

## Differentiation of globular phytoliths in *Arecaceae* and other monocotyledons: morphological description for paleobotanical application

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**Abstract:** Globular phytoliths have been mainly assigned to palms, woody trees, and other monocotyledon families from tropical regions. The lack of detailed descriptions of this phytolith morphology and its correct assignments to particular groups could lead to erroneous interpretations of phytolith records. In order to improve paleobotanical interpretations of phytolith records, we analyzed the phytolith content and described the quantitative and qualitative characters of globular phytoliths of *Arecaceae*, *Bromeliaceae*, *Cannaceae*, *Marantaceae*, *Orchidaceae*, *Strelitziaceae*, and *Zingiberaceae* species from Argentina. Phytoliths were extracted by calcination and multivariate analyses were used to analyze their taxonomic relevance. Phytolith assemblages allowed the differentiation 1) among *Zingiberales* species; 2) among *Arecaceae* species; 3) between *Orchidaceae*, *Arecaceae*, and *Bromeliaceae* families; and 4) between *Zingiberales* and the rest of the groups. The study of distinguishing features of globular morphologies (such as size, roundness, reniformity, number of spines, spine length, and density of spines) allowed the discrimination between two *Arecaceae* subfamilies, and among *Bromeliaceae*, *Cannaceae*, and *Zingiberaceae* families. This work showed the importance of both analyses (phytolith assemblages and phytolith morphometric) in the identification of groups at different levels and represented the first detailed and comparative description of globular phytoliths of palms and other monocotyledons from Argentina.

**Key words:** *Arecaceae*, monocotyledons non-*Arecaceae*, phytoliths, paleobotany, Argentina

### 1. Introduction

Phytoliths are biomineralizations present in cell walls and in extra or intracellular spaces of plant tissues (Parry and Smithson, 1964; Bertoldi de Pomar, 1975). Although the term phytoliths refers to different amorphous and mineral deposits compounds by different elements, this study focuses on biogenic opal silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), also known as opal phytoliths or silicophytoliths (Ollendorf et al., 1987; Mulholland and Rapp, 1992; Osterrieth, 2004; Piperno, 2006). In this study, the term phytolith is employed in reference to silicophytoliths. Once the silica is available as silicic acid from soil solution, it is uptaken by the plant, transported by xylem, and finally it is accumulated for subsequent deposition as amorphous hydrated silica into the plant (Piperno, 1988; Ma and Takahashi, 2002; Exley, 2009). The production of phytoliths in a plant is related to various factors such as the climatic environment of

growth, the amount of water in the soil, the age of the plant, and, most importantly, the taxonomic affinity of the plant itself (Hodson et al., 2005; Piperno, 2006). The patterns of silica accumulation, together with the placement of these deposits in specific tissues and cells of plants, are quite similar in plant species and their most closely related taxa (Tomlinson, 1969; Metcalfe and Chalk, 1979; Kealhofer and Piperno, 1998; Runge, 1999; Hodson et al., 2005; Piperno, 2006).

*Arecaceae* is one of the main silica accumulators, along with the family *Poaceae* (Piperno, 1988; Kealhofer and Piperno, 1998). Schmitt et al. (1995) reported previous studies that described the presence of silica-bodies (stegmata) in various monocotyledons (such as orchids and palms) in unequally thickened cells characteristically disposed next to vascular fibers (subepidermal tissue). Tomlinson (1961) gave information on the variability of

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shape and structure of silica-bodies in the tissues of palms, recognizing mainly 2 types: hat-shaped and spherical outline in longitudinal files of cells adjacent to vascular or nonvascular fibers. According to the ICPN Working Group (International Code for Phytolith Nomenclature), these two types are papillae (or cones) and globular phytoliths (Madella et al., 2005). These phytolith morphologies are highly diagnostic for Arecaceae as a whole, including fossils, and they are quite consistently diagnostic for individual subfamilies, with minor exceptions (Tomlinson, 1961; Bertoldi de Pomar, 1971; Piperno, 1988; Albert et al., 2009).

In Argentina, about 11 species of native palms are distributed from north to south, reaching its maximum extent in the extra tropical latitudes (25–35°S), known as the domain of the pampas (*sensu* Cabrera and Willink, 1973). In this region, including bordering countries, palm phytoliths have been recovered from several archaeological sites (Campos et al., 2001; Zucol et al., 2005; Osterrieth et al., unpublished data), even in sites located further south to parallel 35°S (Brea et al., 2008).

Phytolith studies are a common tool used by many researchers for documenting vegetation changes and disturbance patterns related to human settlement and plant exploitation (Rosen, 2005). In particular, globular echinate phytoliths are commonly mentioned as morphologies resistant to degradation and alteration as well as forms recurrently transport by wind (Osterrieth et al., 2009). Despite the recent advances in detailed morphometric phytolith studies (Ollendorf, 1992, Fahmy, 2008; Albert et al., 2009; Fernández Honaine et al., 2009; Fenwick et al., 2011), investigations (based on quantitative descriptions) of several important plant families has not yet been done, and many phytolith studies are dependent solely on direct comparison of samples with modern reference collections that are usually specific to areas of study or present only a few specimens for performing accurate comparisons. Particularly, the scarcity of quantitative descriptions of modern phytoliths in Arecaceae has precluded more specific taxonomic identification of these phytoliths in archeological and paleobotanical contexts (Albert et al., 2009; Fenwick et al., 2011).

Although some authors have commonly assigned globular morphologies to the family Arecaceae, similar deposits are not unique to palms and occur in a number of monocotyledonous families without obvious close affinity. Tomlinson (1990), Kealhofer and Piperno (1998), and Sandoval-Zapotitla et al. (2010) mentioned the presence of these morphologies in palms as well as in Bromeliaceae, Strelitziaceae, and Orchidaceae; likewise, Pearsall and Dinan (1992), Kealhofer and Piperno (1998), Runge (1999), and Brillhante de Albuquerque et al. (2013) described them in Marantaceae, Cannaceae, and

Zingiberaceae. The redundancy of globular phytoliths in several botanical families could lead to inaccurate paleobotanical reconstructions. Hence, it is important to evaluate if there are significant morphological differences within the globular phytoliths category that would allow the identification of plant taxa.

The objectives of this study were to analyze the phytolith content of different species belonging to the families Arecaceae, Bromeliaceae, Cannaceae, Marantaceae, Orchidaceae, Strelitziaceae, and Zingiberaceae, all of them described as globular producers by other researchers, and to carry out morphometric measurements on the globular phytoliths in order to evaluate how significant the morphological differences are among these globular types. In total we analyzed the phytoliths content of 21 species. These species occur in South America and belong to the families Arecaceae (10 species), Bromeliaceae (4 species), Cannaceae (1 species), Marantaceae (1 species), Orchidaceae (3 species) Strelitziaceae (1 species), and Zingiberaceae (1 species).

## 2. Materials and methods

### 2.1. Plant selection

Based on previous references, which reported the globular phytoliths production in Arecaceae and non-Arecaceae families, and considering the importance of the native Arecaceae species in the paleobotanical studies from Argentina and the bordering countries, 10 species of palms and 11 from 6 families of non-Arecaceae monocotyledons (Bromeliaceae, Cannaceae, Marantaceae, Orchidaceae, Strelitziaceae, and Zingiberaceae) were selected. Native palms were sampled at Instituto de Botánica Darwinion (San Isidro, Buenos Aires, Argentina). The rest of the species were collected from gardens located near Mar del Plata city, Grilli plant nursery of Mar del Plata and the Moconá National Park (Misiones, Argentina) (Table 1).

