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Palynotaxonomy of Neotropical species of *Dioscorea* L. (Dioscoreaceae)

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ABSTRACT
Nineteen Neotropical species of the genus *Dioscorea* L. were subjected to palynological analysis with the objectives of increasing palynological knowledge and identifying characteristics with taxonomic and phylogenetic value. More specifically, the aim was to identify characters that distinguish species and help to resolve relationships among New World clades (i.e. clades NW I, NW II and NW III). Botanical material was obtained from exsiccates deposited in various herbaria. Pollen grains were treated by lactic acetolysis, measured, described, photomicrographed and submitted to statistical analysis. Non-acetolyzed pollen grains were deposited on carbon tape for scanning electron microscopy analysis. The results separate species of *Dioscorea* into six pollen types based on sexine ornamentation: reticulate, perforate, microreticulate, vermiculate, rugulate and spiculate. The pollen grains of species of *Dioscorea* are characterized by having monosulcate and disulcate apertures, sometimes in the same specimen (e.g. *D. anomala*, *D. campestris* and *D. glandulosa*). Traditionally accepted taxonomic sections of *Dioscorea* were not corroborated. Reassessment of established subgenera awaits a better understanding and recognition of phylogenetic lineages.

KEYWORDS
Brazil; monosulcate; disulcate; multivariate analysis; palynology

1. Introduction

Dioscoreaceae comprises about 650 species and has a predominantly Pantropical distribution (Stevens 2001). Species of the family are distributed throughout Brazil, but especially in the Atlantic Forest and Cerrado biomes (BFG 2015). The family is currently accepted as monophyletic but with some uncertainties regarding its relationships with other families of the order Dioscoreales (Caddick et al. 2002), as well as with the position of the genus *Tacca* J.R. Forst. & G. Forst. (Merckx et al. 2006). The family comprises dioecious or monoecious volatile climbing or subshrub plants, most of which have an underground reserve organ (Couto et al. 2018). Their branches are aerial, unarmed, winged or aculeated, and the leaves possess petioles with a pulvinus at both ends. The staminate inflorescences are axillary or rarely terminal, isolated or clustered, pistillate or solitary. The fruits are generally of the capsule, berry or samara (Couto 2015). The family comprises the genera *Stenomeris* Planch (2 spp.), *Trichopus* Gaertn. (2 spp.), *Tacca* (17 spp.) and *Dioscorea* L. (633 spp. = 95% of the species of Dioscoreaceae), with *Dioscorea* representing most of the morphological, chemical and genetic diversity of the family (Govaerts et al. 2007).

Knuth (1924) treated *Dioscorea* as comprising four subgenera: *Dioscorea* subg. *Helmia* (Knuth) Uline (239 spp.); *D.* subg. *Dioscorea* Pax (337 spp.); *D.* subg. *Stenophora* (Uline) R. Knuth (15 spp.); and *D.* subg. *Testudinaria* (Salisb.) Uline (24 spp). The classification of Knuth (1924) included 38.8% of the species of the genus in *D.* subg. *Helmia*, which is characterized by the presence of an expanded wing toward the base of the seeds. The species of this subgenus are distributed throughout the tropical region, but most are in the Neotropics.

Wilkin et al. (2005), Viruel et al. (2016, 2018) and Couto et al. (2018) presented phylogenetic studies with species belonging to different subgenera and several of the sections of Knuth (1924). Few of the sections were found to be monophyletic, while other clades grouped species from several sections generally in concordance with geographic distribution, demonstrating a lack of phylogenetic support for the sections of Knuth (1924).

Couto et al. (2018) carried out the most comprehensive phylogenetic study to date with a greater representation of Neotropical taxa than previous studies. These authors resolved three New World clades – New World I (NW I), New World II (NW II) and New World III (NW III), with one isolated species in the clade New World IV (NW IV). Among these clades of Neotropical species, NW II groups the species of *D.* subg. *Helmia*, which occur in the Neotropics (the rest of the species of this subgenus are positioned so as to make it polyphyletic).

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Couto et al. (2018) asserted that there is still a need for greater sampling of Neotropical clades to achieve a more resolved phylogeny. These authors also noted that the internal relationships of clades NW I and NW II have yet to be fully elucidated and still acknowledge the role of morphological characters, especially those of pollen, in understanding the relationships among the Neotropical species.

Palynological studies involving Dioscoreaceae in the works of Erdtman (1952), Su (1987), Caddick et al. (1998), Xifreda (2000), Schols et al. (2001), Schols, Wilkin et al. (2005), Wilkin et al. (2009) and Couto et al. (2014), verified the possible separation of species of *Dioscorea*, emphasizing the phylogenetic and taxonomic importance of the following characters: size, aperture number, exine ornamentation and the presence of orbicules.

There have been few palynological studies of this genus in the Neotropical Region. Barroso et al. (1974) studied both the taxonomy and the palynology of species of Dioscoreaceae occurring in the municipality of Rio de Janeiro and reported that the pollen grains are alike in form and aperture, yet may vary in the structure of the exine and the appearance of the surface. Recent studies have shown that the species *Dioscorea campestris* Griseb. and *Dioscorea pedalis* (Uline ex R. Knuth) R. Couto & J.M.A. Braga can be separated based on pollen grain size and sexine ornamentation (Couto et al. 2014).

In view of the above, new studies are needed on the pollen morphology of species of *Dioscorea* in the New Word clades NW I, NW III and, especially, NW II, to increase knowledge regarding taxa occurring in the Neotropics. Thus, the present palynological study involved 19 species selected to encompass a broad representation of the morphological diversity of species of these main Neotropical clades, with the aim of identifying taxonomic characters useful for distinguishing species and contributing to a better understanding of the phylogenetic relationships among species of the New World clades NW I, NW II and NW III.

2. Material and methods

Nineteen Neotropical taxa of *Dioscorea* were studied palynologically, with a focus on clade NW II. One specimen of each species was chosen for statistical treatment and illustration, and is indicated by an asterisk (*) after the collector's name in the Supplemental material. Samples were obtained from fertile anthers of pre-anthesis flowers and/or buds from exsiccates deposited in the following herbaria: ESA, HB, NCU, R, RB and RFA (abbreviations in accordance with the Index Herbariorum; Thiers 2018, continuously updated).

The species were selected to account for a wide range of the morphological variability of the genus in the Neotropical Region, as well as its distribution in different habitats (Figure 1). The 19 studied taxa of *Dioscorea* cover all neotropical clades presented in the latest, and the majority of, phylogenetic studies of the genus (Viruel et al. 2016, 2018; Couto et al. 2018).

For analysis by light microscopy (LM), the material was processed according to the 60% lactic acetolysis method established by Raynal and Raynal (1971), because the pollen grains exhibited little resistance to traditional acetolysis. Pollen grains were photomicrographed under LM with a Canon PowerShot G6 digital camera coupled to a Zeiss Axiostar Plus binocular microscope with a 100× objective.

For analysis by scanning electron microscopy (SEM), anthers were separated from herbal material under a stereomicroscope, with the aid of sterilized forceps and stylets, and macerated. The non-acetolyzed pollen grains were then deposited on properly numbered aluminum supports covered with carbon tape (Melhem et al. 2003). The assembly was transferred to a vacuum pump, metalized with a layer of pure gold for about three minutes and examined under a JEOL JSM 6510 SEM located in the Laboratory of Images in Optical and Scanning Microscopy (LABIM) at the Federal University of Rio de Janeiro (UFRJ).

Randomly selected pollen grains in equatorial and lateral equatorial views were measured from at least three slides to homogenize the sample (Salgado-Labouriau 1973). The following measurements were made under a binocular microscope with an ocular micrometer: 25 measurements of maximum diameter (MD) and minimum diameter (mD) in polar view (PV); 10 measurements of polar diameter (PD) and equatorial diameter (ED), in equatorial view (EV); 10 measurements of MD and mD in lateral equatorial view (LEV); 10 measurements of the layers of the exine; and 10 measurements of the aperture (length and width). Classes were established for sulcus length as follows: short = 10.0–14.9 μm; long = 15.0–20.0 μm; and very long = 20.1–31.0 μm.

