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# Pollen morphology of the species of Hernandiaceae, Monimiaceae and Siparunaceae from the Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil

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**ABSTRACT:**

This work describes pollen grains from the species of Hernandiaceae (*Sparattanthelium*), Monimiaceae (*Mollinedia*), and Siparunaceae (*Siparuna*) from a 100 km$^2$ area, Reserva Florestal Adolpho Ducke (03º00'00''S – 59º52'40''W) in the municipality of Manaus, Amazonas, aiming to supply taxonomic studies in Laurales. Pollen material was included in the Amazon Palynogical Database as a reference for other studies. The pollen analysis in light microscopy (LM) and Scanning Eletronic Microscopy (SEM) include the species *Sparattanthelium acreanum* Pilg., *Mollinedia ovata* Ruiz & Pav., *Siparuna cristata* (Poepp. & Endl.) A. DC., *S. depressa* Jangoux, *S. ficoides* S.S. Renner & Hausner, *S. guianensis* Aubl., and *S. poeppigii* (Tul.) A. DC. The pollen of *Mollinedia ovata* is ellipsoidal, catazonasulculate, and rugulate, unlike the pollen of other species. In *Sparattanthelium acreanum* and in the five species of *Siparuna*, the grains appear spheroidal, inaperturate, microechinate, and very uniform in LM, however, details in the ornamentation that allow taxonomical separation are visible by SEM. The results reinforce taxonomic distinction among the three involved families of Laurales and shown importance of SEM in the separation of species.

**Keywords:**
Amazon, Palynology, Taxonomy, Laurales.

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## INTRODUCTION

The families Hernandiaceae, Monimiaceae, and Siparunaceae are assigned to the Order Laurales (Renner & Hausner, 1997; Renner, 1999; APG III, 2009; Judd et al., 2009).

Hernandiaceae has a pantropical distribution, with around 60 species distributed in five genera (Renner & Chanderbali, 2000; Renne,r 2004). It is composed of shrubs, trees, and lianas, with cymose inflorescences and non-showy flowers, unisexual in polygamous plants, monoecious or dioecious, or bisexual. The differentiation of genera is mainly based on the fruits. In Brazil, there are three genera and around ten species; the majority of species are derived from the Amazon and assigned to the genus *Sparattanthelium* (Ribeiro *et al*., 1999; Souza & Lorenzi, 2008).

Hernandiaceae is very phylogenetically and morphologically close to Lauraceae, differing mainly by the presence of an inferior ovary, which is very rare in the Lauraceae (Renner, 1999, Doyle & Endress, 2000).

Monimiaceae is predominantly pantropical and includes around 25 genera and 250 species (Renner, 1998; Renner & Chanderbali, 2000; Renner, 2004). This family is composed of shrubs, trees, and lianas, with cymose inflorescences and non-showy flowers, unisexual flowers in monoecious or dioecious plants. The flowers are pollinated by flies and small beetles, and the fruits are probably scattered by birds. In Brazil, there are five genera and around 70 species. *Mollinedia* is the most common genus, with several species of small trees that are common in forests (Ribeiro *et al*., 1999; Souza & Lorenzi, 2008).

Siparunaceae is Neotropical and has two genera (*Glossocalyx* from West Africa and *Siparuna* from South America) and approximately 75 species, almost all belonging to *Siparuna* (*Glossocalyx* is monospecific). The family is composed of shrubs, trees, and lianas. The inflorescences are axillary or cauliflorous, cymose or fascicular and the flowers are unisexual in monoecious or dioecious plants. *Siparuna* flowers are pollinated by nocturnal flies that visit them for mating and oviposition. In Brazil, there are approximately 40 species, and the majority is from the Amazon, where they are relatively common at the edges of the forest (Ribeiro *et al*., 1999; Souza & Lorenzi, 2008).

Siparunaceae has been recently separated from the Monimiaceae and presents several apomorphies, such as bisporangiate anthers that open in one split, ovules with one integument, and flowers closed by the curved extremities of the receptacle (Renner *et al*., 1997).

The present study aims to obtain more information on the palynology of the Hernandiaceae, Monimiaceae, and Siparunaceae that may contribute to a better understanding of the taxonomic and phylogenetic relations of these families of Laurales. This research is a continuation of the palynological study from the vegetation of the Reserva Florestal Adolpho Ducke, Manaus, AM (RFAD), which is also providing reference material for other palynological studies in the Amazon region.