### 2.2. Phytolith extraction and description

Considering the leaves as the highest accumulator organs of phytoliths in palms (Piperno, 1998) and the importance of them to input of phytoliths in soils, leaves from at least 2 specimens of each species were collected.

Phytoliths were extracted following a calcination technique (Labouriau, 1983). The samples were first placed in an ultrasound bath for 15–20 min and washed with distilled water to remove mineral contaminants. The material was dried at 56 °C for 24 h, and charred at 200 °C for 2 h. Later, it was boiled in 5 N HCl solution for 10 min, washed with distilled water, and filtered with ashless filter paper, until no more chloride ions were detected. Finally, the material was ignited at 760 °C for 3.5 h. Silica content was calculated as a percentage dry weight.

The ashes obtained were mounted with immersion oil and the morphologies were observed and described

with a Zeiss Axiostar Plus microscope at  $\times 400$  and  $\times 1000$  magnification. Photographs were taken with a digital camera Canon Powershot G10. The samples were also gold-coated and observed using a scanning electron microscope (JEOL JSM-6460 LV; Japan) at Universidad Nacional de Mar del Plata, Argentina, to corroborate the measurements. The composition of the ashes was analyzed by X-ray energy dispersive spectroscopy (EDS). The system used was an EDAX Genesis XM4-Sys 60, equipped with a multichannel analyzer EDAX mod EDAM IV, Sapphire Si (Li) detector and super ultra-thin window of Be, and EDAX Genesis version 5.11 software. More than 300 phytoliths were counted in each slide. General phytolith morphologies and specific surface ornamentation features were described according to the ICPN descriptors (Madella et al., 2005).

### 2.3. Analyses of the globular morphologies

In those species where the production of globular echinate phytoliths was abundant (more than 40%), at least 30 globular phytoliths in each slide were observed and the following characters were measured according to the methods proposed by Fenwick et al. (2011) with some modifications (Figure 1):

#### Quantitative variables:

1. Maximum diameter ( $\mu\text{m}$ ) ( $M$ ): the phytolith's largest visible dimension, measured from spine tip to spine tip.

2. Perpendicular diameter ( $\mu\text{m}$ ) ( $D$ ): the maximum diameter of each phytolith perpendicular to the absolute maximum diameter.

3. Sphericity ( $R$ ): the ratio  $M/D$ .

4. Number of spines ( $n$ ): number of spines visible in entirety in the viewing field. Number of spines was regarded as 50% of the globular body total.

5. Mean spine length ( $\mu\text{m}$ ) ( $l$ ): the mean length of spines parallel to the viewing plane.

6. Density of spines ( $d$ ): the number of spines per square micron of surface area. Calculation derived from Fenwick et al. (2011).

#### Qualitative variables:

7. Reniformity (0/1) ( $k$ ): 0 = completely convex body, 1 = kidney-shaped (reniform) body.

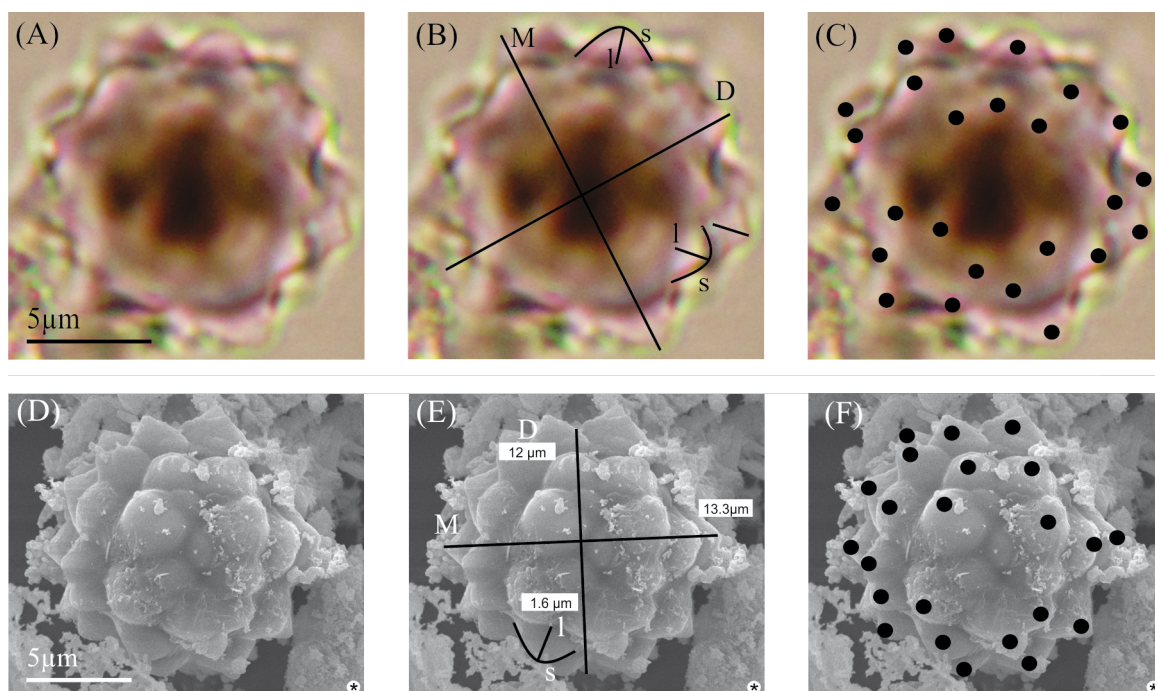
8. Roundness (0/1) ( $u$ ): a dummy variable derived from the quantitative variable of sphericity and indicating whether a phytolith is spherical or ellipsoidal. 0 = ellipsoidal ( $R \geq 1,2$ ); 1 = spherical ( $R < 1,2$ ).

9. Globular edge or surface (1/2/3) ( $c$ ): 1 = psilate; 2 = echinate; 3 = granulate.

10. Spine edge (0/1) ( $s$ ): terminal surface of spines. 0 = sharp-rounded; 1 = sharp-pointed.

### 2.4. Analyses of the cone morphologies

In the leaves of species where cone morphologies were observed, 2 quantitative characters were measured. These



**Figure 1.** (A) and (D) Globular phytoliths observed by light and scanning electron microscopes (\*). (B) and (E) schematic representation of some characters measured on globular phytolith:  $M$  maximum diameter,  $D$  perpendicular diameter,  $l$  spine length,  $s$  spine edge. (C) and (F) black dots represent the counted spines.

characters were measured in a minimum of 30 phytoliths according to the methods proposed by Ollendorf (1992): (L) length of the longest axis of the base; (H) apex height.

### 2.5. Data analyses

Principal component analyses (PCA) were carried out with the purpose of evaluating the relevance of phytolith assemblages and the quantitative and qualitative characters of globular phytoliths in the differentiation of the groups analyzed. PCA based on phytolith assemblages was carried on a variance-covariance matrix and only isolated morphologies were included. PCA based on qualitative and quantitative characters was performed on the basis of a correlation matrix. Three qualitative variables (reniformity, roundness, and spine edge) were submitted as frequency for its analysis.