Ten pollen grains were measured in polar view for comparative purposes. A minimum of three permanent slides of acetolyzed pollen grains was assembled for each specimen to standardize the sample (Salgado-Labouriau et al. 1965). The pollen grains were measured within three days after preparation to avoid any changes in size (Salgado-Labouriau 1973; Wanderley and Melhem 1991). Types of ornamentation of the exine were analyzed and are described later. The slides used in the present study were deposited in the Palynotheca of the Laboratory of Palynology Álvaro Xavier Moreira, of the Department of Botany of the National Museum, Federal University of Rio de Janeiro.

Monocotyledonous pollen grains typically have a single aperture at the distal pole. The majority of species of the present study were found to have disulcate pollen grains. The pollen grains were preferentially positioned in polar view on the slides, making it difficult to obtain pollen grains in equatorial and lateral equatorial views. Pollen grains were recorded in four positions on the slides, as follows (Figure 2): (a) distal polar view: apertures located in the center of the pole; (b) proximal polar view: apertures located near the peripheries of the pollen grain; (c) equatorial view: apertures are partially seen in one of the peripheries of the pollen grain; and (d) lateral equatorial view: the two apertures are visible laterally in equatorial view.

A specific statistical treatment was performed on the results of the samples with 25 measurements, where the following were calculated: arithmetic mean ($\bar{x}$), standard deviation of the mean (s_x), 95% confidence interval (95% CI) and range of variation. The results of the statistical treatment and the morphopollinic analyses are presented in the tables.

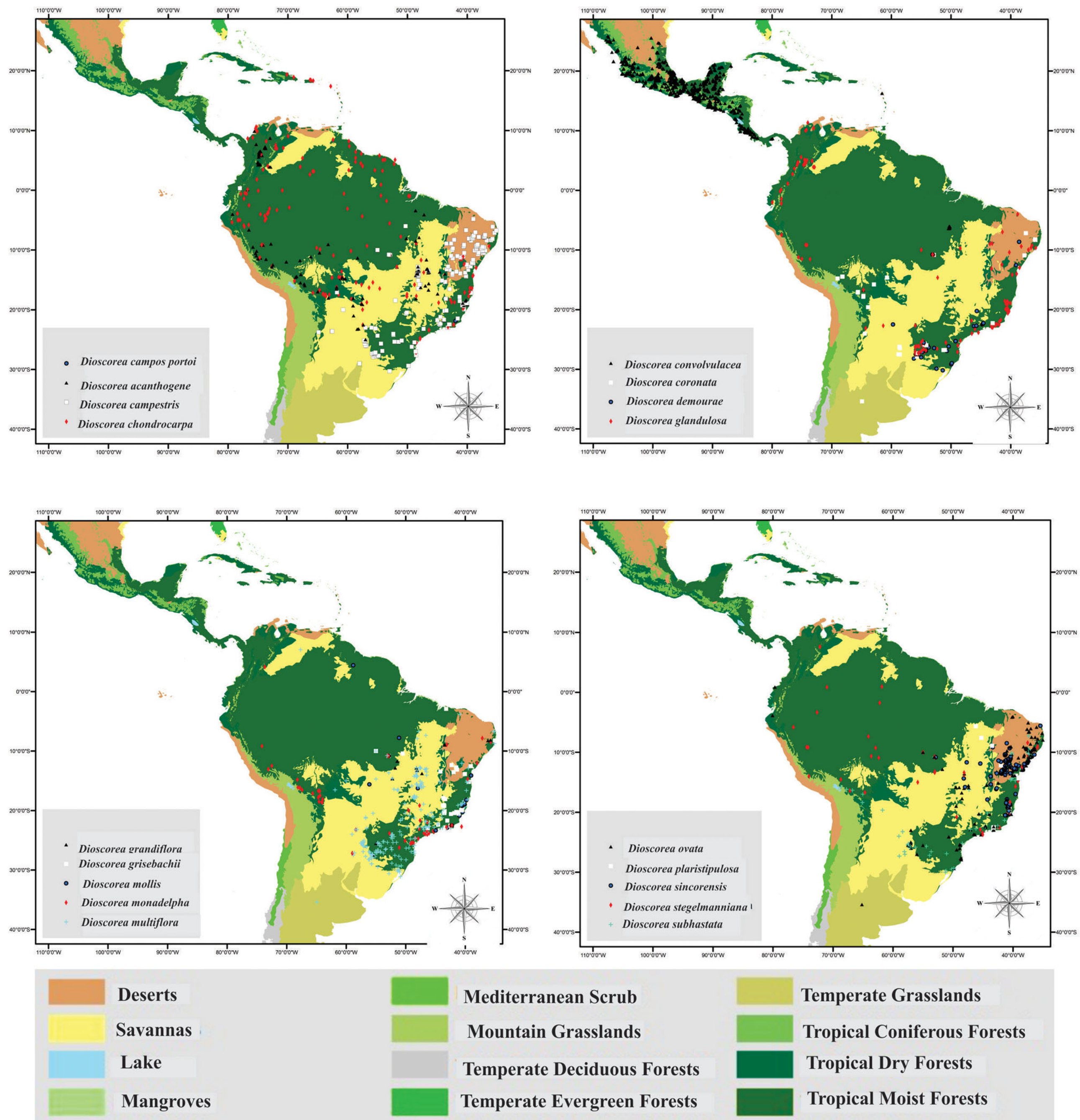

Figure 1. Maps of the distribution of Dioscorea species among different habitats. The distribution map was made using ArcGIS 10.5 (ESRI 2011), based on localities indicated in voucher specimens and further records collected and filtered from GBIF (2019), using the Biome2 vegetation layer from WWF (World Wide Fund for Nature, available in http://services.arcgis.com/BG6nSlhZSAWtExvp/arcgis/rest/services/Biomes2/FeatureServer).

The terminology used in the pollen descriptions follows Erdtman (1952), Punt et al. (2007) and Hesse et al. (2009), who used the following size classes: small, 10–25 μm; and medium, 25–50 μm.

An exploratory analysis of the data was carried out with the aid of PC-ORD version 5.31 (McCune and Mefford 2011). Twenty pollen attributes (variables) of the analyzed species were organized in a matrix. Species names were abbreviated in graphs using the first four letters of the specific epithet while pollen attributes (variables) were expressed as letters.

The following variables (indicated by their respective abbreviations) were included in the multivariate analyses: small – SML; medium – MED; sulcus long – SL; sulcus very long – SVL; two apertures – AP2; one and two apertures – AP1-2; margin – M; operculum – OPER; rugulate-microreticulate – RUG-MICR; reticulate-cristate – RET-CRIS; perforate – PERF; rugulate – RUG; rugulate-reticulate – RUG-RET; rugulate-perforate – RUG-PERF; spiculate – ESP; striate – ESTR; vermiculate-perforate – VER0-PERF; vermiculate – VER; and reticulate – RET; microreticulate – MICRORRET.

Qualitative data were expressed in the matrix as ‘1’ for presence and ‘0’ for absence.

Principal component analysis (PCA) was used to order the variables and determine whether species were grouped by pollen attributes. The variance-covariance (var-cov) was obtained from the means of the morphometric data in the palynological analysis and ordered in a biplot chart based on Euclidean distance. The results are shown in a two-dimensional graph with the first and second principal components. The values of the vectors for each axis and the total cumulative variance are represented in the tables, as is the character matrix for cluster analysis.

Hierarchical cluster analysis (cluster-HCA) was performed to classify the analyzed species into groups that share (similarity) pollen variables. Two aspects were considered in relation to the groups formed and the set of analyzed variables: the percentage of information (variables) needed to form the groups, and the final number of groups formed. A dendrogram was constructed from the cluster analysis using Euclidean distance (Caccavari et al. 2008) and Ward's linkage.