## STUDY AREA

The analysed species are from the Reserva Florestal Adolpho Ducke, with an area of 100 km$^2$, in the municipality of Manaus, Amazonas (03º00'00"S – 59º52'40"W, Figure 1). The vegetation of the reserve is mainly classified as "terra firme" forest and is typical of areas that are not seasonally flooded. On a more detailed scale, different habitats, such as plateau forest, slope forest, tall forest on sandy soil ("campinarana"), and lowland forest, can be recognized. The structure and flora from those formations are especially defined by the type of soil and the topography. The topology is undulating with an altitudinal variation of 80 m between the plateau and the lower land. In the plateaus, the soil is clayey (alic yellow latosol) older sediment, while in the lower-land it is sandy and located in the valleys and at the bottoms of hills (Ribeiro *et al*., 1999). The climate of the region is Af, according to the Köppen classification system (Ribeiro, 1976).

## MATERIAL AND METHODS

The pollen was obtained from floral buds collected from Herbarium exsiccate of the Instituto Nacional de Pesquisas da Amazônia (INPA), with plants from the Reserva Florestal Adolpho Ducke, Manaus, AM.

For analysis by light microscopy (LM), the pollen was prepared according to the standard acetolysis method (Ertdman, 1960) and the slides were mounted in glycerol jelly and sealed with paraffin (Salgado-Labouriau, 1973). The pollen measurements were performed in LM using 25 grains per species randomly distributed in at least three slides, which were later deposited into the reference pollen collection of the Palynology