Finally, those morphometric variables that account for the differentiation between species in the second PCA (PCA based on qualitative and quantitative characters) were subjected to nonparametric analysis of variance (Kruskal–Wallis test) (Zar, 1984).

## 3. Results

### 3.1. Silica content

The mean values of silica content in Areaceae ranged between 1.7% (*Allagoptera campestris*) and 8.0% (*Trachycarpus fortunei*), whereas in non-Areaceae monocotyledons the highest mean value was 2.8%, except *Canna indica*, which presented a mean value of 4.9% (Table 1).

### 3.2. Phytolith description

All of the 21 species analyzed produced phytoliths, and there was a considerable variability in the type and abundance of the morphologies produced. Qualitative and quantitative characters of the globular morphologies in leaves can be observed in Table 2, with globular and nonglobular morphologies photographs of the studied families in Figures 2A through 2P and Figures 3A through 3G, respectively. The abundance of the most important morphologies is shown in Figure 4 and in the supplementary material (on the journal's website).

#### 3.2.1. Areaceae

##### 3.2.1.1. Arecoideae

In all except one (*Acrocomia aculeata*) of the six studied species, phytolith assemblage was mainly represented by articulated and isolated globular echinate phytoliths. The globular echinate morphologies showed a range of mean values of M from 6.27 to 8.94  $\mu\text{m}$  and an ellipsoidal and kidney shaped body. The number of spines ranges from 11 to 15; the length of spines ranges between 1.13 and 1.22  $\mu\text{m}$  and the spine edge is mostly rounded, except in *E. edulis*, which showed a strong tendency towards having

sharp-pointed spines. Other phytolith morphologies were elongate psilate and elongate with fusiform edges articulated to globular echinate phytoliths; tabular sublobate phytoliths; and stomatal complexes. Phytolith assemblage in *Acrocomia aculeata* was mainly represented by isolated cones with values of L (length of the base) and H (apex height) between 6 and 14  $\mu\text{m}$  and 4 and 6  $\mu\text{m}$ , respectively, and cones articulated to elongate phytoliths with fusiform edges.

##### 3.2.1.2. Coryphoideae

The most abundant morphologies in the phytolith assemblages of the 4 studied species were articulated and isolated globular echinate. Globular echinate morphology presented mean values of maximum diameter (M) from 9.25 to 14.26  $\mu\text{m}$ , a strong tendency to be spherical (82%–87%), and a mean *n* and *l* quite high (from 17 to 21 and from 1.42  $\mu\text{m}$  to 1.84  $\mu\text{m}$ , respectively). Spine edge in *P. canariensis* and *T. fortunei* showed a weak tendency towards having sharp-rounded spines, while *C. alba* and *T. campestris* showed only sharp-rounded spines. The spine density in this group is low with respect to Arecoideae and the rest of the species analyzed. Moreover, other phytoliths were observed: elongate psilate and elongate with fusiform edges in isolated form and articulated to globular echinate phytoliths; tabular sublobate phytoliths in isolated and articulated form; stomatal complexes; and cylindrical sulcate tracheid in isolated and articulated form.

#### 3.2.2. Bromeliaceae

##### 3.2.2.1. Bromelioideae

In the two studied species, globular echinate phytoliths were the dominant morphologies in the assemblages. Globular echinate phytoliths from *Billbergia* sp. had a mean M of 3.32  $\mu\text{m}$  and a strong tendency toward sphericity (85%), while *A. comosus* presented a mean M of 4.90  $\mu\text{m}$  and a weak tendency towards sphericity (58% ellipsoidal forms and a smaller amount of kidney-shapes). The mean *n* values range between 7 and 9, with values of *l* approximately of 2  $\mu\text{m}$  and sharp-pointed spine edges. Other phytolith morphologies such as articulated tabular sublobate and cylindrical sulcate tracheid were observed.

##### 3.2.2.2. Tillandsioideae

In these species, articulated and isolated globular echinate phytoliths were dominant. Globular morphologies presented a mean of M of 2.65 (*Guzmania* sp.) and 3.43  $\mu\text{m}$  (*T. aëranthos*) and a strong tendency to be spherical (80%–90%). Both species had an *n* approximately of 6 with spines of 0.95  $\mu\text{m}$  and sharp-pointed spine edges. Oblong elongate phytoliths arranged in radiated form and elongate with fusiform edges were also described in their assemblages.

**Table 1.** Species selected for the study and silica content calculated as % dry weight. (N) native palm to the region, (E) exotic palm to the region, (SI) San Isidro, Darwinion Herbarium, (SPF) Herbarium from the University of Sao Paulo, (UNNO) Universidad Nacional del Nordeste, (IBN) Instituto de Botánica del Nordeste, (JBA) Jardín Botánico de Asunción, (JBCT) Jardín Botánico Carlos Thays, (UNMdP) Laboratorio de Geocología de suelos, Universidad Nacional de Mar del Plata.

Family/ Subfamily Species	Code	Specimen number/ Herbarium code	Silica content (% dry weight)
Arecaceae / Arecoideae			
<i>Acrocomia aculeata</i> (Jacq.) Lood. ex Mart. (N)	Aa	no. 326 (SPF) no. 239 (SI) no. 314 (UNNO)	4.9
<i>Allagoptera campestris</i> (Mart.) Kuntze (N)	Ac	no. 1046 (SI) no. 3724 (SI) no. 1295 (SI)	1.7
<i>Butia capitata</i> (Mart.) Becc. (N)	Bc	no. 82093 (SI) no. 15284 (SI) no. s/n (JBCT)	4.6
<i>Butia paraguayensis</i> (Barb. Rodr.) C. H. Bailey (N)	Bp	no. 278059 (SI) no. 32583 (SI) no. 9905 (SI)	2.2
<i>Euterpes edulis</i> Mart. (N)	Ee	no. 25077 (SI) no. 875 (SI)	5.5
<i>Syagrus romanzoffiana</i> (Cham.) Glassman (N)	Sr	no. 28911 (SI) no. 4940 (IBN)	2.8
Arecaceae / Coryphoideae			
<i>Copernicia alba</i> Moroni (N)	Ca	no. 5286 (IBN) no. 41629 (JBA) no. 325 (SI)	5.1
<i>Phoenix canariensis</i> Chabaud (E)	Pc	no. 568 (UNMdP) no. 569 (UNMdP)	5.0
<i>Trachycarpus fortunei</i> (Hook.) H. Wendl. (E)	Tf	no. 566 (UNMdP) no. 567 (UNMdP)	8.0
<i>Trithrinax campestris</i> (Burmeister) Drude & Grises (N)	Tc	no. 277 (SI) no. 276 (SI) no. 6167 (SI)	3.8
Bromeliaceae / Bromelioideae			
<i>Ananas comosus</i> (L.) Merr. (N)	Aco	no.B1.123 a, b (UNMdP)	1.2
<i>Billbergia</i> sp.	Bsp	no.122B2 a, b (UNMdP)	1.6
Bromeliaceae / Tillandsioideae			
<i>Guzmania</i> sp.	Gsp	no.121B3 a, b (UNMdP)	2.0
<i>Tillandsia aëranthos</i> (Loisel.) L. B.Sm. (N)	Ta	no.B4 573 (UNMdP) no.B4 572 (UNMdP)	0.7
Cannaceae			
<i>Canna indica</i> L. (N)	Ci	no.570 (UNMdP) no.571 (UNMdP)	4.9
Marantaceae			
<i>Calathea makoyana</i> E. Morren (E)	Cm	no.M1 120 a, b. (UNMdP)	0.4
Orchidaceae / Epidendroideae			
<i>Gomesa planifolia</i> Klotzsch & Rchb. F. (N)	Gp	no. O1 117 a, b. (UNMdP)	0.8
<i>Isochilus linearis</i> (Jacq.) R. Br. (N)	IL	no. O2 118 a, b (UNMdP)	0.4
<i>Oncidium</i> sp.	Osp	no. O3 119 a, b (UNMdP)	2.8
Strelitziaceae			
<i>Strelitzia reginae</i> Banks (E)	St	no. 576 (UNMdP) no. 577 (UNMdP)	2.5
Zingiberaceae			
<i>Zingiber</i> sp.	Zsp	no. 574 (UNMdP) no. 575 (UNMdP)	1.6

**Table 2.** Qualitative and quantitative characters of the globular morphologies in leaves. Min: minimum value, max: maximum value, mean: mean value of 2–3 individuals.