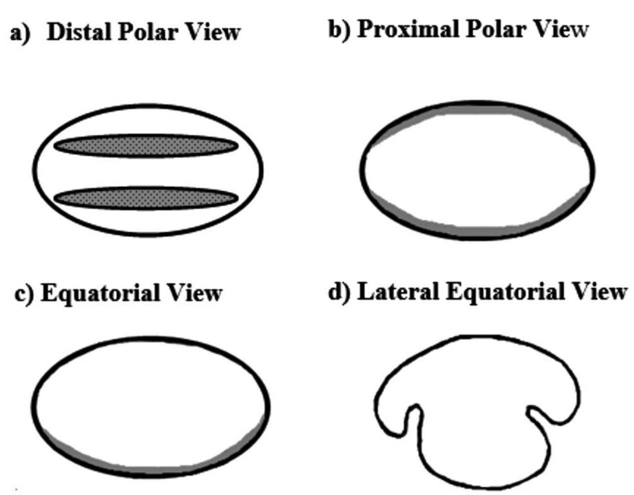

Figure 2. Position of the pollen grains.

3. Results

The following characteristics were used in the morphological analysis of pollen grains of *Dioscorea* (Plates 1–4; Figures 2–4; Tables 1–4): dispersion unit, polarity, contour, size, number and type of aperture(s), exine ornamentation and operculum presence/absence.

3.1. Dispersion unit, polarity and contour

The pollen grains of all the studied species of *Dioscorea* were heteropolar monads with, in most species, an elliptical contour in polar view, the exceptions being *D. anomala*, which had a circular contour with one of the faces being approximately acute, and *D. monadelpha*, which varied from elliptical to triangular (Plate 3, figure 4). The equatorial view was difficult to observe for most of the species. The contour in lateral equatorial view was circular-lobate (Plate 2, figure 4; Plate 3, figure 6; Plate 4, figure 6).

3.2. Size

Pollen grains of the analyzed species of *Dioscorea* varied from small (nine species) to medium (10 species). In polar view (Table 2) the smallest maximum diameter was for *D. campestris* (20.5 μm), while the greatest maximum diameter was for *D. anomala* (35.5 μm). Pollen fallen in equatorial view was found for only four species: *D. campestris*, *D. glandulosa*, *D. monadelpha* and *D. ovata*; *D. campestris* had the smallest while *D. campestris* had the largest (Table 3). In lateral equatorial view, *D. campestris* had the smallest maximum diameter while *D. multiflora* had the greatest (Table 4). Measurement of the maximum diameter in polar view revealed differences in the ends of the pollen grains, which were classified as the following contours (shapes): truncate for 16 taxa (*D. acanthogene*, *D. chondrocarpa*, *D. anomala*, *D. campos-portoi*, *D. coronata*,

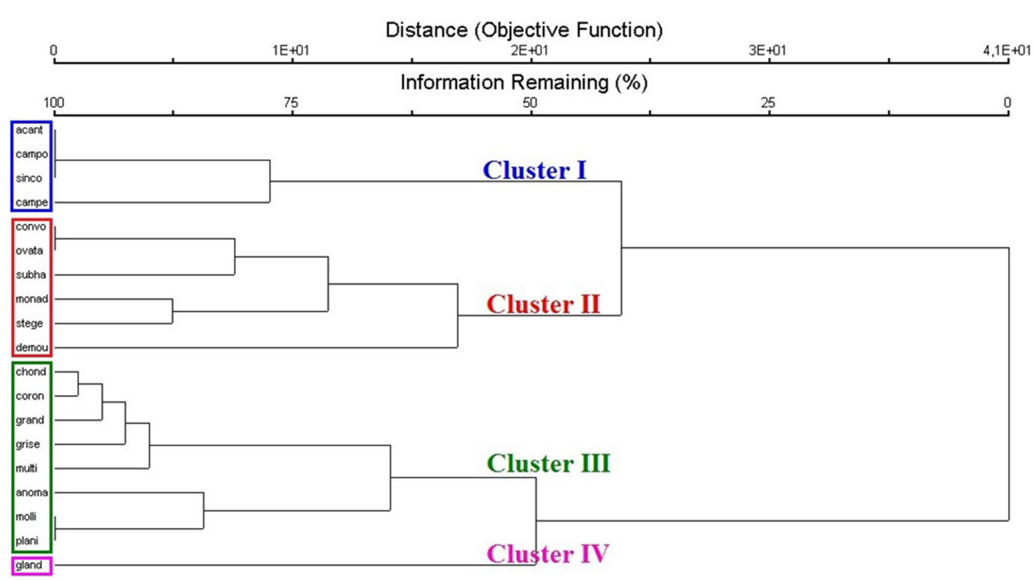

Figure 3. Cluster analysis performed with the measured variables of pollen from species of *Dioscorea* L. acant - D. acanthogene; anoma - D. anomala; campe - D. campestris; campo - D. campos-portoi; chond - D. chondrocarpa; convo - D. convolvulacea; coron - D. coronata; demou - D. demourae; gland - D. glandulosa; grand - D. grandiflora; grise - D. grisebachii; molli - D. mollis; monad - D. monadelpha; multi - D. multiflora; ovata - D. ovata; plani - D. planistipulosa; sinco - D. sincorensis; stege - D. stegelmanniana; subha - D. subhastata