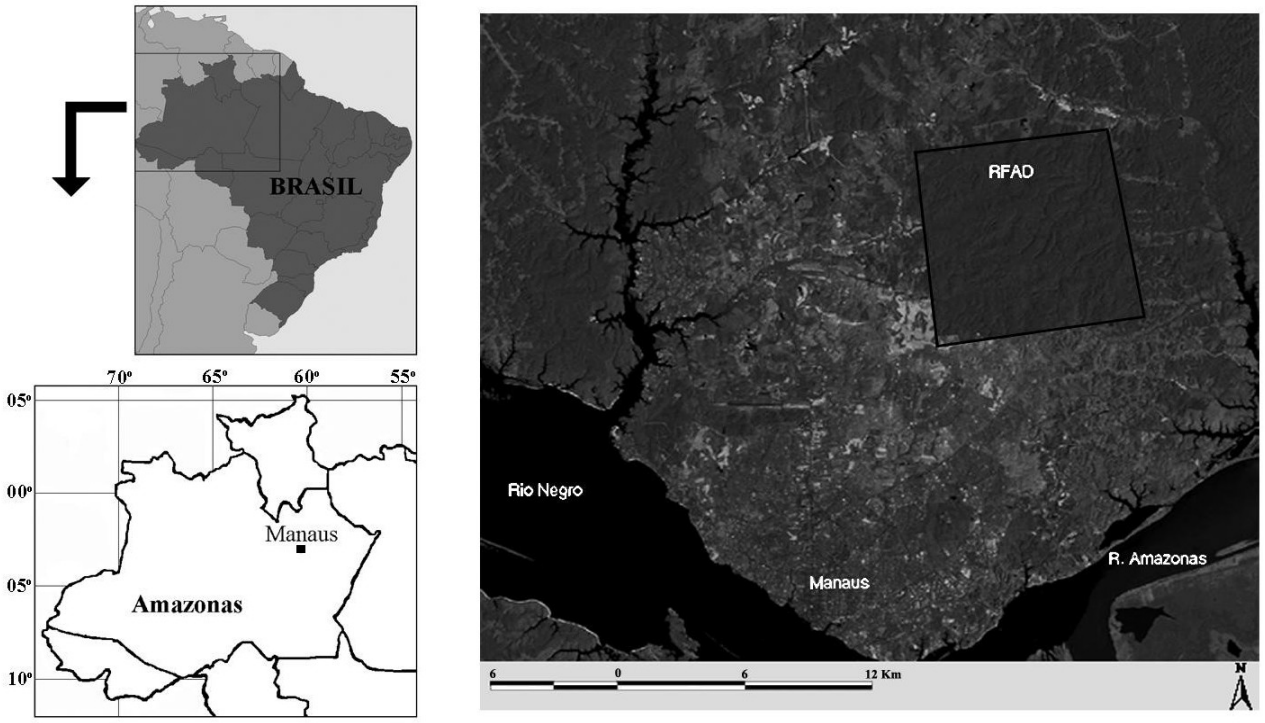



**Figure 1. Study site, Reserva Florestal Adolpho Ducke (RFAD), 03º00'00''S – 59º52'40''W, Manaus, Amazonas, Brazil.**

Laboratory of INPA. For each species, the arithmetic mean ($x$) of the axis size was calculated, as well as the standard deviation of the mean ($s_x$) and a Confidence Interval of 95% (CI). The exine thickness was also measured. The Scanning Electronic Microscopy (SEM) was used to observe the details of the ornamentation.

The pollen morphology of each species was described with an emphasis on the characteristics that allow the identification of the material. The terminology used was that of Punt et al. (2007) and Ertdman (1960), taking into consideration the grain size and shape, the presence or absence of aperture, and the ornamentation pattern. Photomicrographs from both LM and SEM and ecological data are included in the descriptions. The SEM was performed on non-acetolysed grains.

## RESULTS

Descriptions from the species:

**Magnoliideae**

**Laurales**

**Hernandiaceae**

*Sparattanthelium acreanum* Pilg. (**Figures 2A–G**)

Spheroidal, inaperturate, microechinate. Echinae densely distributed. Obscure stratification. Small to medium size grain.

In SEM: A microverrucate portion encircles the base of the echinae and continuous for all irregular surfaces.

Size: 20-26 µm (**Table 1**).

Exine thickness: 1µm.

Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 11/VII/1996, Assunção, P.A.C.L. 338 (INPA).

Ecological data: Woody liane, until 8 m high, in aquifer depressions of sand soils of forest in central and occidental Amazonian (Ribeiro *et al*., 1999).

**Monimiaceae**

*Mollinedia ovata* Ruiz & Pav. (**Figures 2H–O**)

Ellipsoidal, oblate, very delicate and some times creased. Catazonasulculate, rugulate. Prominent psilate bandlike aperture. Obscure stratification. Small to medium size pollen.

In SEM: Distinct rugulate surface and psilate bandlike aperture.

Equatorial major axis: 22-27 µm (Table 1).

Equatorial small axis: 16-20 µm (Table 1).

Exine thickness: 1 µm.

Observation: according to Walker (1976), TEM micrographs showed that the band is composed of ektexine and endexine, while the rest of the pollen wall is made up entirely of endexine. Thus, although the bandlike area must be designated the

**Table 1. Size (µm) of the pollen grains.**

| Species | Axis interval (n = 25) (µm) | Mean ($x \pm s_x$) (µm) | CI (95%) |
|---|---|---|---|
| *Sparattanthelium acreanum* | 20-26 | 23.16±1,77 | 0.35 |
| *Mollinedia ovata* (Ema) | 22-27 | 24.8±1,76 | 0.34 |
| *Mollinedia ovata* (Esa) | 16-20 | 18.12±1,72 | 0.67 |
| *Siparuna cristata* | 13-35 | 21.28±4,7 | 0.92 |
| *Siparuna depressa* | 13-25 | 18.55±3,59 | 0.7 |
| *Siparuna ficoides* | 13-27 | 21.88±3,07 | 0.6 |
| *Siparuna guianensis* | 11-32 | 26.01±5,21 | 1,02 |
| *Siparuna poeppigii* | 13-19 | 15.76±1,54 | 0.3 |

**(Abbreviations: n – number of pollen grains, $x$ – arithmetic mean, $s_x$ – average standard deviation, CI –confidence interval, Ema – Equatorial major axis, Esa – Equatorial small axis).**

aperture morphologically (at least from light studies alone), in reality the bulk of the surface of the pollen grains in this genus apparently functions aperturally (at least in terms of being composed of endexine rather than ektexine).
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 21/VIII/1998, Souza, M.A.D. et al. 564 (INPA).
Ecological data: Tree or shrubs, chance in plateau aquifer forests soil of large areas from Amazonian region (Ribeiro *et al*., 1999).

