

## Pollen of colombian magnolias

### Polen de magnolias colombianas

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#### ABSTRACT

The family Magnoliaceae has been of great interest to scientists seeking to understand flowering plant evolution. Morphological characters have been investigated in several studies, especially in China, the country with the highest number of species. Colombia, with 36 species, has the highest number of species in South America. In spite of the family's evolutionary importance and significant threats to species survival, information is still lacking about Colombian Magnoliaceae due to a paucity of research. In this article, the pollen morphology of fourteen *Magnolia* species from Colombia is described based on size, shape, apertures, exine and sculpture. Pollen grains from fresh material and herbaria collections were processed using standard acetolysis methods, and morphological descriptions were elaborated based on both light and scanning electron microscopy observations. In this study the pollen grains were considered large (from 68.2 to 115  $\mu\text{m}$  in the longest axis and from 41.4 to 69.3  $\mu\text{m}$  in the shortest axis), boat-shaped and anasulcate. Exine sculpture patterns such as rugulate, psilate, perforate and combinations of these types were found in the species analyzed. Although there is high uniformity in the shape of the pollen grains, some differences were found among species, not only in size but also in ornamentation. The high uniformity of pollen morphology among the Colombian species supports the most recent classification of the American magnolias.

**Key words.** *Dugandiodendron*, Neotropics, pollen morphology, *Talauma*.

#### RESUMEN

La familia Magnoliaceae ha sido de gran interés para los científicos que buscan entender la evolución de las plantas en flor. Los caracteres morfológicos han sido investigados en varios estudios, especialmente en China, el país con mayor número de especies. Colombia, con 36 especies, tiene el mayor número de especies en América del Sur. A pesar de la importancia evolutiva de la familia y las amenazas significativas a las especies supervivientes, todavía falta información sobre las Magnoliaceae colombianas debido a la falta de investigaciones. En este artículo se describe la morfología del polen de catorce especies de *Magnolia* de Colombia con base en su tamaño, forma, aberturas, exina y escultura. Los granos de polen de material fresco y colecciones de herbario fueron estudiados usando métodos de acetólisis estándar, y las descripciones morfológicas fueron elaboradas a partir de las observaciones de microscopía de luz y microscopía electrónica de barrido. En este estudio los granos de polen se consideraron grandes (de 68,2 a 115  $\mu\text{m}$  en el eje más largo y de 41,4 a 69,3  $\mu\text{m}$  en el eje más corto), en forma de barco y anasulcado. En las especies analizadas se encontraron patrones de escultura en exina tales como rugoso, psilado, perforado y combinaciones de estos tipos. Aunque existe una gran uniformidad en la forma de los granos de polen, se encontraron algunas diferencias entre las especies, no sólo en tamaño sino también en ornamentación. La alta uniformidad de la morfología del polen entre las especies colombianas apoya la clasificación más reciente de las magnolias americanas.

**Palabras clave.** *Dugandiodendron*, Neotropics, morfología del polen, *Talauma*.

## INTRODUCTION

Magnoliaceae Juss. comprises approximately 300 species distributed mainly in tropical and subtropical areas of Asia and America (Rivers *et al.* 2016). The family is characterized by trees and shrubs with an annular scar around the nodes and floral organs spirally arranged. Most species have bisexual flowers except for a few Asian species with unisexual flowers (Chen & Nootboom 2003). Two monophyletic subfamilies have been well recognized: Liriodendroideae and Magnolioideae (Azuma *et al.* 2001). However, the classification of their members has been very controversial (Azuma *et al.* 1999, 2001, Dandy 1927, 1978, Chen & Nootboom 1993, Figlar & Nootboom 2004, Frodin & Govaerts 1996, Keng 1978, Kim *et al.* 2001, 2002, Law 1984, Li & Conran 2003, Lozano 1975, 1983, Nootboom 1984, 1985, 1987, 1993, 2000, Vázquez-García 1994).

According to the most recent classification of neotropical species based on morphological and molecular studies, Magnolioideae comprises only the genus *Magnolia* L. and the other genera are currently considered sections and subsections of this genus (Figlar & Nootboom 2004). Currently, all the Colombian species are included in Section *Talauma* (Figlar & Nootboom 2004). However, these species belong to two different subsections based on morphological characters: *Talauma* Juss. and *Dugandiodendron* Lozano (Figlar & Nootboom 2004, Lozano 1975, 1983, 1994).