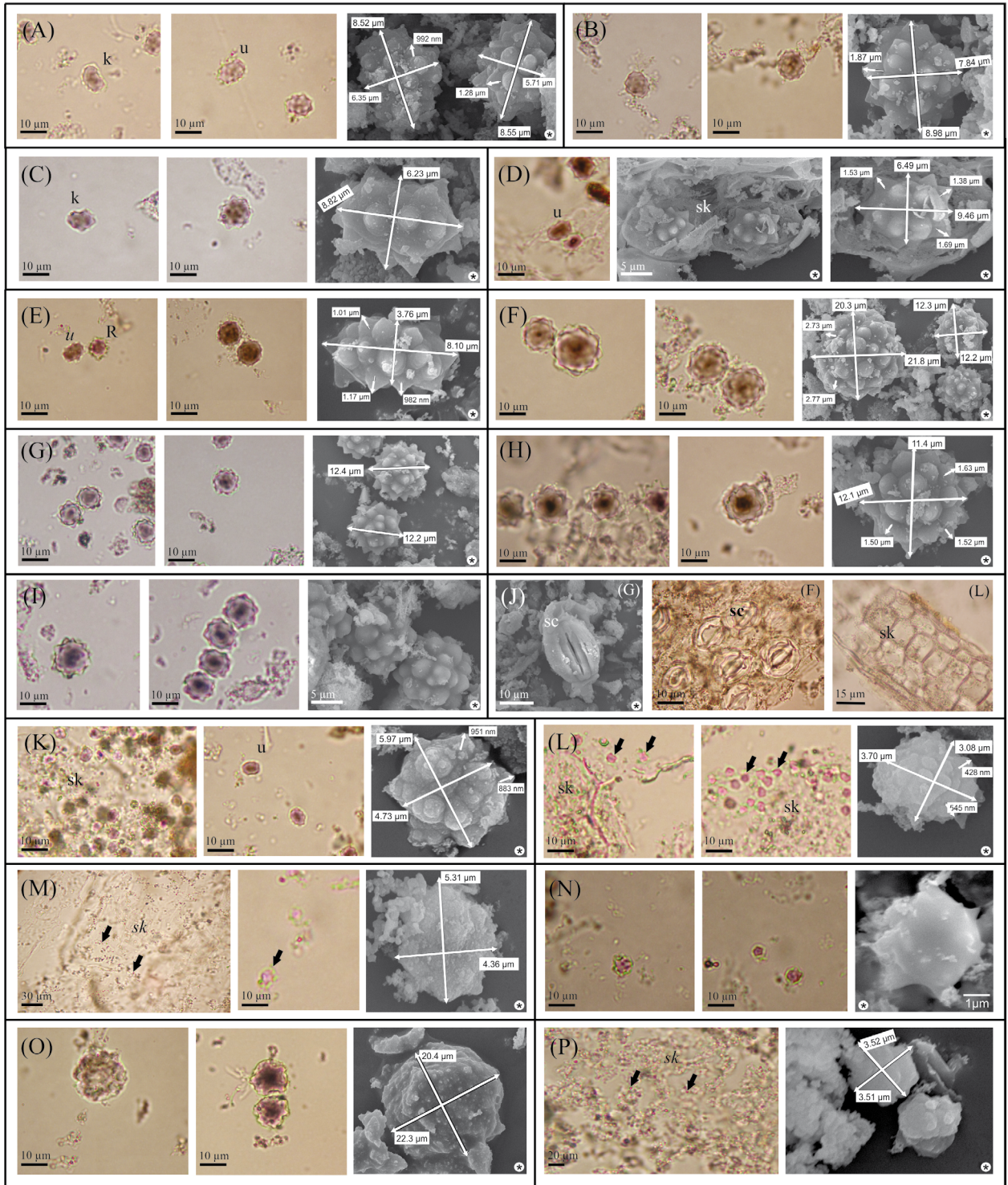
Species	Max. diameter (µm)			Perp. diameter (µm)			Sphericity (µm)			<i>f</i> (round)*	<i>f</i> (reniform)*
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Mean	Mean
<b>Arecaceae</b>											
<i>Allagoptera campestris</i>	5.00	7.57	11.00	4.00	5.72	9.00	1.00	1.32	2.25	0.19	0.22
<i>Butia capitata</i>	5.00	8.94	14.00	4.00	7.28	14.00	1.00	1.24	2.00	0.36	0.12
<i>Butia paraguayensis</i>	4.00	7.11	11.00	3.00	5.43	20.00	1.00	1.31	2.33	0.21	0.17
<i>Copernicia alba</i>	7.00	14.26	27.00	6.00	13.24	21.00	1.00	1.08	1.43	0.82	0.00
<i>Euterpes edulis</i>	5.00	6.27	9.00	4.00	5.29	7.00	1.00	1.18	1.75	0.29	0.00
<i>Phoenix canariensis</i>	7.00	9.25	13.00	6.00	8.63	12.00	1.00	1.07	1.29	0.85	0.00
<i>Syagrus romanzoffiana</i>	4.00	8.03	18.00	3.00	6.33	17.00	1.00	1.27	1.83	0.26	0.20
<i>Trachycarpus fortunei</i>	9.00	12.07	16.00	8.00	11.23	15.00	0.91	1.07	1.33	0.87	0.00
<i>Trithrinax campestris</i>	8.00	14.00	18.00	8.00	13.06	18.00	1.00	1.07	1.50	0.86	0.00
<b>Bromeliaceae</b>											
<i>Ananas comosus</i>	3.00	4.90	6.00	3.00	4.43	6.00	1.00	1.11	1.67	0.58	0.02
<i>Billbergia</i> sp.	2.00	3.32	6.00	2.00	3.10	4.00	1.00	1.07	2.00	0.85	0.00
<i>Guzmania</i> sp.	1.00	2.65	5.00	1.00	2.58	4.00	0.67	1.03	1.33	0.90	0.00
<i>Tillandsia aëranthos</i>	2.00	3.43	5.00	1.00	3.23	5.00	1.00	1.06	2.00	0.80	0.00
<b>Cannaceae</b>											
<i>Canna indica</i> (G)	10.00	17.92	23.00	10.00	16.69	20.00	0.92	1.07	1.31	0.81	0.00
<b>Zingiberaceae</b>											
<i>Zingiber</i> sp.	1.00	1.38	3.00	1.00	1.38	3.00	1.00	1.00	1.00	1.00	0.00