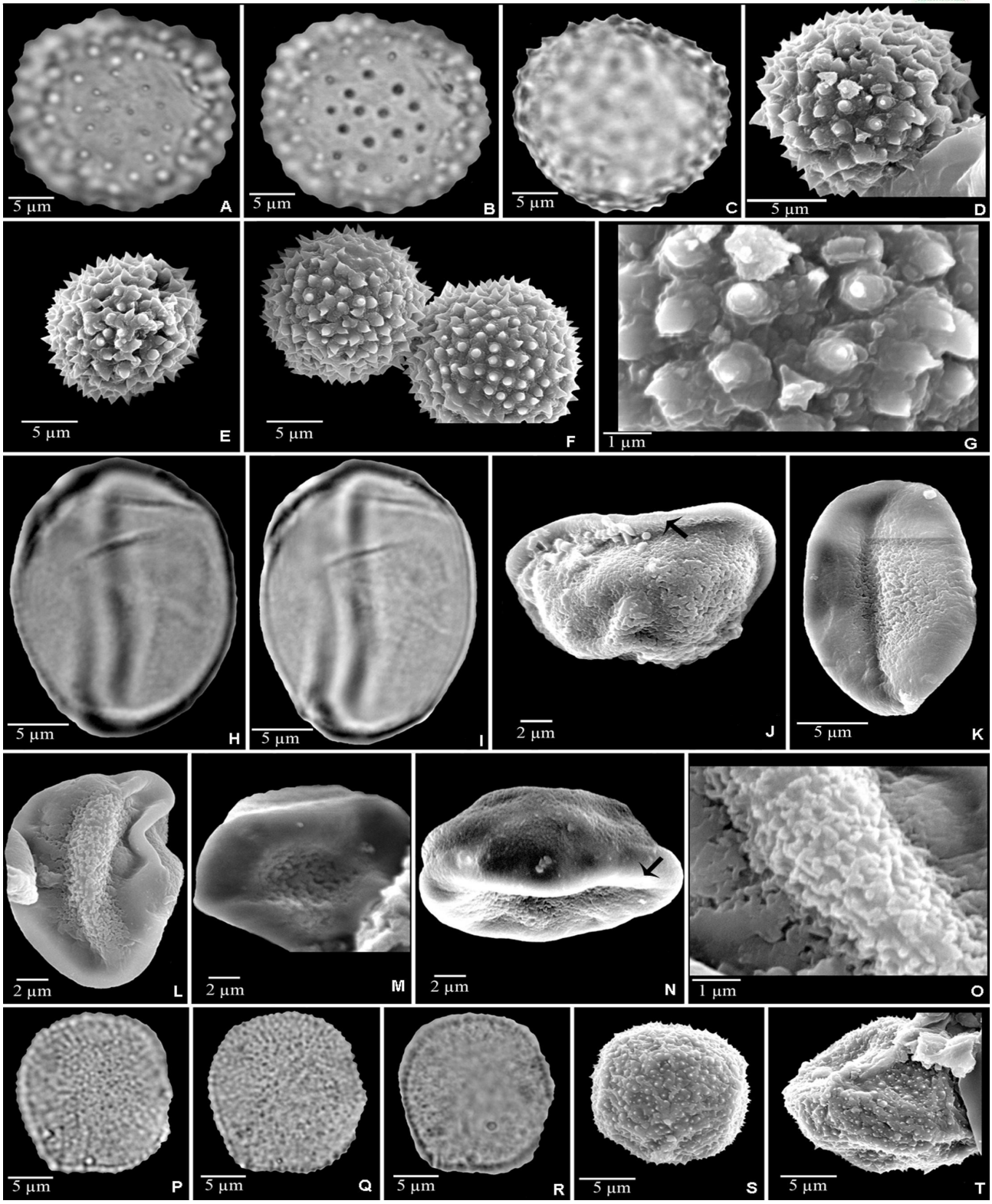
**Siparunaceae**

*Siparuna cristata* (Poepp. & Endl.) A. DC. (**Figures 2P–T, Figure 3A**)
Spheroidal, inaperturate, very delicate and hyaline, microechinate. Very small echinae, scattered distributed. Obscure stratification. Small to medium size pollen.
In SEM: Echinae of two distinct sizes, projected from an irregular surface.
Size: 13-35 µm (Table 1).
Exine thickness: 1µm.
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 13/X/1995, Sothers, C.A. & Pereira, E. da C. 628 (INPA).
Ecological data: Shrub or small tree until 9 m high, in clay or sandy-clay humid soils in subforest in the "igarapé" margin in "terra firme" forest. Chance in large areas from Amazonian region (Ribeiro *et al*., 1999).