*Dugandiodendron*, with 14 species restricted to Colombia, northern Ecuador, and eastern Venezuela, was initially described at generic rank by Lozano (1975) based mainly on two morphological characters: heliciconvolute prefoliation and the pseudoaxillary position of the flowers. Although the characters used for generic circumscription have

been questioned (Nootboom 1985), the monophyly of *Dugandiodendron* has been suggested based on fruit dehiscence and stamen morphology (Serna 2005).

Several morphological characters still need to be explored in order to clarify the systematics of Colombian magnolias. However, these species are difficult to study because of their rarity, restricted geographical distribution and high level threats to their survival. For all these reasons, Magnoliaceae was chosen as a research and conservation priority in Colombia in its National Strategy for Plant Conservation (Samper & García 2001).

In light of the controversy about the taxonomy of Magnoliaceae and the paucity of phylogenetically informative characters (Nie *et al.* 2008), a study of pollen morphology was undertaken in order to contribute additional character data. Magnoliaceae pollen is stenopalynous in shape and aperture type. Some variations are common in the sporoderm structure, sculpture and size (Praglowksi 1974). Agababian (1972) described pollen grains with a fine granular exine and perforated tectum. Similarly, Praglowski (1974) considered that most pollen grains in this family have a perforated or slightly rugulate tectum, and occasionally a microreticulate exine. Other recent studies have described several ornamentation types in Asian species (Xu 1999, Xu & Kirchoff 2008). In Colombia, pollen grains of Magnoliaceae have been described as monocolpate, prolate and subprolate. Smooth pollen grains with few and scattered perforations have been described in *Talauma* species, while *Dugandiodendron* pollen grains have been described as microreticulate or reticulate (Lozano 1975).

Although Lozano (1975) provides some information for a limited sample of colombian species, the pollen morphology of neotropical magnolias is still largely

unknown. The goal of this study was to describe the pollen grains of a broader sample of *Magnolia* species from Colombia and evaluate the contribution of pollen morphology to the classification and systematics of Colombian magnolias. To characterize the pollen grains, a description of size, shape and ornamentation was carried out on available material of fourteen species obtained from herbaria and field collections.

## MATERIALS AND METHODS

Pollen grains of Magnoliaceae species from Colombia were collected from herbarium specimens deposited in COL and JAUM (Table 1). Material fixed in alcohol was also available for some species from Antioquia department. Pollen grains were subjected to standard acetolysis standard (Erdtman 1960) in the laboratory of Paleocology at the National University of Colombia, Medellín. Light microscopic analyses were performed using an Olympus BMX 40 microscope, and

SEM analyses were performed using a JEOL JSM 5910 LD Scanning Electron Microscope. The accelerating voltage was 15 kV.

For each species, at least 25 pollen grains were measured under light microscopy at 100X with an Olympus OMS4 micrometer, except in *Magnolia silvioi* (Lozano) Govaerts and *Magnolia sambuensis* (Pittier) Govaerts, which were measured at 40X because of their size. Exine was always measured at 100X. After acetolysis, the pollen voucher specimens were mounted on slides with glycerine and deposited in the collection of the laboratory of paleoecology of the Universidad Nacional de Colombia, Medellín (Table 1). The terminology of Punt *et al.* (2007) and Zhang *et al.* (2014) has been adopted here to describe pollen morphological characters, except those related to pollen grain shape, for which terminology was adopted from Walker and Doyle (1975). The shape of pollen grains was described as the product of division

**Table 1.** Voucher specimens deposited in the laboratory of Paleocology (LP), Universidad Nacional de Colombia, Medellín.

