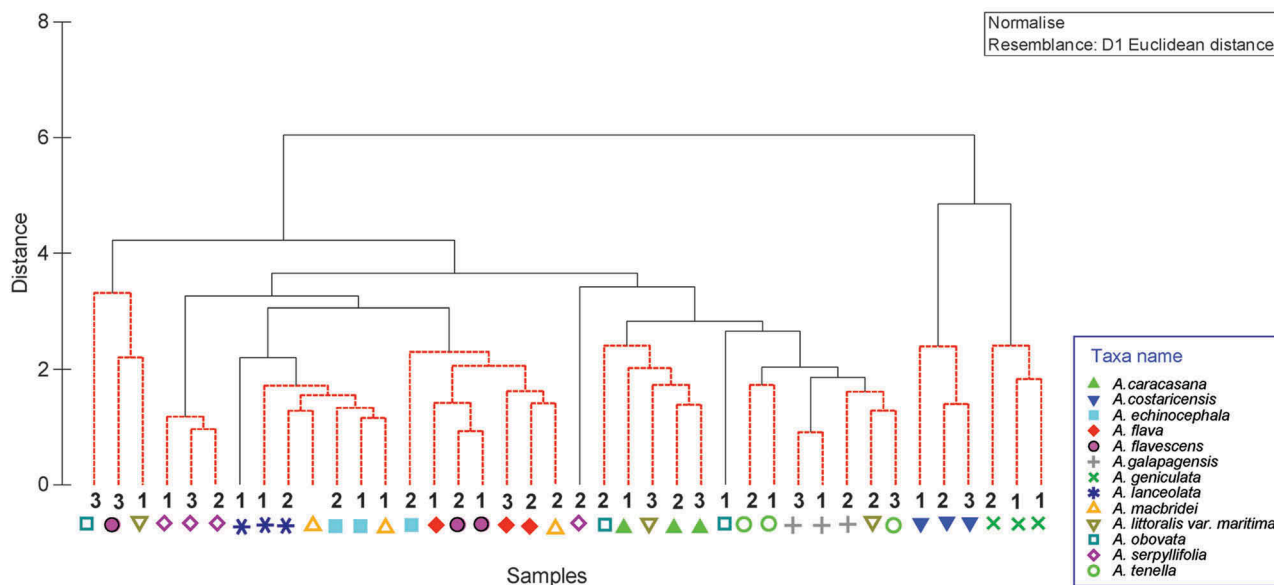


Figure 11. UPGMA tree from cluster analyses using Euclidean distance matrices.

(Table II), but multivariate analyses indicate that the variation helps to differentiate three pollen types within the genus. Pollen type III has the thinnest sexine compared to pollen types I and II (Table III).

*Pollen morphology useful in the circumscription of species of Alternanthera*

*Intraspecific pollen variation.* — The most evident variation seen within *Alternanthera* species was related to pollen form and pollen number. For example *A. littoralis* var. *maritima* had both dodecahedral and cubic pollen, the latter less in abundance. Similar cubic pollen and pollen abundance has also been reported for *Tidestromia suffruticosa* Standl. var. *suffruticosa* (Sánchez-del Pino 2001; Sánchez-del Pino & Flores Olvera 2006). *Alternanthera echinocephala* and *A. serpyllifolia* had dodecahedral and spheroidal pollen grains in less number.

*Interspecific pollen variation.* — Our analysis of pollen grains suggested 25 informative characters; 22 were quantitative (Tables I–IV) and three qualitative (Table IV). The combination of a number of characters rather than individual pollen features helped in recognising groups or species. Borsch (1998) already mentioned that the number of punctae and microspines and their ratio to each other is characteristic at species level. We confirm the importance of these characters and include additional characters. The quantitative characters were variable enough to allow us to recognise and identify groups of species based on pollen diameter, pore membrane diameter,

flat ring, and exine ultrastructure and ornamentation (Figures 10, 11, Table IV).