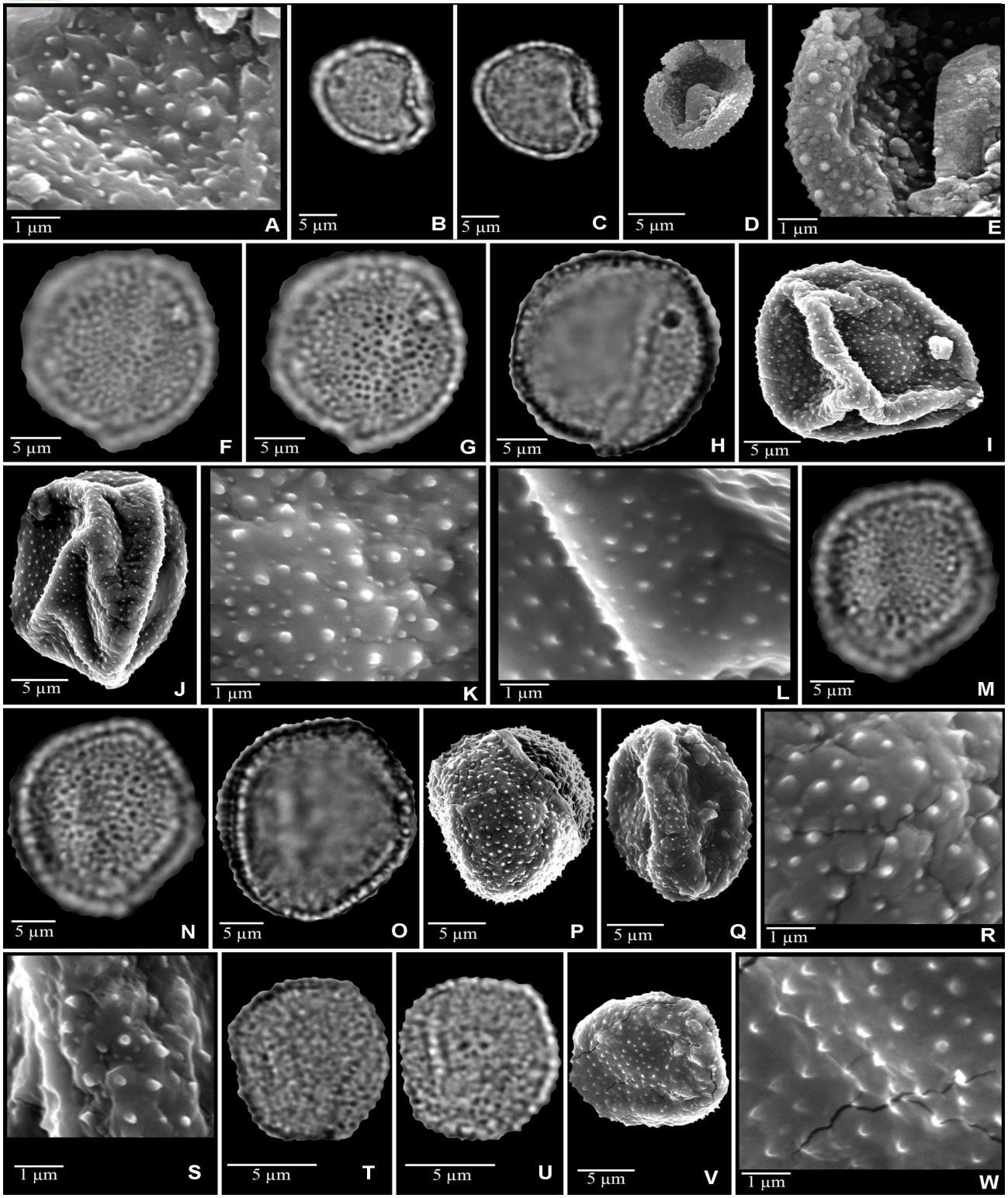
***Siparuna depressa*** Jangoux (**Figures 3B–E**)
Spheroidal, inaperturate, very delicate and hyaline. Microechinate-microverrucate. Very small projections scattered distributed. Obscure stratification. Small size pollen.
In SEM: Scattered echinae in a dense irregular microverrucate surface.
Size: 13-25 µm (Table 1).
Exine thickness: 1µm.
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 02/IX/1968, Souza, J.A. 125 (INPA).
Ecological data: tree, until 20 m high, in clayed soils of forest or brushwood (Ribeiro *et al*., 1999).