\*frequency. (G) globular granulate phytoliths

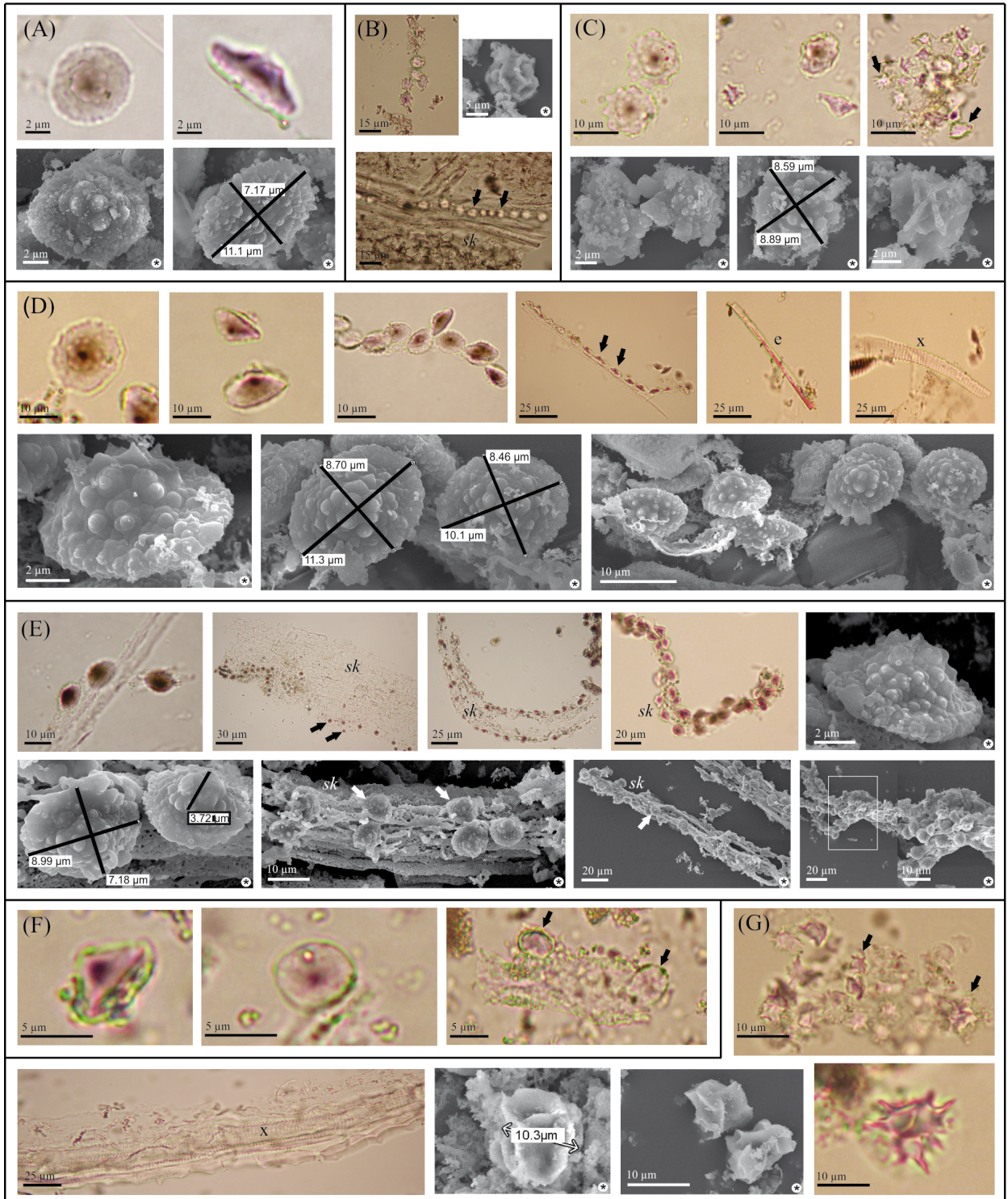
**Table 2.** (Continued).

Species	Globular edge	Number of spines (no.)			Spine length (µm)			<i>f</i> (spine edge)*	Spine density (no./µm <sup>2</sup> )		
		Min	Mean	Max	Min	Mean	Max	Mean	Min	Mean	Max
<b>Arecaceae</b>											
<i>Allagoptera campestris</i>	2.00	7.00	10.81	18.00	0.50	1.13	2.20	0.00	0.07	0.16	0.31
<i>Butia capitata</i>	2.00	9.00	14.74	23.00	1.00	1.22	1.60	0.00	0.05	0.15	0.41
<i>Butia paraguayensis</i>	2.00	7.00	12.33	20.00	0.90	1.23	2.00	0.06	0.08	0.20	0.62
<i>Copernicia alba</i>	2.00	10.00	20.63	30.00	1.20	1.84	2.50	0.00	0.03	0.07	0.27
<i>Euterpes edulis</i>	2.00	7.00	11.48	17.00	1.00	1.12	1.50	1.00	0.10	0.22	0.32
<i>Phoenix canariensis</i>	2.00	14.00	18.20	23.00	1.10	1.53	2.00	0.37	0.07	0.14	0.26
<i>Syagrus romanzoffiana</i>	2.00	9.00	13.54	30.00	0.90	1.21	2.00	0.00	0.06	0.17	0.47
<i>Trachycarpus fortunei</i>	2.00	14.00	16.50	19.00	1.00	1.42	1.80	0.17	0.05	0.08	0.16
<i>Trithrinax campestris</i>	2.00	11.00	16.38	23.00	1.00	2.21	3.20	0.00	0.03	0.06	0.19
<b>Bromeliaceae</b>											
<i>Ananas comosus</i>	2.00	6.00	8.70	12.00	0.70	0.94	1.00	1.00	0.15	0.25	0.78
<i>Billbergia</i> sp.	2.00	5.00	6.48	9.00	0.70	0.95	1.00	1.00	0.20	0.40	0.95
<i>Guzmania</i> sp.	2.00	4.00	6.03	11.00	0.40	0.49	0.50	1.00	0.20	0.59	2.55
<i>Tillandsia aëranthos</i>	2.00	5.00	6.55	10.00	0.50	0.63	0.70	1.00	0.13	0.38	1.98
<b>Cannaceae</b>											
<i>Canna indica</i> (G)	3.00	–	–	–	–	–	–	–	–	–	–
<b>Zingiberaceae</b>											
<i>Zingiber</i> sp.	1.00	–	–	–	–	–	–	–	–	–	–