Our study indicated that pollen shape in *Alternanthera* between single species can be dodecahedral or spherical and as proposed by Borsch (1998) closely related to changes in pore number. Similar patterns of pollen shape–aperture number variation has been seen in other species of Amaranthaceae (Borsch 1998; Chin & Lim 2011). Most of the species sampled in the present study have dodecahedral and spheroidal pollen (e.g. *A. echinocephala*, *A. serpyllifolia*). Only grains of *A. costaricensis* are all spheroidal and, according to Chin and Lim (2011), *A. philoxeroides* has also been described with all spheroidal pollen (Li et al. [1993] cited by Chin & Lim 2011). The variation of pollen shape suggest that this character is evolutionary labile and thus in conjunction with pore number with minimal phylogenetic signal.

The palynological work of Chin and Lim (2011) on *Alternanthera* considered features such as microspines size, shape, number and arrangement as well as number of ectexinous bodies and perforation distribution that have not been commonly reported for the genus. In this study, the number of ectexinous bodies help to differentiate pollen at species level and pollen types whereas the two arrangements of nanopores on structural elements (relatively ordered in a row and relatively ordered in two rows) helped to distinguish pollen at species level. Therefore, we agree with Chin and Lim (2011) about the relevance of the characters ‘number of ectexinous bodies’ and

‘distribution of perforations at the mesoporia’ to differentiate species. In addition to these characters, we characterised variation for nine additional characters with potential usefulness for understanding and summarising pollen morphological changes among species of *Alternanthera*. These characters include ‘metareticulum width (MW)’, ‘distances between two conjunction points (DBTP)’, ‘flat ring (FR)’, ‘sexine width in structural elements (SSE)’, ‘sexine width in conjunction points (SCP)’, ‘tectum on structural elements (TSE)’, ‘tectum on conjunction points (TCP)’, ‘columellae width’ and ‘number of columellae per mesopodium’.

Cluster analysis using a Euclidian distance of 4.5 (Figures 10, 11) identified three major pollen groups. One group comprises most of the species included in the present study (Figure 11). Our data also indicate that subgroups within pollen type III (Figure 11) could be recognised based on the ten discriminative characters (Figure 10A) and perhaps delimitation of pollen groups and subgroups within pollen type III would closely reflect phylogenetic relationships proposed by Sánchez del-Pino et al. (2012). However given our sampling and extent of the variation we preferred to describe and formally recognise here pollen groups.

A cluster analyses allowed to distinguish interspecific variation based on rare pollen grains observed in the sampling such as in *Alternanthera littoralis* var. *maritima*, which has been considered an hybrid in other analyses (Sánchez del-Pino et al. 2012), and *A. echinocephala*, a Galapagos endemic based on a population of pollen grains with amorphous metareticula and skewed structural elements absent of spines.

The pollen characters useful to characterise species and by level of importance were ‘pollen ornamentation’, ‘pollen form’ and ‘pores number’, ‘spines length’, ‘number of ektexinous bodies on pore membranes’, ‘arrangement of nanopores on structural elements’, ‘number of row of spines per structural element’, ‘number of nanopores on ektexinous bodies’ and ‘metareticula form’. The less informative characters to distinguish species were ‘number of nanopores’, ‘sculpture type’, ‘number of spines on tectum’, ‘number of columellae per structural element’ and ‘number of rows of columellae per structural element’ (Table IV).

**Conclusions**

Pollen observations, images of pollen morphology and characterisation of diversity of 13 species of *Alternanthera* are presented here. Multivariate analyses show that a seemingly subtle morphological variation of 14 pollen characters in representative species of

Table IV. Pollen characters of 13 species of *Alternanthera* observed and measured using SEM.