Species	Collector	Herbarium	Origin	Voucher
Subsection <i>Dugandiodendron</i>				
<i>M. coronata</i> Serna, Velásquez & Cogollo	Velásquez 3357	JAUM	Barbosa, Antioquia	LP 657
<i>M. guatapensis</i> (Lozano) Govaerts	Velásquez 3380	JAUM	Yarumal, Antioquia	LP 640
<i>M. lenticellata</i> (Lozano) Govaerts	Cogollo 3385	COL	Urrao, Antioquia	LP 647
<i>M. urraoensis</i> (Lozano) Govaerts	Lozano 2971	COL	Caicedo, Antioquia	LP 663
<i>M. yarumalensis</i> (Lozano) Govaerts	Tuberquia 1730	JAUM	Jardín, Antioquia	LP 665
Subsection <i>Talauma</i>				
<i>M. arcabucoana</i> (Lozano) Govaerts	Mahecha 9	COL	Cabrera, Cundinamarca	LP 631
<i>M. caricifragans</i> (Lozano) Govaerts	Lozano 993	COL	Fusagasugá, Cundinamarca	LP 634
<i>M. espinalii</i> (Lozano) Govaerts	Velásquez 3383	JAUM	Envigado, Antioquia	LP 637
<i>M. hernandezii</i> (Lozano) Govaerts	Tuberquia 1633	JAUM	Buriticá, Antioquia	LP 642
<i>M. polyhyphophylla</i> (Lozano) Govaerts	Velásquez 3396	JAUM	Yarumal, Antioquia	LP 652
<i>M. sambuensis</i> (Pittier) Govaerts	Valencia 103	MEDEL	Bajirá, Chocó	LP 1469
<i>M. santanderiana</i> (Lozano) Govaerts	Gentry 20138	COL	Tona, Santander	LP 654
<i>M. silvioi</i> (Lozano) Govaerts	Velásquez 3387	JAUM	Yalí, Antioquia	LP 656
<i>M. wolfii</i> (Lozano) Govaerts	Wolf 333	COL	Santa Rosa de Cabal, Risaralda	LP 664

of the longest axis (LA) by the shortest equatorial axis (SEA) (Zhang *et al.* 2014).

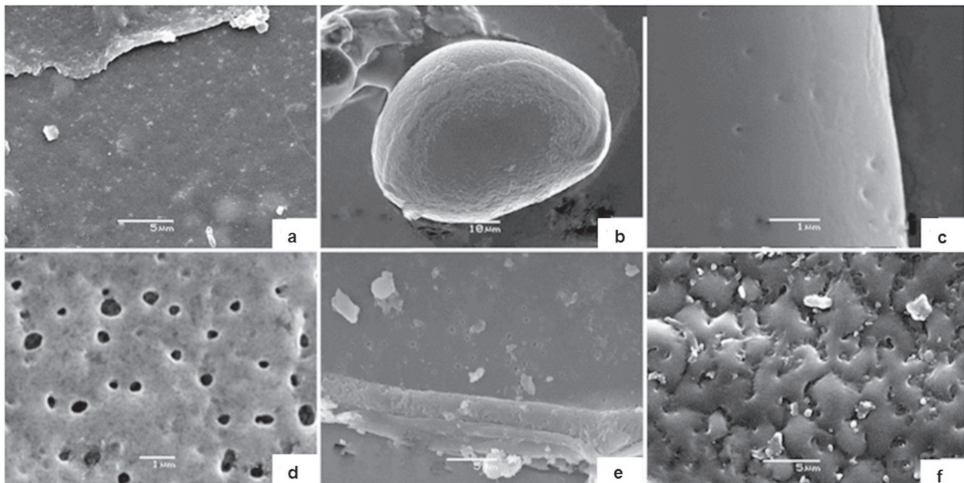
Pollen type was defined according the classification proposed by Praglowski (1974) based on tectum surface and grain size: large pollen grains, 45  $\mu\text{m}$  or larger, smooth tectum surface (Type 1); small pollen grains, <45  $\mu\text{m}$ , smooth tectum surface (Type 2); large pollen grains, sculpture slightly rugulate (Type 3); small pollen grains, sculpture slightly rugulate (Type 4), sculpture coarsely rugulate (Type 5); pollen grains semitectate, exine structure microreticulate, large (Type 6) and pollen grains semitectate, exine structure microreticulate, small (Type 7).