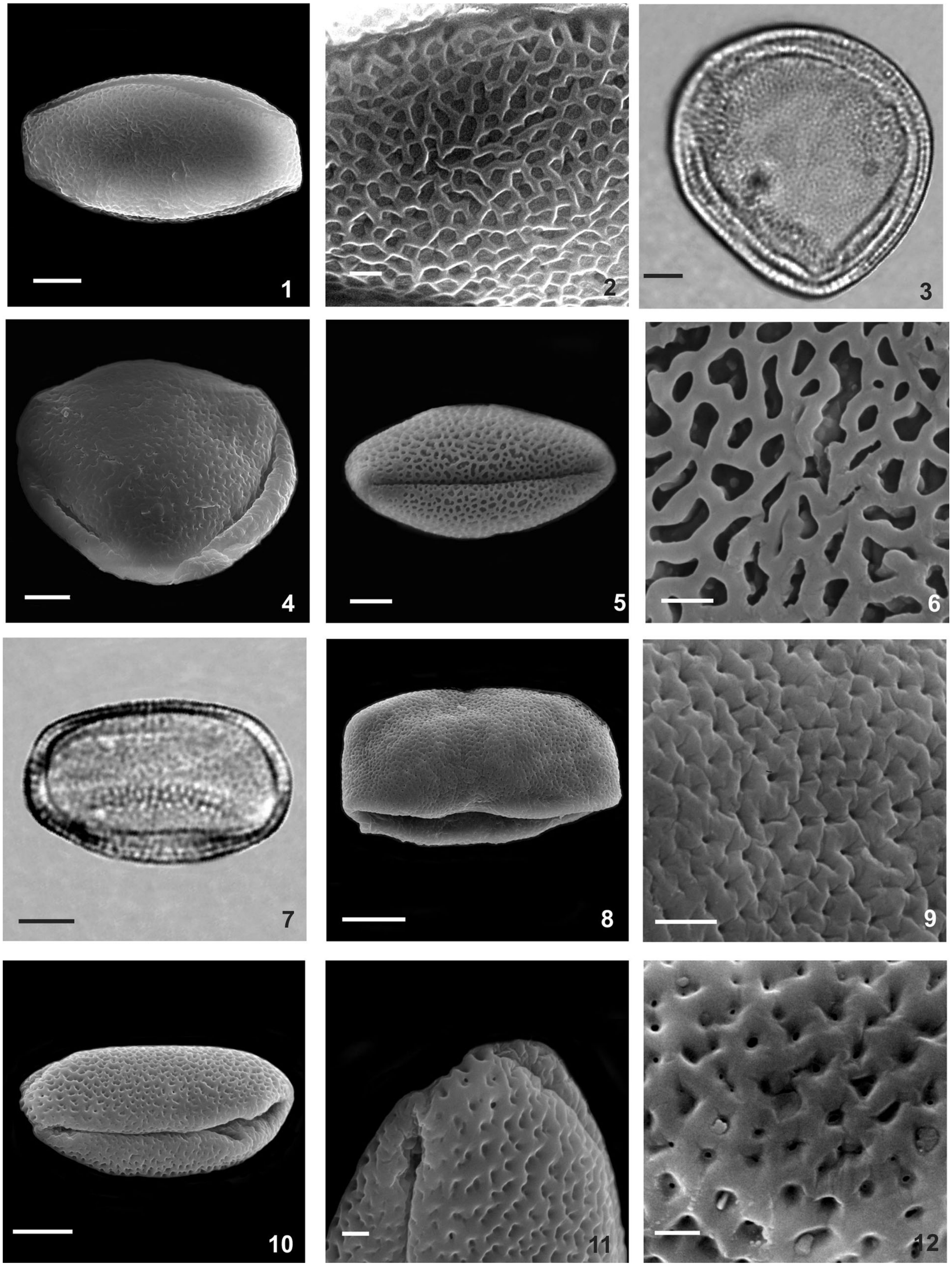

Plate 1. Photomicrographs and electromicrographs of *Dioscorea* species: *D. acanthogene* – 1. polar view: general aspect (SEM), 2. detail of the surface (SEM). *D. anomala* – 3. polar view: optical section (LM), 4. general aspect (SEM). *D. campestris* – 5. polar view: general aspect and aperture (SEM), 6. detail of the surface (SEM). *D. campos-portoi* – 7. polar view: general aspect (LM). *D. chondrocarpa* – 8. polar view: general aspect (SEM), 9. detail of the surface (SEM). *D. convolvulacea* – 10. polar view: general aspect (SEM), 11. detail of the ends of the aperture (SEM), 12. detail of the surface (MEV). Scale bars: 1, 3–5, 7, 8, 10 = 5 μm; 2, 6, 9, 11, 12 = 1 μm.

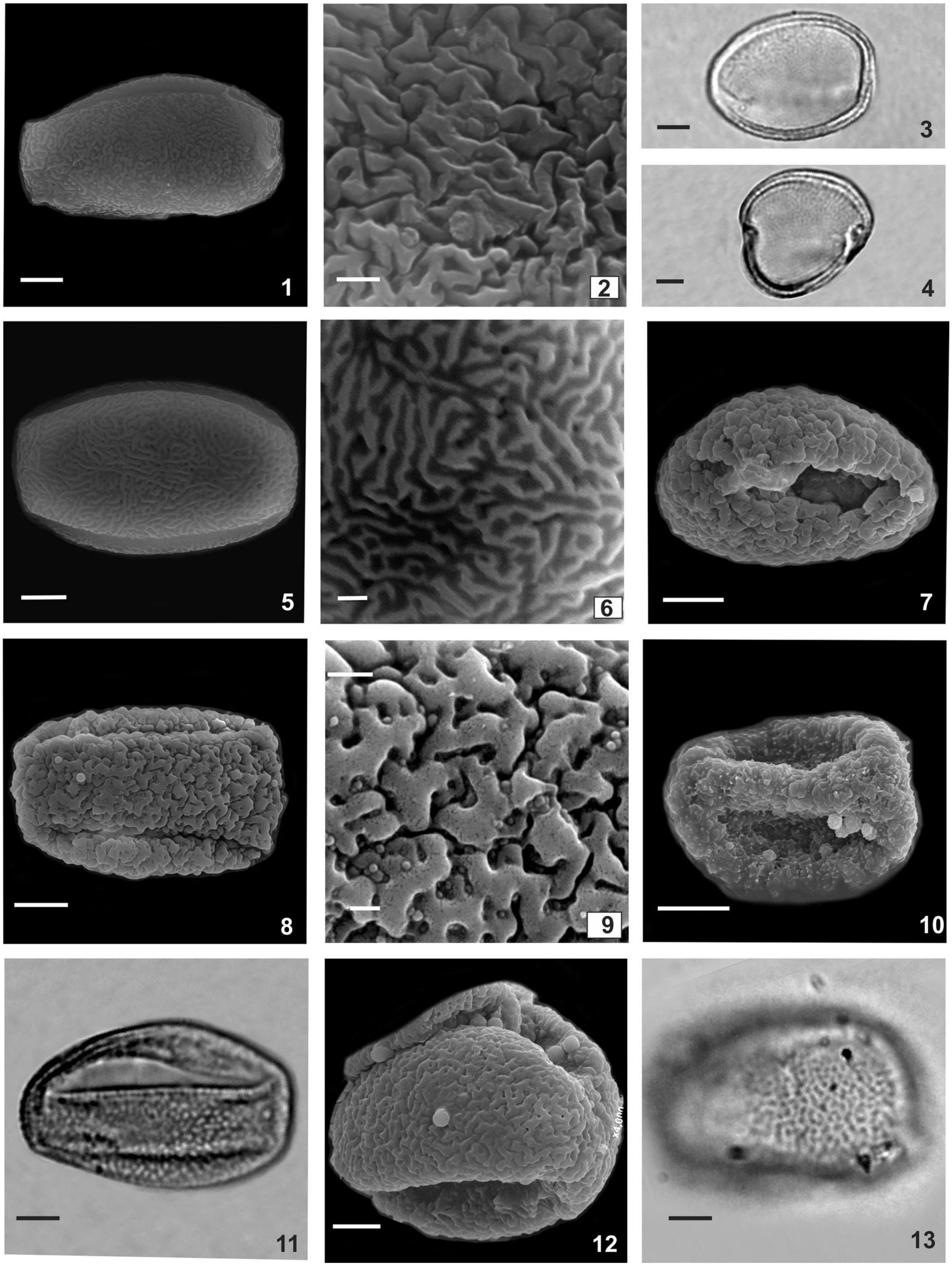

Plate 2. Photomicrographs and electromicrographs of *Dioscorea* species: *D. coronata* – 1. polar view: general aspect (SEM), 2. detail of the surface (MEV). *D. demourae* – 3. polar view: outline (LM), 4. equatorial lateral view: optical section (LM), 5. polar view: general aspect (SEM), 6. detail of the surface (SEM). *D. glandulosa* – 7. polar view: general aspect and one aperture (SEM), 8. general aspect and two aperture (SEM), 9. detail of the surface (MEV). *D. grandiflora* – 10. polar view: general aspect and aperture (SEM). *D. grisebachii* – 11. polar view: aperture (LM), 12. general aspect and aperture (SEM), 13. surface (LM). Scale bars: 1, 3–5, 7, 8, 10–13 = 5 μm; 2, 6, 9 = 1 μm.