***Siparuna ficoides*** S.S. Renner & Hausner (**Figures 3F–L**)
Spheroidal, inaperturate, very delicate and hyaline, sometimes creased. Microechinate. Very small echinae, scattered distributed. Obscure stratification. Small to medium size pollen.
In SEM: Echinae of two distinct sizes, projected from a plane surface.
Size: 13-27 µm (Table 1).
Exine thickness: 1µm.
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 29/IX/1995, Sothers, C.A. et al. 591 (INPA).
Ecological data: tree, until 20 m high, in clayed soils of subforest, in plateau forest. Ocasional in Brazilian Amazon (Ribeiro *et al*., 1999).

***Siparuna guianensis*** Aubl. (**Figures 3M–S**)
Spheroidal, inaperturate, very delicate and hyaline, sometimes creased. Microechinate. Very small echinae, scattered distributed. Obscure stratification. Small to medium size pollen.
In SEM: Echinae with more or less uniform size.
Size: 11-32 µm (Table 1).
Exine thickness: 1 µm.
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 29/IX/1995, Sothers, C.A. et al. 592 (INPA).
Ecological data: tree until 12 m high, clayed, sandy-clay or stony, humid or dry soil. Occurs in "terra firme" forest, grassland, brushwood and margin of




**Figures 2. A – T. A – G. *Sparattanthelium acreanum* Pilg. A – C (LM): 1°-3° pl., D – G (SEM): D – F. General view, G. Detail of microechinate surface. A microverrucate portion encircles the base of the echinae and continuous for all the irregular surface; H – O. *Mollinedia ovata* Ruiz & Pav. H – I. Distal face (LM): 1°-2° pl., J – O. More or less creased grains (SEM): J – N: general view: J. Distal face, bandlike (arrow), K – M. Proximal face, N. Equatorial view, bandlike (arrow), O. Detail of the rugulate surface; P – T. *Siparuna cristata* (Poepp. & Endl.) A. DC. P – R (LM): 1°-3° pl., S – T (SEM): general view. (LM = light microscopy, SEM = scanning electronic microscopy, pl. = planes).**




**Figures 3. A –W: A. *Siparuna cristata* (Poepp. & Endl.) A. DC. (SEM): detail of microechinate surface with two sizes of very small echinae; B – E. *Siparuna depressa* Jangoux. B – C (LM): 1º-2º pl., D – E (SEM): D: general view, E: detail of scattered very small echinae in a dense irregular microverrucate surface; F – L. *Siparuna ficoides* Renner & Hausner. F – H (LM): 1º-3º pl., I – L (SEM): I – J: general view, K – L: detail of very small echinae of two distinct sizes, projected from a plane surface; M – S. *Siparuna guianensis* Aubl. M – O (LM): 1º-3º pl., P – S (SEM): P – Q: general view, R – S: detail of very small echinae, scattered distributed; T – W. *Siparuna poeppigii* (Tul.) A. DC. T – U (LM): 1º-2º pl., V – W (SEM): V: general view, W: detail of very small echinae, scattered distributed. (LM = light microscopy, SEM = scanning electronic microscopy, pl. = planes).**

forest. Frequent since Panamá until Bolívia (Ribeiro *et al*., 1999).

***Siparuna poeppigii*** (Tul.) A. DC. (**Figure 3T–W**)
Spheroidal, inaperturate, very delicate and hyaline, sometimes creased. Microechinate. Very small echinae, scattered distributed. Obscure stratification. Small size pollen.
In SEM: Echinae with uniform size.
Size: 13-19 µm (Table 1).
Exine thickness: about 1µm.
Examined material: BRASIL. Amazonas: Manaus-Itacoatiara Km 26, 09/VIII/1995, Sothers, C.A. et al. 555 (INPA).
Observation: Echinate pattern is very similar to *S. guianensis* but with smaller pollen size.
Ecological data: Small tree until 4 m high, in sandy soil. In brushwood or more open areas, in "terra firme" forest. Chance, in Brasil, Equador and Peru (Ribeiro *et al*., 1999).

## DISCUSSION

The pollen of *Mollinedia ovata* is catazonasulculate, a very different morphology from the other studied species.