\*frequency. (G) globular granulate phytoliths

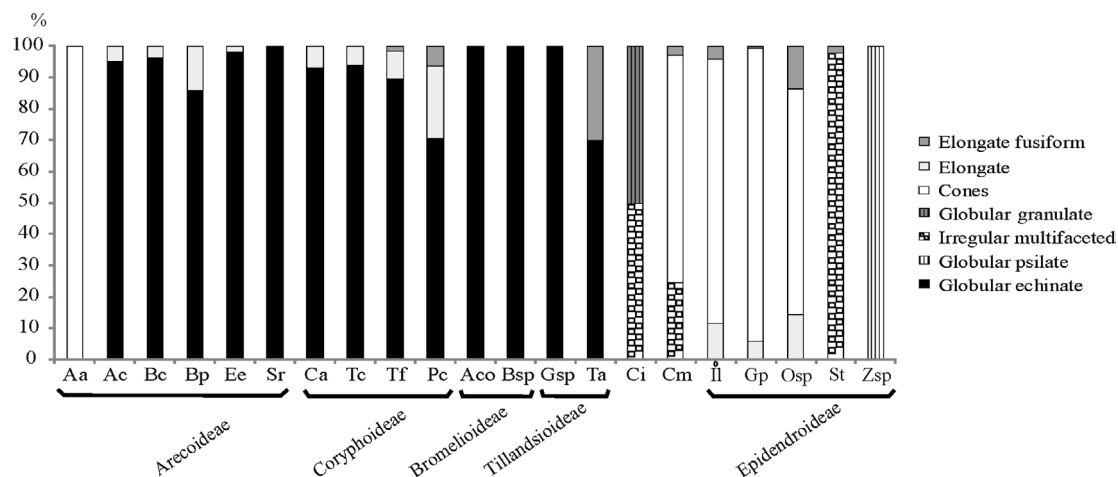


**Figure 2.** Globular phytoliths found in leaves of Arecaceae, Bromeliaceae, Cannaceae, and Zingiberaceae. (A) *Allagoptera campestris*, (B) *Butia capitata*, (C) *Butia paraguayensis*, (D) *Euterpes edulis*, (E) *Syagrus romanzoffiana*, (F) *Copernicia alba*, (G) *Phoenix canariensis*, (H) *Trithrinax campestris*, (I) *Trachycarpus fortunei*, (J) other nonglobular morphologies, (K) *Ananas comosus*, (L) *Billbergia* sp., (M) *Guzmania* sp., (N) *Tillandsia aëranthos*, (O) *Canna indica*, (P) *Zingiber* sp., (k) kidney-shaped body, (R) spherical body, (sc) stomatal complexes, (sk) skeleton phytoliths, (u) ellipsoidal phytolith. The black arrows indicate globular phytoliths less visible. (\*) SEM photographs.



**Figure 3.** Nonglobular morphologies found in leaves of *Arecaceae*, *Cannaceae*, *Marantaceae*, *Orchidaceae*, and *Strelitziaceae*. (A) *Acrocomia aculeata*, (B) *Canna indica*, (C) *Calathea makoyana*, (D) *Gomesa planifolia*, (E) *Isochilus linearis*, (F) *Oncidium* sp., (G) *Strelitzia reginae*, (e) elongate phytolith. (sk) skeleton phytoliths. (x) cylindrical sulcate tracheid or xylem. The black and white arrows indicate globular phytoliths less visible. (\*) SEM photographs.





**Figure 4.** Percentages of the most abundant isolated phytoliths in leaves. Abbreviations of species are as given in Table 1. Below species abbreviations are subfamilies categories.

### 3.2.3. Cannaceae

#### 3.2.3.1. *Canna indica*

The most important morphology observed in this species belonged to globular granulate phytoliths and irregular multifaceted phytoliths in articulated and isolated form. The globular granulate presented a mean  $M$  of 17.92  $\mu\text{m}$ , a tendency towards sphericity of 81%, and unlike previous globular echinate morphologies this species presented a globular edge granulated without spines. Other phytolith morphologies described were articulated cylindrical sulcate tracheid and isolated tabular sublobate.

### 3.2.4. Marantaceae

#### 3.2.4.1. *Calathea makoyana*

The dominant morphologies observed in this species were: cones (in isolated form or articulated with elongate psilate) with values of  $L$  between 5 and 15  $\mu\text{m}$  and  $H$  between 3 and 4  $\mu\text{m}$ ; and irregular multifaceted in articulated and isolated form. Elongate phytoliths with fusiform edges were found articulated.

### 3.2.5. Orchidaceae

#### 3.2.5.1. Epidendroideae

In the three studied species, articulated and isolated cones were the dominant morphologies. The values of  $L$  and  $H$  ranged between 5 and 13  $\mu\text{m}$  and 3 and 5  $\mu\text{m}$ , respectively. Elongate oblong phytoliths associated with fibers and cones, and psilate elongate in isolated form and articulated with fusiform edges were also described. Cylindrical sulcate tracheid and prickles or hooks were observed in smaller amounts (less than 1.5%).

### 3.2.6. Strelitziaceae

#### 3.2.6.1. *Strelitzia reginae*

Irregular multifaceted phytoliths in isolated form and articulated with elongates with fusiform edges were the

most abundant morphologies in this species. Elongates with fusiform edges in isolated form and articulated with cylindrical sulcate tracheid were also observed.

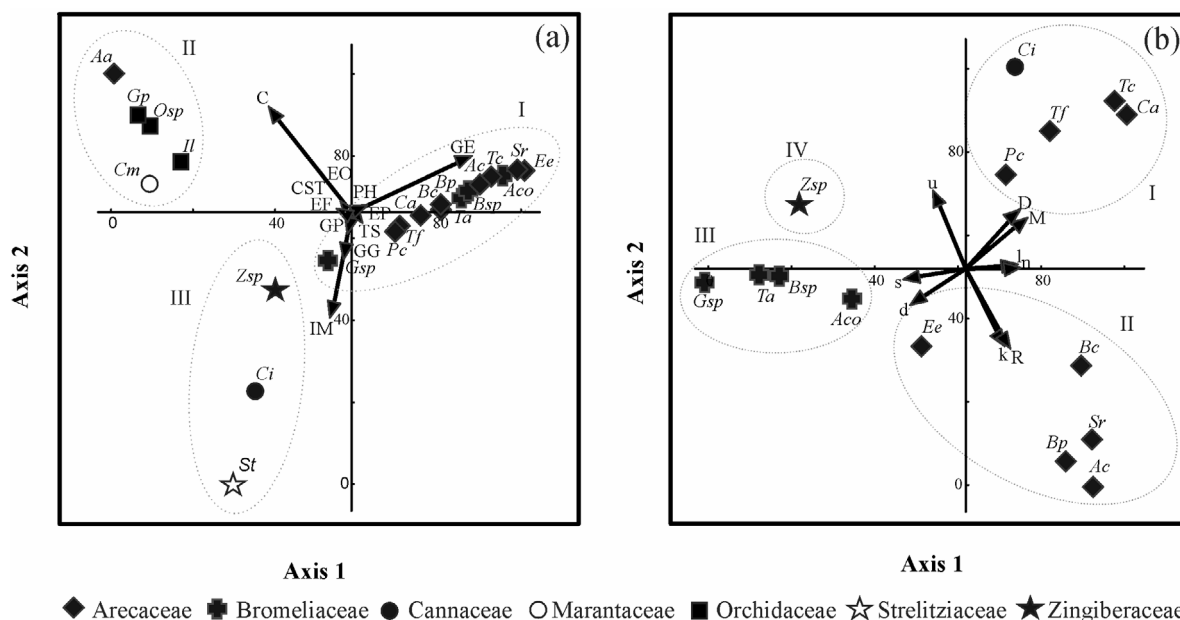
### 3.2.7. Zingiberaceae

#### 3.2.7.1. *Zingiber sp.*

Articulated and isolated globular psilate phytoliths were the only dominant morphology observed in this species. This morphology had the smallest mean of  $M$  (1.38  $\mu\text{m}$ ), a spherical shape, and it was described as globular psilate (without visible spines).