## RESULTS

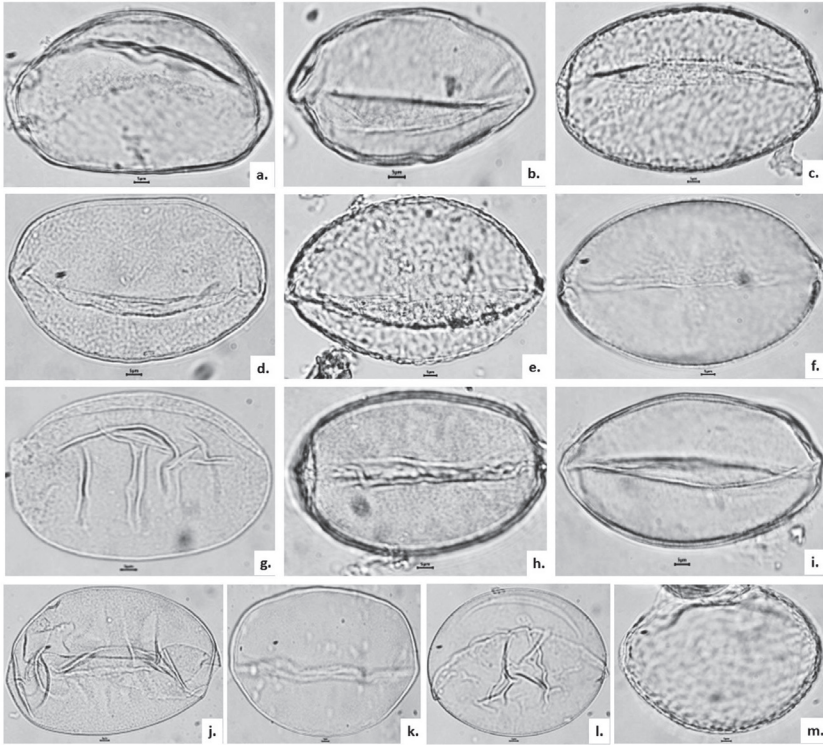
The species studied are listed in Table 1. All the pollen grains were anasulcate, bilateral symmetric, heteropolar, and folded in boat shape. In polar view, the pollen grains were ellipsoidal and in lateral view, biconvex or plane-convex. The size of the pollen grains

varied from  $68.20 \pm 7.1 \mu\text{m}$  to  $115.0 \pm 7.7 \mu\text{m}$  in the longest axis (LA) and  $41.4 \pm 2.3 \mu\text{m}$  to  $69.3 \pm 10.0 \mu\text{m}$  in the shortest equatorial axis (SEA) (n=25), and are considered large or medium in size. Three types of pollen shapes in the species analyzed were found: prolate, perprolate and subprolate. The ornamentation was also variable: psilate-perforate, slightly rugulate, rugulate and rugulate-perforate (Figs. 1-2). Measurements and ornamentation information for each species are shown in Table 2.

The pollen grain size was proportional to the flower size, especially for the species with large flowers. For instance, grains from *M. silvioi* and *M. sambuensis* were the largest, corresponding to the largest flowers of the Magnoliaceae in Colombia (tepals 7.5–8.5 cm long x 4.0–4.5 cm wide and 5.0–6.5 cm long x 4.0–4.5 cm wide, respectively). The smallest pollen grains were found in the species *M. caricifragans* and *M. polyhyposphylla* (3.5–4.5 cm long x 1.5–2.5 cm wide and 2.0–2.3 cm long x 1.5–2.6 cm wide, respectively). However, the smallest



**Figure 1.** Scanning Electron Microscopy images of pollen grains from Subsection *Dugandiodendron*: **a.** *Magnolia coronata*, **b.** *M. urraoensis*. Subsection *Talauma*: **c.** *M. arcabucoana*, **d.** *M. espinalii*, **e.** *M. hernandezii*, and **f.** *M. silvioi*.



**Figure 2.** Light Microscopy images of pollen grains of the species studied from Subsections *Dugandiodendron* (D) and *Talauma* (T): **a.** *M. arcabucoana* (T), **b.** *M. caricifragans* (T), **c.** *M. coronata* (D), **d.** *M. espinalii* (T), **e.** *M. guatapensis* (D), **f.** *M. hernandezii* (T), **g.** *M. lenticellata* (D), **h.** *M. polyhypsophylla* (T), **i.** *M. santanderiana* (T), **j.** *M. silvioi* (T), **k.** *M. urraoensis* (D), **l.** *M. wolfii* (T) and **m.** *M. yarumalensis* (D).

flowers among the Colombian magnolias are found in *M. coronata* (2.5 – 3.0 cm long and 1.0 – 1.5 cm wide).

Two general types of ornamentation were observed: rugulate and psilate with perforations. In the rugulate type, some variations were found: slightly rugulate, rugulate and rugulate with perforations. In this study, based on size and type of ornamentation, the pollen grains correspond to three out of the seven pollen types according to Pragłowski (1974) (Table 2).

Pollen grains of the Subsection *Dugandiodendron* were considered large, varying

from  $70.5 \pm 5.7$  to  $89.1 \pm 6.0$  in the LA and  $49.1 \pm 5.1$  to  $69.3 \pm 10.0$  in their SEA. Based on sculpture, pollen grains were Type 3 and 5: pollen grains slightly rugulate and rugulate with or without perforations (Table 2).