Taxon	PN	M	Pore membrane				Tectum				Columellae		
			Ektexinous bodies				ANP	NS	ST	SRSE	CSE	CRSE	
			NEB	NNS	NNP	NNS							
<i>A. caracasana</i>	12-14	Pentagonal-Hexagonal	15-40	70-130	15-20	Relatively forming two rows	8-10	Nano-,microspines	1,2	6-9	1, 2		
<i>A. costaricensis</i>	24-30	Pentagonal-Hexagonal	7-10	10-16	8-15	Relatively forming two rows	4-7	Nano-, microspines or echinae	1	3-4	1		
<i>A. echinocephala</i>	12; 20-24	Pentagonal-Hexagonal	36-54	68-148	14-30	Relatively forming two rows	6-7	Nano-, microspines	1	6-12	1, 2		
<i>A. flava</i>	12	Pentagonal	37-84	80-112	15-22	Relatively ordered in a row	7-8	Nano-, microspines	1, 2	6-12	1, 2		
<i>A. flavescens</i>	12	Pentagonal	46-64	146-174	14-30	Relatively forming two rows	6-8	Microspines	1	7-13	1, 2		
<i>A. galapagensis</i>	12	Pentagonal	27-42	60-93	6-13	Relatively ordered in a row	7-9	Nano-, microspines	1	7-8	1		
<i>A. geniculata</i>	12	Pentagonal	20-22	27-29	10-25	Relatively forming two rows	4-9	Microspines or echinae	1	7-14	1, 2		
<i>A. lanceolata</i>	12	Pentagonal	29-30	140-164	14-19	Relatively forming two rows	6-10	Nano-, microspines	1,2	7	1		
<i>A. macbridei</i>	12	Pentagonal-Hexagonal	40	77-128	15-20	Relatively forming two rows	7-8	Nano-, microspines	1,2	6-9	1, 2		
<i>A. littoralis</i> var. <i>maritima</i>	12	Pentagonal-Hexagonal	28-29	65-85	6-13	Relatively ordered in a row	8-10	Nano-, microspines	1,2	6-9	1, 2		
<i>A. obovata</i>	12	Pentagonal	26-36	85-151	22-43	Relatively forming two rows	14-20	Nano-, microspines	1,2	6-9	1, 2		
<i>A. serpyllifolia</i>	12; 14-16	Pentagonal-Hexagonal	20-22	32-56	10-13	Relatively forming two rows	0	Abscent	0	7-8	1, 2		
<i>A. tenella</i>	12-14	Pentagonal-Hexagonal	74-82	26-33	11-25	Relatively ordered in a row	8-16	Nano-, microspines	1,2	7-12	1, 2		

Note: ANP, arrangement of nanopores on structural element; CRSE, number of rows of columellae per structural element; CSE, number of columellae per structural element; M, metareticulum; NEB, number of ektexinous bodies on pore membrane; NNP, number of nanopores on structural element; NNS, number of nanopores on ektexinous bodies; NS, number of spines on tectum; PN, pore number; SRSE, number of rows per structural element; ST, sculpture type.

Table V. Correlation of the matrix of pollen variables with the canonical axis projected in the CAP plot.