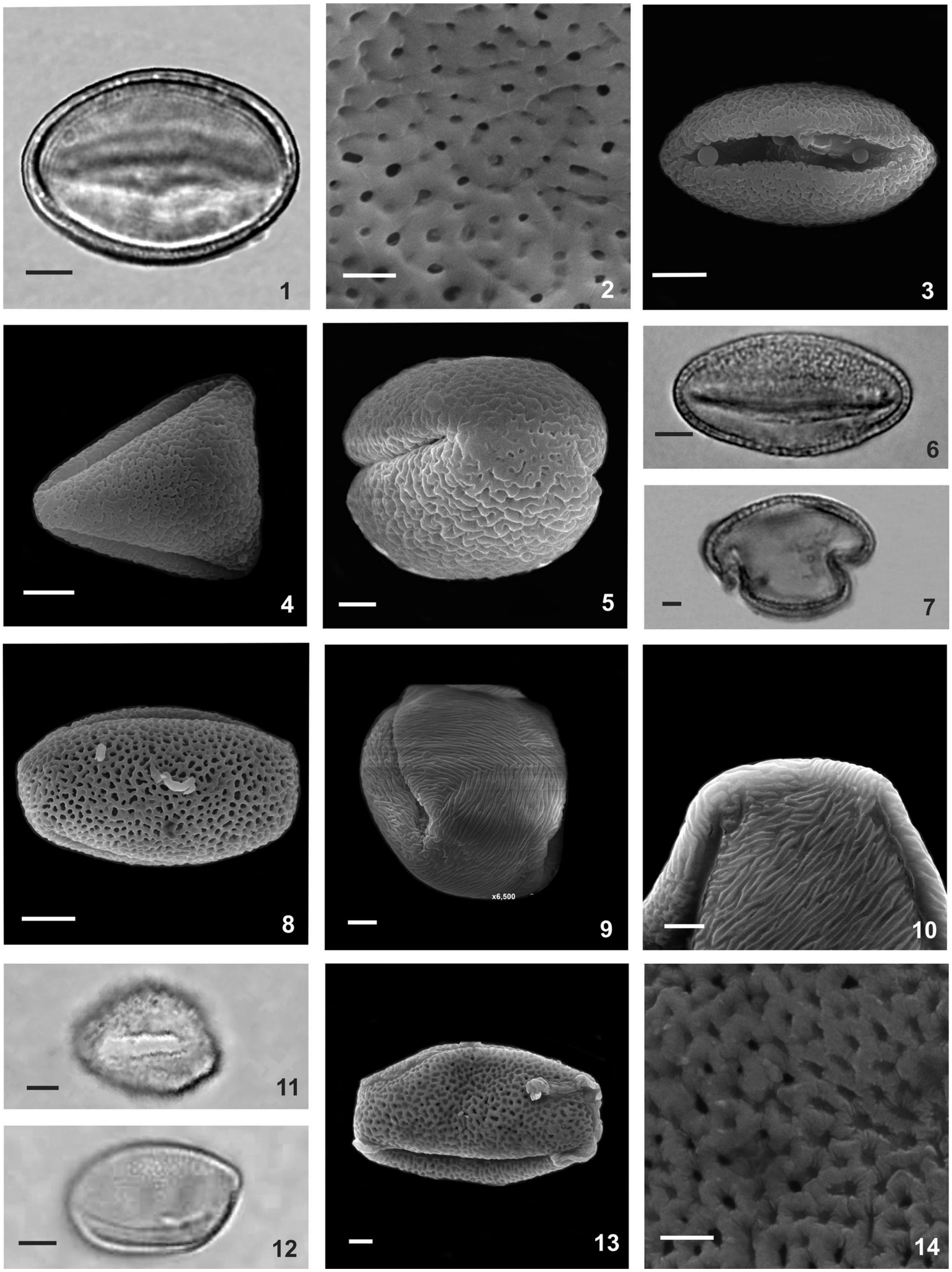

Plate 3. Photomicrographs and electromicrographs of *Dioscorea* species: *D. mollis* – 1. polar view: outline (LM), 2. detail of the surface (SEM). *D. monadelpha* – 3. polar view: aperture (SEM), 4. polar view: general aspect (SEM), 5. equatorial lateral view: general aspect and surface (SEM). *D. multiflora* – 6. polar view: aperture (LM), 7. equatorial lateral view: optical section (LM), 8. polar view: general aspect and surface (SEM). *D. olfersiana* – 9. equatorial lateral view: general aspect and ends of the aperture (SEM). 10. detail of the surface and ends of the aperture (SEM). *D. ovata* – 11. polar view: outline (LM), 12. aperture (LM), 13. general aspect (SEM), 14. detail of the surface (SEM). Scale bars: 1, 4, 6–13 = 5 μm; 2, 3, 14 = 1 μm; 5 = 2 μm.

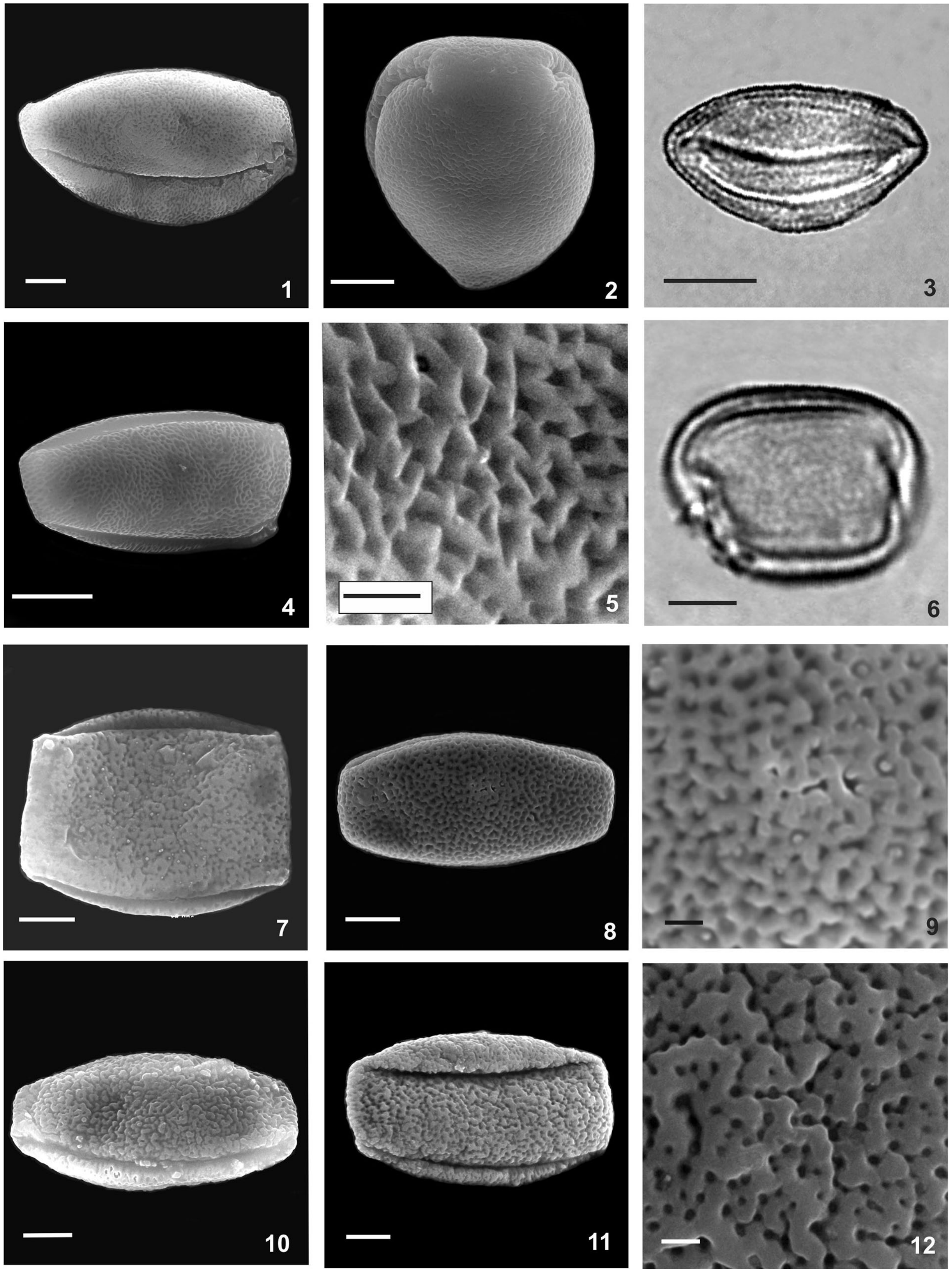

Plate 4. Photomicrographs and electromicrographs of *Dioscorea* species: *D. perdicum* – 1. polar view: general aspect and aperture (SEM), 2. general aspect: surface (SEM). *D. planistipulosa* – 3. polar view: aperture (LM). *D. sincorensis* – 4. polar view: general aspect and aperture (SEM), 5. detail of the surface (SEM). *D. sinuata* – 6. lateral view: outline (LM), 7. polar view: general aspect and surface (SEM). *D. stegelmanniana* – 8. polar view: general aspect (SEM), 9. detail of the surface (SEM). *D. subhastata* – 10. polar view: general aspect and surface (SEM). *D. therezopolensis* – 11. polar view: general aspect and aperture (SEM), 12. detail of the surface (SEM). Scale bars: 1, 2, 4, 6, 8, 10, 11 = 5 μm; 3 = 10 μm; 5, 9, 12 = 1 μm; 7 = 2 μm.