### 3.3. Principal component analyses

The PCA based on phytolith assemblages accounted for 87.38% of the total variance (65.87% for axis 1 and 21.51% for axis 2) (Figure 5a). The sample and vector plots clearly showed three main groups: Group I: species of Arecoaceae (without *Acrocomia aculeata*) and Bromeliaceae; Group II: *Acrocomia aculeata*, species of Marantaceae and Orchidaceae; and Group III: species of Cannaceae, Strelitziaceae, and Zingiberaceae. Group I was differentiated by the presence of globular echinate phytoliths, Group II was characterized by the production of cones, and Group III presented species with irregular multifaceted phytoliths (*C. indica* and *S. reginae*), globular granulate (*C. indica*), and globular psilate morphologies (*Zingiber sp.*). The second PCA based on the quantitative and qualitative characters of globular phytoliths (Figure 5b) revealed the same differences as the previous PCA. In this analysis, the two first axes accounted for 73.30% of the total variance (41.74% for axis 1 and 31.56% for axis 2). The sample and vector plots showed the following four groups: Group I: species of the subfamily Coryphoideae and *C. indica* (Cannaceae); Group II: species of the subfamily Arecoideae; Group III: species of Bromeliaceae; and Group IV: *Zingiber sp.* The members of Group I, except *C. indica*,



**Figure 5.** Simple scatterplot and vector plot of the principal components analysis of species based on the relative frequencies of isolated phytolith morphologies (a) and based on qualitative and quantitative characters of globular phytoliths (b). Abbreviations of species are as given in Table 1. (C) cones, (CST) cylindrical sulcate tracheid, (EF) elongate fusiform, (EO) elongate oblong, (EP) elongate psilate and sinuate, (GE) globular echinate, (GG) globular granulate, (GP) globular psilate, (IM) irregular multifaceted, (PH) prickles and hooks, and (TS) tabular sublobate.

were most easily differentiated by presenting mean values of *M*, *D*, *n*, and *l* highest and the lowest values of spine density. *C. indica* was integrated to this group by presented values of *M* and *D* similar to the Coryphoideae species. Group II presented mean values of *M*, *D*, *n*, and *l* higher than the values obtained from groups III and IV. Moreover, this group presented kidney globular morphologies. Group IV was characterized by the lowest means values of *M* and *D*, the highest values of *u*, and absence of spines.

Significant differences between mean values of *M* (maximum diameter) ( $H = 28.681, P = 0.012$ ), *D* (perpendicular diameter) ( $H = 28.755, P = 0.011$ ), *n* (number of spines) ( $H = 28.554, P = 0.012$ ), *l* (mean spine length) ( $H = 28.208, P = 0.013$ ), and *d* (density of spines) ( $H = 28.347, P = 0.013$ ) were found after Kruskal–Wallis tests.

#### 4. Discussion

##### 4.1. Silica content

The silica content in the analyzed leaves reflected an important variability within a genus, subfamily, and family, as reported by many other researchers (Hodson et al., 2005). The values for native palms are included in the range of values obtained for palms (3.8% and 10%) and other monocotyledons such as grasses (3% and 13%) and sedges (1.3% and 12.8%) by other researchers (Gallego et al., 2004; Fernández Honaine et al., 2008; Fernández

Honaine et al., 2009; Patterer, unpublished data). Although silica content cannot be considered as taxonomically useful (e.g., Henriot et al., 2006; Piperno, 2006), the quantitative analysis could be relevant if they are addressed from other disciplines, such as biogeochemistry and plant anatomy and physiology. The quantification of the silica content in plants constitutes a relevant aspect for the understanding of the silica cycle, where plants have an important role (Borrelli et al., 2008).

##### 4.2. Phytolith assemblages

Although the most abundant phytolith morphologies in leaf assemblages are the cones and globular phytoliths, the silicification process is also common in xylem (cylindrical sulcate tracheid phytoliths), in fibers (described as elongate with fusiform edges), parenchyma tissue (tabular sublobate), and in epidermal cells (elongate psilate, prickles/hooks, and stomatal complexes). Even though the dominant phytolith morphologies of the reported assemblages in this work had already been described by several authors in species from other regions, some differences were observed (Tomlinson, 1961; Piperno, 1988; Tomlinson, 1990; Pearsall and Dinan, 1992; Kealhofer and Piperno, 1998; Runge, 1999; Sandoval-Zapotitla et al., 2010; Brilhante de Albuquerque et al., 2013). In this work, in contrast to other reports (Tomlinson, 1990; Kealhofer and Piperno, 1998) the globular phytoliths were observed in all families analyzed, except in Marantaceae

and Orchidaceae. The quantitative characters measured on leaf cones phytoliths (length and height) in species of Arecaceae, Marantaceae, and Orchidaceae showed a strong overlapping among them and did not allow family differentiation. Furthermore, these values are similar to those described for Cyperaceae species (Ollendorf, 1992; Fernández Honaine et al., 2009). These results suggest that other features such as the apex and base ornamentations of the cone should be taken into account to distinguish among different taxa. Tomlinson (1961) noted that members of Arecaceae (such as *Latania* sp.) can characteristically produce two types of siliceous bodies (hat-shaped or conical forms with a flattened or irregular base, and irregular spherical bodies with spinulose surface). In all palms species analyzed in this study, only one of these two morphology types was observed in each species. Other authors reported the presence of cones and globular morphologies in Marantaceae leaves (Piperno, 1988; Kealhofer and Piperno, 1998; Brillhante de Albuquerque et al., 2013), and mention the typical spherical phytoliths with an irregularly angled or folded surface in members from Cannaceae, Marantaceae, and Zingiberaceae (Piperno, 1988; Runge, 1999; Brillhante de Albuquerque et al., 2013). These descriptions coincide with the results reported in this work as regards to the presence of globular morphology in *Canna indica* and *Zingiber* sp. but not in *Calathea makoyana* (Marantaceae). The presence of cones in Marantaceae is consistent with these results. The irregular multifaceted phytoliths observed in the species of Cannaceae, Marantaceae, and Strelitziaceae analyzed in this study could represent the spherical phytoliths with an irregularly angled or folded surface described by Piperno (2006). However, the observation under light and electronic microscope made in the present study did not allow us to describe them as spherical.

PCA based on phytolith assemblages allowed us to arrange the species in three groups resembling the systematic association between them. In general, species belonging to the same family were grouped together (for example, palms and Bromeliaceae species) and families from the same order (Zingiberales) were integrated to the same group (Cannaceae, Strelitziaceae, and Zingiberaceae). The most important phytolith morphologies responsible for the division of the groups were the cones, and the globular and the irregular multifaceted phytoliths. However, the incorporation of more species of these families in future analyses would be necessary to corroborate our findings.