*Sparattanthelium acreanum* and the species of *Siparuna* present spherical, inaperturate and microechinate pollen. However, the pollen of *S. acreanum* has thicker and more densely distributed echinae than the pollen of the *Siparuna* species, which is evident even by LM. In *S. acreanum* SEM allowed detailed analysis of the microverrucate base of these echinae. The results also showed that no clear differences are apparent in the pollen of *Siparuna* species by LM, which corroborates the observations of Pignal *et al*. (1999) on the morphological uniformity of the Siparunaceae pollen. However, in SEM the differences in shape, size and pattern of projections distribution and the appearance of the surface from which the echinae grow allow the separation of *Siparuna cristata*, *S. depressa*, and *S. ficoides*. *S. guianensis* and *S. poeppigii* did not show significant differences in their patterns of ornamentation distribution, but *S. poeppigii* presents a smaller pollen size.
The results are according to the interrelationships of Laurale families and the modern classification of flowering plants presented in the APG III (2009).

## CONCLUSIONS

The palynological results obtained reinforce the taxonomic differences between the Hernandiaceae, Monimiaceae, and Siparunaceae in Laurales. They also show the importance of SEM in the separation of the species, especially in the genus *Siparuna,* which appears morphologically uniform by LM.

The material studied is available in the Amazon Palynological Database, giving access to information on the pollen of the region, which is an important tool for taxonomic, phylogenetic and environmental research.

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## REFERENCES

**APG [Angiosperm Phylogeny Group]. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society,* 161: 105-121.

**Doyle JA and Endress PK. 2000.** Morphological phylogenetic analyses of basal angiosperms:comparison and combination with molecular data. *International Journal of Plant Sciences* 161:(6Suppl.):S121-S153.

**Erdtman G. 1960.** The acetolysis method. A revised description. *Svensk Botanik Tidskrift* 54:561 -564.

**Judd WS, Campbell CS, Kellogg EA, Stevens PF and Donoghu MJ. 2009.** Sistemática Vegetal: Um Enfoque Filogenético. 3 ed. Artmed, Porto Alegre.

**Pignal M, Lugardon B, Jérémie J and Le Thomas A. 1999.** Morphologie et ultrastructure du pollen des Siparunaceae (Laurales). *Grana* 38:210-217.

**Punt W, Hoen PP, Blackmore S, Nilsson S and Thomas A. 2007.** Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143:1-81.

**Renner SS. 1998.** Phylogenetic affinities of Monimiaceae based on cpDNA gene and spacer sequences. *Perspectives in Plant Ecology, Evolution and Systematics* 1:61-77.

**Renner SS. 1999.** Circumscription and phylogeny of Laurales: evidence from molecular and morphological data. *American Journal of Botany* 86:1301-1315.

**Renner SS. 2004.** Variation in diversity among Laurales, Early Cretaceous to Present. Biologiske Skrifter 55:441-458.

**Renner SS and Chanderbali A. 2000.** What is the relationship among Hernandiaceae, Lauraceae, and Monimiaceae, and why is this question so difficult to answer? *International Journal of Plant Sciences* 161(6 Suppl.):109-119.

**Renner SS and Hausner G. 1997.** *Siparuna* (Siparunaceae) and *Mollinedia* (Monimiaceae). *Flora del Ecuador* 59:1-125.

**Renner SS, Schwarzbach AE and Lohmann L. 1997.** Phylogenetic position and floral function of Siparuna (Siparunaceae: Laurales). *International Journal of Plant Sciences* 158(6 Suppl.):89-98.

**Ribeiro MNG. 1976.** Aspectos Climatológicos de Manaus. *Acta Amazonica* 6:229-233.

**Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR and Procópio LC. 1999.** Flora da Reserva Ducke: guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central. INPA, Manaus.

**Salgado-Labouriau ML. 1973.** Contribuição à palinologia dos Cerrados. Academia Brasileira de Ciências, Rio de Janeiro.

**Souza, VC and Lorenzi H. 2008.** Botânica Sistemática: guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas no Brasil, baseado em APG II. 2 ed. Instituto Plantarum, Nova Odessa.

**Walker JW. 1976.** Evolutionary significance of the exine in the pollen of primitive angiosperms. In: Ferguson IK, Muller J. (Eds). The evolutionary significance of the exine. Linnean Society: Academic Press, London. 251-308.