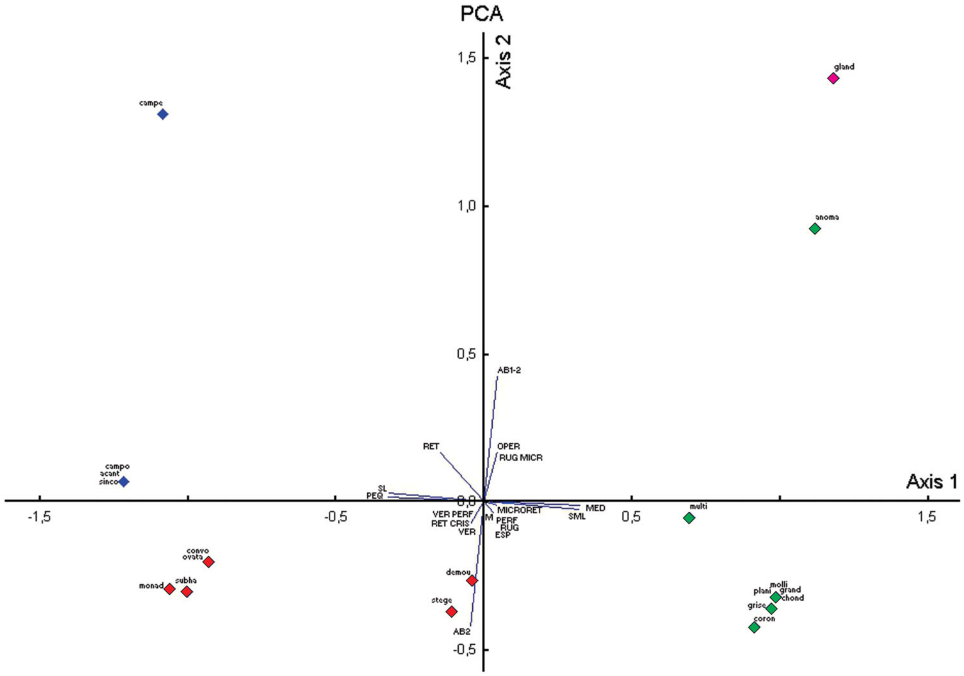

Figure 4. Principal component analysis (PCA) performed with the measured variables of pollen from species of *Dioscorea L.*

D. demourae, *D. glandulosa*, *D. grandiflora*, *D. grisebachii*, *D. mollis*, *D. multiflora*, *D. ovata*, *D. planistipulosa*, *D. sincorensis*, *D. stegelmanniana* and *D. subhastata*); acute-truncate for two taxa (*D. convolvulacea* and *D. monadelpha* – Plate 1, figure 10; Plate 3, figure 4); and acute for one taxon (*D. campestris* – Plate 1, figure 5).

3.3. Aperture

The majority of species had disulcate pollen grains, with the exceptions being *D. anomala*, *D. campestris* and *D. glandulosa*, for which the pollen grains were monosulcate (Plate 1, figures 3, 5; Plate 2, figures 7, 8). Sulci were long or very long (Table 4) and with margins. Sulci are better observed under SEM, but due to rupturing during acetolysis they were observed only under LM, which made measuring them difficult. The presence of an operculum distinguished *D. glandulosa* (Plate 2, figure 7).

3.4. Stratification and ornamentation of the exine

The species exhibited variation in the thickness of the layers of the exine. The sexine was as thick as the nexine in eight species, thicker than the nexine in six species and thinner than the nexine in five species (Table 4).

The majority of species had a reticulate or microreticulate sexine, while the sexine of of *D. monadelpha* and *D. stegelmanniana* was reticulate-cristate (Plate 3, figures 4, 5). An entirely perforated sexine surface was observed only in *D. chondrocarpa* (Plate 3, figure 9). The sexines of *D. coronata* and *D. subhastata* were vermiculate (Plate 2, figure 2; Plate 4, figure 10), that of *D. demourae* was vermiculate-perforate (Plate 2, figure 6), and those of *D. grisebachii* rugulate and *D. glandulosa* were rugulate-microreticulate (Plate 2, figures 9, 12). A spiculate sexine was observed only in *D. grandiflora* (Plate 2, figure 10).

3.5. Multivariate analysis

3.5.1. Hierarchical cluster analysis (HCA)

Relationships among the analyzed species were depicted with a dendrogram that explained about 42.32% of the data. Four clusters were formed when 50% of the remaining information (variables) was analyzed (Figure 3):

- - Cluster I: *D. acanthogene*, *D. campos-portoi*, *D. sincorensis* and *D. campestris.*
- - Cluster II: *D. convolvulacea*, *D. ovata*, *D. subhastata*, *D. monadelpha*, *D. stegelmanniana* and *D. demourae*.
- - Cluster III: *D. chondrocarpa*, *D. coronata*, *D. grandiflora*, *D. grisebachii*, *D. multiflora*, *D. anomala*, *D. mollis* and *D. planistipulosa*.
- - Cluster IV: *D. glandulosa.*

3.5.2. Principal component analysis (PCA)

The PCA was significant and explained 59.35% of the total variance. The first two axes (components) were significant and explained 44.87% and 14.48% of the variation, respectively. The significant variables for axis 1 were disulcate aperture, long sulci, small size and reticulate ornamentation of the sexine. The significant variables for axis 2 were very long sulcus, monosulcate and disulcate apertures, small size and reticulate ornamentation of the sexine (Figure 4).

The species are fairly well dispersed between the negative and positive poles of axes 1 and 2, with few apparent groupings because there are few characteristics that differentiate these species.

The species *D. planistipulosa*, *D. mollis*, *D. grandiflora*, *D. chondrocarpa*, *D. grisebachii* and *D. coronata* are located toward the positive pole of axis 1 because they possess medium-sized pollen grains with a very long sulcus. The species *D. glandulosa* and *D. anomala* are positioned near each other and distanced from the others because they possess pollen grains with both monosulcate and disulcate apertures, and *D. glandulosa* has an operculum. The species *D. campestris*, *D. sincorensis*, *D. campos-portoi* and *D. acanthogene* are close to each other at the negative pole of axis 1 because they have small pollen grains with a long sulcus. The species *D. campestris* is distanced from the others by possessing one and two apertures.

The species *D. glandulosa*, *D. anomala* and *D. campestris* were differentiated toward the positive pole of axis 2 based on having monosulcate and disulcate pollen. Among these, *D. campestris* differs in having a small size, revealing that this character is very significant in the analysis of similarity of the pollen grains. The species *D. convolvulacea* and *D. ovata* are similar; *D. subhastata* and *D. monadelpha* are close to each other, differing only in the ornamentation of the exine; and the species *D. stegelmanniana* and *D. demourae* share only a disulcate aperture. These six species are located on the same pole in both axes. At the negative pole of axis 2 and the positive pole of axis 1, the species *D. chondrocarpa*, *D. coronata*, *D. grandiflora* and *D. grisebachii* are differentiated only by the ornamentation of the sexine; *D. planistipulosa* and *D. mollis* are similar to each other, and *D. multiflora* is isolated from the others because of the medium size of its pollen and its very long sulcus and reticulate sexine.

Table 1. Morphologic characters of *Dioscorea* pollen grains.

Species	Size	No. of apertures	Sexine ornamentation
Dioscorea acanthogene	Small	2	Reticulate
D anomala	Medium	1–2	Microreticulate
D. campestris	Small	1–2	Reticulate
D. campos-portoi	Small	2	Reticulate
D. chondrocarpa	Medium	2	Perforate
D. convolvulacea	Small	2	Microreticulate
D. coronata	Medium	2	Vermiculate
D. demourae	Medium	2	Vermiculate-perfurate
D. glandulosa	Medium	1–2	Rugulate-microrreticulate
D. grandiflora	Medium	2	Spiculate
D. grisebachii	Medium	2	Rugulate
D. mollis	Medium	2	Microreticulate
D. monadelfa	Small	2	Reticulate-cristate
D. multiflora	Medium	2	Reticulate
D. ovata	Small	2	Microreticulate
D.planistipulosa	Medium	2	Microreticulate
D. sincorensis	Small	2	Reticulate
D. stegelmanniana	Small	2	Reticulate-cristate
D. subhastata	Small	2	Vermiculate

4. Discussion

Palynological analysis of 19 taxa of *Dioscorea* revealed important pollinic attributes that contribute to its delimitation, such as size, contour (shape), number of apertures and sexine ornamentation.