For Subsection *Talauma*, pollen grains were considered large, varying from  $68.2 \pm 7.1$  to  $115.0 \pm 7.7$  in the LA and  $41.4 \pm 2.3$  to  $65.9 \pm 11.9$  in the SEA. Two general types of ornamentation were found: psilate-perforate (2 species) and rugulate (8). In the rugulate type, two subtypes of grains were observed: slightly rugulate (4) and slightly rugulate-perforate (4).



**Table 2.** Pollen morphological features of the species of *Magnolia* (Subsections *Dugandiodendron* and *Talauma*) examined from Colombia.

Subsection	Species	LA (µm)	SEA 2 (µm)	LA/SEA	Shape	Colpe wide (µm)	Exine (µm)	Ornamentation	Pollen Type*
<i>Dugandiodendron</i>	<i>M. coronata</i>	89.1 ± 6.0	54.3 ± 4.5	1.64	Prolate	4.1 ± 2.4	1.72 ± 0.24	slightly rugulate-perforate	3
	<i>M. guatapensis</i>	82.0 ± 7.6	58.4 ± 8.5	1.40	Prolate	8.6 ± 6.3	1.72 ± 0.37	rugulate	5
	<i>M. lenticellata</i>	70.5 ± 5.7	49.1 ± 5.1	1.44	Prolate	6.07 ± 5.5	1.34 ± 0.19	slightly rugulate	3
	<i>M. urraoensis</i>	88.8 ± 12.3	54.6 ± 9.8	1.63	Prolate	6.58 ± 2.3	2.07 ± 0.25	rugulate-perforate	5
	<i>M. yarumalensis</i>	85.5 ± 8.6	69.3 ± 10.0	1.23	Subprolate	7.14 ± 5.4	1.60 ± 0.31	rugulate	5
<i>Talauma</i>	<i>M. arabucoana</i>	79.7 ± 6.1	49.6 ± 5.1	1.61	Prolate	7.52 ± 4.6	1.34 ± 0.17	slightly rugulate-perforate	3
	<i>M. caricifragans</i>	69.6 ± 6.2	44.1 ± 5.7	1.58	Prolate	8.93 ± 3.9	1.36 ± 0.19	psilate-perforate	1
	<i>M. espinalli</i>	73.2 ± 4.7	41.4 ± 2.3	1.77	Prolate	6.88 ± 2.1	1.59 ± 0.23	slightly rugulate-perforate	3
	<i>M. hernandezii</i>	82.0 ± 5.8	61.0 ± 7.1	1.34	Prolate	0.79 ± 2.6	1.22 ± 0.32	psilate-perforate	1
	<i>M. polyhypsophylla</i>	68.20 ± 7.1	44.0 ± 3.2	1.41	Prolate	10.08 ± 1.4	1.74 ± 0.23	slightly rugulate	3
	<i>M. sambuensis</i>	115.0 ± 7.7	46.0 ± 3.1	2.50	Perprolate	2.51 ± 1.2	1.76 ± 0.21	slightly rugulate	3
	<i>M. santanderiana</i>	74.8 ± 4.7	42.4 ± 3.3	1.77	Prolate	4.53 ± 2.0	1.55 ± 0.25	slightly rugulate	3
	<i>M. silvoti</i>	114.2 ± 12.5	45.7 ± 5.0	2.50	Perprolate	7.82 ± 1.9	1.88 ± 0.22	slightly rugulate-perforate	3
	<i>M. wolffi</i>	87.8 ± 7.2	65.9 ± 11.9	1.33	Subprolate	7.84 ± 9.8	1.27 ± 0.37	slightly rugulate	3

Abbreviations: LA = longest axis; SEA = shortest equatorial axis. \* According to Pragłowski (1974).

## DISCUSSION

The main pollen characters described in this work such as sculpture, structure, and shape, were similar to the information previously published for species of Magnoliaceae (Pragłowski 1974, Xu & Kirchoff 2008). The sulcate aperture, related to heteropolarity in Magnoliaceae, was found in all species analyzed. All the observed grains were large, corresponding to Pragłowski's (1974) Types 1, 3 or 5. In this study, the dominant shape was boat-shaped, which is characteristic of Magnoliaceae (Xu & Kirchoff 2008). Boat-shaped elliptic, boat-shaped oblong and boat-shaped elongate variations were also found in all Colombian species.