Axis	D	PMD	FR	MW	SL	DBTP	NSE
CAP1	-0.479493132	-0.607748858	0.457559165	-0.177357534	-0.859806886	-0.314571512	0.412039222
CAP2	0.466607527	0.050608826	-0.541035749	-0.335053703	-0.27241842	0.009955611	-0.15672122
CAP3	-0.353255477	-0.181654596	-0.542640829	-0.470395877	-0.389286056	-0.171479229	-0.536398723
CAP4	-0.117556773	-0.553838935	0.085357969	-0.6832162	0.062647948	-0.619273312	0.061097289
CAP5	-0.523664245	-0.218513072	-0.262644692	-0.227351493	-0.159628577	-0.466221531	-0.039535086
CAP6	-0.144828127	-0.177965604	0.124307003	0.044433333	0.020082317	-0.216562709	0.096624096
CAP7	-0.010296596	0.102188206	0.25437138	0.116596117	0.025947363	0.117729938	0.664979492
CAP8	0.302347137	0.415442566	0.114392123	0.25636653	0.022530648	0.43844726	-0.184637499
Axis	SSE	TSE	CSE	NCP	SCP	TCP	CCP
CAP1	-0.502096934	0.204725302	-0.573338683	0.549187657	-0.503264502	-0.035522641	-0.589964066
CAP2	0.413715945	-0.065981508	0.316117277	-0.33489142	0.432402795	-0.048772898	0.327582862
CAP3	-0.640421202	-0.730995363	-0.643491074	-0.625141627	-0.598542205	-0.26008962	-0.679170698
CAP4	-0.266501085	-0.517163358	-0.259116669	0.068679812	-0.177740385	-0.699042817	-0.146182177
CAP5	-0.155742631	-0.257403745	-0.180655185	-0.07004993	-0.250817049	-0.563107973	-0.105356451
CAP6	-0.141761739	0.083981897	-0.104593092	-0.326014947	-0.034133151	-0.137279796	0.120118003
CAP7	-0.111716607	-0.065263327	-0.122406217	0.233328076	0.173168008	0.235389508	-0.045637269
CAP8	0.14074567	-0.059811448	0.127651173	-0.109287021	0.119102358	-0.183865884	0.073453951

Note: CCP, columellae on conjunction points; CSE, columellae on structural elements; D, diameter; DBTP, distance between two pores; FR, flat ring; MW, metareticulum width; NCP, nexine of conjunction points; NSE, nexine of structural elements; PMD, pore membrane diameter; SCP, sexine of conjunction points; SL, spine length; SSE, sexine of structural elements; TCP, tectum on conjunction points; TSE, tectum on structural elements.

*Alternanthera* is useful to recognise species and three main pollen types within the genus. These pollen types further subdivide the *Pffafia*-type pollen characteristic of *Alternanthera* assigned by Borsch (1998). The subdivision of the *Pffafia*-type pollen into three pollen types is sustained by ten characters that were discriminative based on the multivariate analyses and corresponds to the pollen diameter (D), sexine width in structural elements (SSE) and in the conjunction points (SCP), columellae on structural elements (CSE) and on conjunction points (CCP), pore membrane diameter (PMD), spine length (SL), nexine width in structural elements (NSE) and in conjunction points (NCP) and flat ring (FR). These characters are thoroughly described here for the genus.

Pollen of *Alternanthera* consists of a complex morphological structure. Here, we have revised the terminology used in previous works to determine, which characters are of taxonomic importance and to clearly define the terminology.

Pollen grains of *Alternanthera* are metareticulate, of the *Pffafia*-type and dodecahedral or spheroidal. Mesoporia are tectate, with perforations (nanopores) and mostly spinulate except for *A. serpyllifolia*. Mesoporia are formed by structural elements and conjunction points. Pantoporate pollen has poral membranes covered with ektexinous bodies arranged toward the centre of the pore and having nanopores. Pollen grains have a psilate flat ring surrounding the poral membrane.

Pollen morphology helped in taxonomically circumscribing the *Alternanthera* taxa. The nine quan-

titative pollen characters that were useful to characterise species of *Alternanthera* are 'pollen form and ornamentation', 'pore number', 'spines length and arrangement on mesoporus' (or one structural element), 'number of ektexinous bodies on poral membrane', 'number of nanopores on poral membrane', 'arrangement of nanopores on mesoporus' (or one structural element) and 'metareticula form'.

Pollen type I, as defined in this study and represented by *Alternanthera costaricensis*, and pollen type II, represented by *A. geniculata*, show notable changes in diameter, distance between two pores, pore membrane diameter, metareticulum width, spine length and tectum and sexine thickness.

With more studies like the one presented here, our knowledge about pollen variation in *Alternanthera* is increasing and laying the basis for evolutionary studies. Our reports in conjunction with previous studies show that pore number varies from the commonly reported 12–14 that has been considered a constant number for the pollen of the genus and that could be informative to separate species groups.

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