Pollen grains with a fine exine, as found for the species of *Disocorea* analyzed here, do not allow the traditional acetolytic treatment of Erdtman (1952), and only the methodology for fragile or very fragile pollen grains of Raynal and Raynal (1971) could be used. This confirmation of exine fragility allows the inference to be made that it would be difficult to preserve these pollen grains in their natural habitat.

Table 3. Measures (in μm) of pollen grains in equatorial view of *Dioscorea* species (n = 10): polar diameter (PD) and equatorial diameter (ED). x – arithmetic mean.

	Polar diameter		Equatorial diameter	
Species	Range	$\bar{x}$	Range	$\bar{x}$
D. campestris	12.5–15.0	14.2	17.5–20.0	19.6
D. glandulosa	20.0–25.0	22.5	25.0–30.0	26.8
D. monadelpha	15.0–20.0	17.3	22.5–27.5	25.0
D. ovata	12.5–20.0	17.5	22.5–25.0	24.3

Table 2. Measures (in μm) of pollen grains in polar view of *Dioscorea* species (n = 25): larger diameter (LD) and smaller diameter (SD). x – arithmetic mean; sx – standard deviation of the mean; C.I. – confidence interval.

	Largest diameter			Smallest diameter		
Species	Range	$\bar{x} \pm s_{\bar{x}}$	CI 95%	Range	$\bar{x} \pm s_{\bar{x}}$	CI 95%
D. acanthogene	17.5–25.0	21.6 ± 0.4	20.8–22.4	10.0–20.0	16.1 ± 0.5	15.1–17.1
D. anomala	22.5–45.0	35.5 ± 1.1	33.1–37.9	12.5–32.5	23.4 ± 1.0	21.3–25.5
D. campestris	17.5–25.0	20.5 ± 0.4	19.7–21.3	10.0–15.0	13.2 ± 0.3	12.6–13.8
D. campos-portoi	20.0–27.5	24.2 ± 0.3	23.5–24.9	15.0–17.5	16.2 ± 0.3	15.7–16.7
D. chondrocarpa	22.5–30.0	25.8 ± 0.4	25.0–26.6	12.5–22.5	16.5 ± 0.5	15.5–17.5
D. convolvulacea	20.0–27.5	22.5 ± 0.5	21.5–23.5	15.0–20.0	18.0 ± 0.3	17.3–18.7
D. coronata	22.5–35.0	28.9 ± 0.5	27.8–30.0	17.5–27.5	21.5 ± 0.5	20.5–22.5
D. demourae	22.5–30.0	25.8 ± 0.4	24.9–26.7	12.5–22.5	17.7 ± 0.4	16.8–18.6
D. glandulosa	22.5–32.5	27.2 ± 0.5	26.1–28.3	17.5–27.5	23.2 ± 0.5	22.2–24.2
D. grandiflora	22.5–30.0	25.5 ± 0.4	24.6–26.4	17.5–25.0	21.3 ± 0.5	20.4–22.2
D. grisebachii	27.5–40.0	32.7 ± 0.5	31.6–33.8	12.5–22.5	18.5 ± 0.6	17.3–19.7
D. mollis	22.5–35.0	30.1 ± 0.5	29.0–31.2	15.0–25.0	20.4 ± 0.5	19.4–21.4
D. monadelpha	22.5–30.0	24.8 ± 0.4	23.9–25.7	15.0–22.5	18.2 ± 0.5	17.2–19.2
D. multiflora	27.5–35.0	32.2 ± 0.5	31.1–33.3	15.0–25.0	19.7 ± 0.5	18.7–20.7
D. ovata	20.0–27.5	23.9 ± 0.4	23.0–24.8	10.0–20.0	14.1 ± 0.4	13.2–15.0
D. planistipulosa	25.0–32.5	28.8 ± 0.5	27.7–29.9	12.5–20.0	16.7 ± 0.5	15.6–17.8
D. sincorensis	17.5–25.0	21.2 ± 0.4	20.4–22.0	15.0–20.0	16.7 ± 0.3	16.1–17.3
D. stegelmanniana	22.5–27.5	24.9 ± 0.3	24.3–25.5	12.5–20.0	16.2 ± 0.5	15.1–17.3
D. subhastata	20.0–25.0	22.2 ± 0.3	21.7–22.7	12.5–20.0	15.3 ± 0.4	14.4–16.2

The studied species of *Dioscorea* exhibited morphometric variation with both small and medium-sized pollen grains. This variation was also observed by Erdtman (1952), Su (1987), Schols et al. (2001, 2003), Schols, Wilkin et al. (2005), Wilkin et al. (2009) and Couto et al. (2014). Large pollen grains were recorded for species from Africa (*D. buchananni* Benth, *D. nipponica* Makino) and the species *D. pyrenaica* Bubani & Bordère ex Gren of Europe (Su 1987; Schols et al. 2001; Schols, Wilkin et al. 2005).

Barroso et al. (1974) studied both the palynology and the taxonomy of 24 species of *Dioscorea* and affirmed that the pollen grains are equal in shape and aperture. The results obtained here revealed a difference in the number of apertures because *D. glandulosa* exhibited a pattern of 1(-2) sulcate. The above authors did not record monosulcate pollen grains, which may have been due to their low percentage in the slides analyzed.

Table 4. Measures (in μm) of pollen grains in equatorial lateral view of *Dioscorea* species (n = 10): larger diameter (LD) and smaller diameter (SD). x – arithmetic mean.

	Larger diameter		Smallest diameter	
Species	Range	$\bar{x}$	Range	$\bar{x}$
D. acanthogene	15.0–22.5	19.8	12.5–20.0	17.0
D. anomala	24.5–25.0	24.8	20.0–22.5	21.3
D. campestris	12.5–15.0	13.8	5.0–7.5	6.3
D. campos-portoi	17.5–22.5	19.3	10.0–17.5	13.8
D. chondrocarpa	20.0–25.0	23.3	10.0–20.0	16.7
D. convolvulacea	7.5–20.0	16.9	12.5–22.5	20.6
D. coronata	17.5–20.0	19.0	5.0–15.0	8.8
D. demourae	20.0–27.5	23.8	15.0–20.0	18.0
D. glandulosa	20.0–25.0	23.2	10.0–19.0	14.8
D. grandiflora	22.5–27.5	25.5	17.5–22.5	21.0
D. grisebachii	24.5–25.0	24.8	17.5–22.5	20.0
D. mollis	20.0–25.0	22.8	12.5–22.5	19.5
D. monadelpha	17.5–22.5	19.5	12.5–20.0	15.0
D. multiflora	27.5–37.5	32.5	25.0–27.5	26.3
D. ovata	17.5–20.0	18.5	15.0–17.5	15.5
D. planistipulosa	14.5–15.0	14.8	9.5–10.0	9.8
D. sincorensis	17.5–20.0	18.0	10.0–15.0	13.0
D. stegelmanniana	17.5–22.5	20.6	15.0–20.0	18.1
D. subhastata	17.5–20.0	18.8	12.5–15.0	13.1

Su (1987) described the pollen morphology of 33 species of *Dioscorea* distributed among five sections and emphasized variation in the number of apertures, with the majority of the pollen grains being disulcate, with the exception of *Stenophora*, which had one aperture, and *Opsophyton*, which had two or three. Other authors reported the occurrence of monosulcate and disulcate pollen grains, including Caddick et al. (1998), Schols et al. (2001), Schols et al. (2003), Schols, Furness et al. (2005), Schols, Wilkin et al. (2005), Wilkin et al. (2009) and Viruel et al. (2010). The results obtained here corroborate those of the aforementioned authors but disagree with Su (1987) who recorded trisulcate pollen grains.