Other studies have described several ornamentation types in Asian species in Magnoliaceae as follows: foveolate in *Michelia* L. (Xu 1999, Xu & Kirchoff 2008); microperforate in *Alcimandra* Dandy, *Manglietia* Blume, *Michelia* (Xu & Kirchoff 2008), *Lirianthe* Spach, *Houpoëa* N. H. Xia & C. Y. Wu, *Parakmeria* Hu and Cheng and *Talauma* (Zhang *et al.* 2014); perforate in some species of *Manglietia*, *Michelia* and *Lirianthe* (Zhang *et al.* 2014); rugulate in *Woonyoungia* Law (Zhang *et al.* 2014); coarsely rugulate in *Kmeria* (Pierre) Dandy (Xu & Kirchoff 2008), and rugulate in *Michelia* and with elaborated projections in *Talauma* (Zhang *et al.* 2014). In the case of *Magnolia grandiflora* L., Xu & Kirchoff (2008) described the pollen grains as microperforate, while Zhang *et al.* (2014) described the pollen grains as perforate and microperforate. These results show the high variability among species of the same genus. The results presented here demonstrate the high variability among species from the same subsection.

Lozano (1983) characterized pollen grains of four Colombian magnolias. He described the pollen grains of these species

as monocolpate, prolate and subprolate. Apparently, he found some differences in the exine sculpture between *Talauma* and *Dugandiodendron*, and reported grains of Subsection *Talauma* species such as *M. caricifragans* and *M. santanderiana* as smooth with small and scattered perforations. On the contrary, he described *M. argyrothricha* and *M. mahechae* from Subsection *Dugandiodendron* as having microreticulate or reticulate pollen grains.

Later, Lozano (1994) described pollen grains of Magnoliaceae as ellipsoidal with rounded ends and classified the species based on Pragłowski's types (1974). *Magnolia calophylla* (Lozano) Govaerts, *M. caricifragans* and *M. santanderiana*, were described as Type 2. Pollen grains of *M. mahechae* and *M. urraoense* were classified as Type 4, while *Magnolia argyrothricha* (Lozano) Govaerts, *Magnolia colombiana* (Little) Govaerts and *M. yarumalensis* grains belonged to Type 5. Our results concur with those of Lozano (1983, 1994) for *M. yarumalensis* but not for *M. caricifragans* (Type 2 in Lozano vs. Type 1 in this work), *M. santanderiana* (Type 2 vs. Type 3) and *M. urraoensis* (Type 4 vs. Type 5) (Table 2). These differences can be explained by the maturity level of grains, considering that Lozano (1983) observed immature pollen.

According to this study, two species from Subsection *Talauma* presented Type 1, the same type reported in species from the West Indies, United States and South America (Pragłowski 1974). Seven species from Subsection *Talauma* and three species from Subsection *Dugandiodendron*, exhibited the same pollen type as *M. grandiflora* (Type 3), while three species from Subsection *Dugandiodendron* share the same type as *Liriodendron tulipifera* L. (Type 5), native species from North America.

The Colombian species from Subsection *Dugandiodendron* and Subsection *Talauma* (Section *Talauma*) share similar morphological patterns. Despite morphological characters such as exine structure, sculpture and pollen size that have some taxonomic significance, it is not possible to establish clear differences between these groups (Pragłowski 1974, Nooteboom 1984).

The shape size, ornamentation, polarity and symmetry of pollen in colombian species of *Magnolia* are consistent with descriptions of pollen previously described in Asian and American species. Although some variability in pollen shape, ornamentation, and size was observed among the species examined in this study, the variability does not correspond to the previous taxonomic classification. For instance, Type 3 pollen grains were found in both subsections. The uniformity of pollen grain morphology among the Colombian species supports the classification proposed by Figlar and Nooteboom (2004) for neotropical Magnoliaceae, in which all Colombian species are included in Section *Talauma*.

The scarcity of available material and the acetolysis process limited the number of species and the total amount of pollen that could be sampled to obtain good photographic evidence for this study. Future studies could include increased sampling from herbarium collections and ideally new field work, in order to characterize the pollen of the remaining species.

#### AUTHOR CONTRIBUTIONS

MSG collected material from field and herbarium specimens, performed laboratory work and measurements. MSG and CVR described the pollen grain shape and ornamentation, obtained photographs and wrote the draft of the manuscript. Both

authors improved the final manuscript based on the reviewers' comments and suggestions.

#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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