The results of the PCA based on quantitative and qualitative characters of globular phytoliths allowed us to distinguish some families that had been previously gathered together in the first PCA. Arecaceae and Bromeliaceae were separated and the two subfamilies (Arecoideae and Coryphoideae) could be differentiated

within the Arecaceae group. *Canna indica* and *Zingiber* sp. were also separated and *C. indica* was incorporated into the palms group due to its mean value of maximum diameter. The most important characters in the division of the groups were maximum diameter (M), perpendicular diameter (D), number of spines (*n*), and mean spine length (*l*); however, the rest of the quantitative variables were very useful in the differentiation to family, subfamily, and genus level. For example, the species from Arecaceae presented spines that tend to be larger, more frequent, and better defined than those from Bromeliaceae species (Piperno, 1988). Coryphoideae species showed the most highest values of M, D, *n*, and *l* but also showed a strong tendency to be spherical (*u*) and have lower values of density of spines (*d*) than Arecoideae. Other authors found both ellipsoidal and spherical globular phytoliths in the palms analyzed (Patterer et al., 2011). However, in this work, the Arecoideae subfamily presented a higher tendency towards ellipsoidal (*u*) and reniform (*k*) forms than Coryphoideae. In many genera of palms, the presence of rounded and pointed terminal surfaces of the spines (*s*) is commonly described (Piperno, 1988). However, in our work, pointed terminal surfaces were more frequent in Bromeliaceae than in Arecaceae except for one species of palm (*E. edulis*) that presented the same frequency as Bromeliaceae. The results obtained for palms represent the first data for this region and, in agreement with Fenwich et al. (2011), although it was not possible to identify palm phytoliths at species level, some differences at other levels (genus or subfamily) may be observed. The detailed analyses of globular phytoliths can represent a complementary tool for distinguishing among different kinds of globular forms produced by relatively close taxa (Kress, 1990; Fenwich et al., 2011).

A strong overlapping among values of maximum diameter (M) of globular morphology was observed in all species analyzed (4–27  $\mu\text{m}$ , Arecaceae; 1–6  $\mu\text{m}$ , Bromeliaceae; 10–23  $\mu\text{m}$ , Cannaceae; and 1–3  $\mu\text{m}$ , Zingiberaceae), which is consistent with other reports carried out by several authors (Tomlinson, 1969; Piperno, 1988; Wallis, 2003; Patterer, unpublished data). These overlappings could indicate different stages in the development of the phytoliths (Runge, 1999). However, the study of other features (especially *n*, *l*, and *d*) in globular morphologies might aid in the taxonomic identification of these forms beyond the measured size (Piperno, 1988; Fenwich et al., 2011).

In summary, the paper presents the first detailed and comparative description of globular phytoliths of palms and other monocotyledons of this region. Some variables described, such as sphericity, roundness, reniformity, number of spines, spine length, spine edge, and density of spines, constitute the first data of the species analyzed.

The study of other distinguishing features beyond the size of globular morphologies allowed the taxonomic differentiation between the phytoliths of some taxa of palms and monocotyledon nonpalms. These results also showed the importance of both analyses based on assemblages and quantitative and qualitative characters in the identification of groups at different levels. Finally, the morphological differences observed between globular phytoliths may be a useful tool for paleobotanical and/or archaeological studies, especially in those instances where

phytoliths represent the only source of information of paleocommunities or human activities.

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**Supplementary material.** Percentages of isolated and articulated phytoliths in leaves. Values are the means of 2–3 individuals. Abbreviations of species are as given in Table 1.

	Areaceae						Bromeliaceae							
	Arecoideae			Coryphoideae			Bromelioideae			Tillandsioideae				
Morphologies	Aa	Ac	Bc	Bp	Ee	Sr	Ca	Tc	Tf	Pc	Aco	Bsp	Gsp	Ta
<b>Articulated</b>														
Globular echinate	0	0	1.8	0	0	0	0	5.4	2.5	0	0	0	77.0	0
Globular psilate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Globular granulate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Irregular multifaceted	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Globular + elongate psilate	0	0.8	0	2.9	0	3.6	12.0	8.5	0	5.7	0	0	0	0
Elongate	0	0.5	5.4	0	4.2	0	3.1	9.2	7.1	4.3	0	0	0	0
Elongate oblong	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabular sublobate	0	0.5	0	0	0	0	0	1.4	1.7	0	0	26.9	0	0
Oblong cells arranged in radiated form	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0
Cones + elongate psilate	11.1	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomatal complexes	0	0	0	0	2.3	0	17.1	0	0.8	3.1	0	0	0	0
Elongate fusiform	0	0	0	0	0	0	0	0	0	0	0	0	0	30.1
Cylindrical sulcate tracheid	0	0	0	0	0	0	4.0	0	0	2.9	1.2	0	0	0
<b>Isolated</b>														
Globular echinate	0	79.0	60.5	59.6	91.5	92.7	54.1	82.0	43.7	44.2	84.9	73.1	21.2	69.9
Globular psilate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Irregular multifaceted	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Globular granulate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elongate	0	4.0	2.3	10.0	2.0	0	4.0	5.2	4.2	14.3	0	0	0	0
Elongate oblong	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabular sublobate	0	0	0	14.2	0	0	0	0	34.7	19.2	0	0	0	0
Cones	88.9	0	0	0	0	0	0	0	0	0	0	0	0	0
Elongate fusiform	0	0	0	0	0	0	0	0	0.8	4.0	0	0	0	0
Cylindrical sulcate tracheid	0	0	0	0	0	0	0	0	1.3	0	0	0	0	0
Prickles/hooks	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Non-ID	0	17.5	30.1	13.3	0	3.6	5.7	6.8	3.2	2.4	13.9	0	0	0
<b>Total phytoliths</b>	<b>369</b>	<b>569</b>	<b>559</b>	<b>450</b>	<b>355</b>	<b>468</b>	<b>351</b>	<b>434</b>	<b>476</b>	<b>421</b>	<b>410</b>	<b>346</b>	<b>331</b>	<b>335</b>

**Supplementary material.** (Continued).

	Cannaceae	Marantaceae	Orchidaceae			Strelitziaceae	Zingiberaceae
			Epidendroideae				
Morphologies	Ci	Cm	Gp	Il	Osp	St	Zsp
<b>Articulated</b>							
Globular echinate	0	0	0	0	0	0	0
Globular psilate	0	0	0	0	0	0	75.0
Globular granulate	16.5	0	0	0	0	0	0
Irregular multifaceted	1.3	3.6	0	0	0	7	0
Globular + elongate psilate	0	0	0	0	0	0	0
Elongate	0	0	0	0	0	0	0
Elongate oblong	0	0	5.5	10.1	0	0	0
Tabular sublobate	0	0	0	0	0	0	0
Oblong cells arranged in radiated form	0	0	0	0	0	0	0
Cones + elongate psilate	0	8.0	11.4	30.9	4.7	0	0
Stomatal complexes	0	0	0	0	0	0	0
Elongate fusiform	0	2.7	0	0	0	0	0
Cylindrical sulcate tracheid	6.5	0	0	0	0	0	0
<b>Isolated</b>							
Globular echinate	0	0	0	0	0	0	0
Globular psilate	0	0	0	0	0	0	25.0
Irregular multifaceted	37.5	21.4	0	0	0	84.9	0
Globular granulate	37.7	0	0	0	0	0	0
Elongate	0	0	0	0	0	0	0
Elongate oblong	0	0	4.3	6.8	13.5	0	0
Tabular sublobate	0.5	0	0	0	0	0	0
Cones	0	63.7	70.2	49.7	67.7	0	0
Elongate fusiform	0	0	0.5	2.5	12.7	2.1	0
Cylindrical sulcate tracheid	0	0	0.5	0	1.4	0	0
Prickles/hooks	0	0	0.5	0	0	0	0
Non-ID	0	0.6	7.1	0	0	6.0	0
<b>Total phytoliths</b>	<b>387</b>	<b>336</b>	<b>420</b>	<b>398</b>	<b>362</b>	<b>485</b>	<b>400</b>