Couto et al. (2014) analyzed the pollen morphology of *D. campestris* and *D. pedalis* (Uline ex R. Knuth) R. Couto & J.M.A. Braga, but did not observe disulcate pollen grains. The palynological analysis of the present study found that *D. campestris* varied in the number of openings, with both monosulcate (7%) and disulcate (93%) pollen grains.

Barroso et al. (1974) divided species of *Dioscorea* into groups based on the ornamentation of the sexine: (A) reticulate-ornamented, (B) reticulate, (C) ornamented and (D) striated. Of the 26 species analyzed by these authors, six are included in the present study, none of which had pollen grains with ornamentation that can be classified into the groups of Barroso et al. (1974). This divergence in results is possibly due to the previous authors' lack of resources related to SEM, which brings clarity regarding the type of sexine ornamentation.

Zavada (1983), Caddick et al. (1998) and Schols et al. (2001) described different patterns of sexine ornamentation: finely reticulate, reticulate, striate, gemmate and rugulate-reticulate. In addition to the ornamentation patterns defined by theses authors, nine more were recorded in the present study: perforate, microreticulate, reticulate-cristate, spiculate, rugulate, rugulate-perforate, rugulate-microreticulate, vermiculate and vermiculate-perforate. However, the present study did not find gemmate or finely reticulate patterns. These differences, according to Couto et al. (2018), may be

Table 5. Measures (in μm) of the apertures and layers of the exine of pollen grains of the *Dioscorea* species (n = 10).

	Sulcus				Thickness of exine		
Species	Length	Width	Margin	Distance between apertures	Total	Sexine	Nexine
D. acanthogene	16.1	2.2	0.9	9.5	1.5	0.8	0.7
D. anomala	30.8	7.8	0.5	11.8	1.9	1.0	0.9
D. campestris	17.2	4.0	1.0	11.0	1.9	1.0	0.9
D. campos-portoi	19.6	2.6	0.6	7.3	1.8	0.9	0.9
D. chondrocarpa	20.1	4.5	0.6	7.6	1.7	0.8	0.9
D. convolvulacea	16.8	1.8	0.7	6.1	1.4	0.6	0.8
D. coronata	22.1	4.1	1.0	13.0	1.8	0.9	0.9
D. demourae	18.9	3.4	0.4	9.0	1.9	0.9	1.0
D. glandulosa	22.8	9.0	2.0	12.3	2.0	1.0	1.0
D. grandiflora	24.0	9.8	2.0	9.0	2.0	0.9	1.1
D. grisebachii	24.6	3.8	1.0	9.9	1.9	1.0	0.9
D. mollis	22.7	4.8	1.1	4.8	1.8	0.9	0.9
D. monadelpha	18.5	4.8	1.0	9.0	1.8	0.9	0.9
D. multiflora	27.0	4.6	0.9	8.6	1.6	0.8	0.8
D. ovata	19.7	3.8	1.0	11.5	2.0	1.0	1.0
D. planistipulosa	24.2	5.5	1.0	6.8	1.7	0.9	0.8
D. sincorensis	15.0	2.0	0.3	10.2	1.7	0.9	0.8
D. stegelmanniana	20.3	3.5	1.0	8.8	1.5	0.7	0.8
D. subhastata	17.3	7.0	1.0	9.0	2.0	1.0	1.0

because the species they studied were not Neotropical and, therefore, are among different clades than are the species studied here.

Schols et al. (2003) and Schols, Wilkin et al. (2005) analyzed 61 species of *Dioscorea*, of which 54% were perforate, 28% were striate, 6% varied from perforate to microreticulate (characteristics of both ornamentations are observed in the same pollen grain), 6% were cerebroid-perforate, 3% were rugulate, 2% were gemmate and 1% were perforate to rugulate. The taxa analyzed here exhibited similar characteristics of pollen grain ornamentation.

Couto et al. (2014) palynologically analyzed *D. campestris* and *D. pedalis* and concluded that there are differences in details of the sexine (lumina with or without ornamentation). The present study corroborated these findings of Couto et al. (2014).

According to Fraga (2016), the species *D. piperifolia* Humb & Bompl. ex Will., *D. glandulosa* and *D. grandiflora* are part of a taxonomic complex of critical species, differentiated only by a few macromorphological characters and exhibiting little variation. Couto et al. (2018) considered *D. piperifolia* and *D. grandiflora* to form a clade with strong support in all their analyses. The pollinic attributes recorded here revealed a distinction between these two species, with *D. grandiflora* having only disulcate pollen grains with spiculate ornamentation while *D. glandulosa* has monosulcate and disulcate pollen grains in the same specimens, and is the only species with an operculum and rugulate ornamentation, microreticulate with the presence of granules, showing that palynology did not corroborate the suggested phylogeny.

According to Tenorio et al. (2017), the species *D. mollis*, *D. chondrocarpa* and *D. planistipulosa* are anatomically similar to species of the Old World with two phloem units between great vessels of meta-xylem, while taxa of the clades NW I and NW II possess only one unit of phloem. Palynologically, *D. mollis* and *D. planistipulosa* grouped very close in the hierarchical cluster analysis due to possessing a microreticulate sexine, which differs from the perforate sexine of *D. chondrocarpa*.

The hierarchical cluster analysis formed four large groups. Cluster I contained *D. acanthogene*, *D. campos-portoi* and *D. sincorensis*, which were all very similar, and *D. campestris*, which was separated from the others, which were only disulcate, by being 1–2-sulcate.

Cluster II contained: *D. convolvulacea* and *D. ovata*, which were very similar; *D. subhastata* and *D. monadelpha*, which differed only in ornamentation; and *D. stegelmanniana* and *D. demourae*, which were linked by a single character (disulcate aperture). Cluster III was subdivided into two groups, with *D. mollis* and *D. planistipulosa* being very similar.

Cluster IV contained only *D. glandulosa*, which was differentiated by having 1–2 apertures and the presence of an operculum, the latter standing out in comparison to the other species.

The PCA revealed that the species located at the negative pole of axis 1 were those of clusters I and II, while those at the positive pole belonged to clusters III and IV. The species *D. convolvulacea*, *D. ovata*, *D. monadelpha* and *D. subhastata*, belonging to Cluster I (at negative poles of axes 1 and 2), were similar due to the strong characteristic of small size, along with being disulcate and having long sulci. The three species *D. campestris*, *D. anomala* and *D. glandulosa* were distinguished because they had pollen grains with both aperture patterns (monosulcate and disulcate), which was supported by their location at the positive pole of axis 2.

The pollinic attributes analyzed here partially corroborate the phylogenetic analysis of Couto et al. (2018) since the pollen data in the multivariate analysis separated *D. subhastata* from *D. monadelpha*, while in the phylogeny they were in the same clade. This divergence may be due to the fact that microcharacters, including those of pollen, were not used in the phylogenetic analysis.

5. Conclusion

The results obtained by the present work allowed the analyzed species to be organized into six groups based on sexine ornamentation: reticulate, microreticulate, perforate, vermiculate, rugulate and spiculate.

Fifty-three percent of the studied species had their pollen grains characterized for the first time: *D. acanthogene*, *D. campos-portoi*, *D. convolvulacea*, *D. demourae*, *D. grandiflora*, *D. grisebachii*, *D. monadelpha*, *D. planistipulosa*, *D. sincorensis* and *D. stegelmanniana.*

Some pollen grains of species of *Dioscorea* (*D. anomala*, *D. campestris* and *D. glandulosa*) were characterized as having both monosulcate and disulcate aperture patterns in the same specimen.

The present study did not corroborate the sections of the taxonomic classification of Knuth (1924), indicating that a reassessment of established subgenera is needed, as well as a better understanding of phylogenetic relationships. Both the principal component analysis and the cluster analysis confirmed this result, since species of different sections were grouped in the same clusters and dispersed on axes 1 and 2.

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Disclosure statement

The authors declare that they have no conflict of interest.

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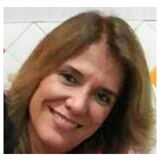

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