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

ABSTRACT:
This work describes pollen grains from the species of Hernandiaceae (*Sparattanthelium*), Monimiaceae (*Mollinedia*), and Siparunaceae (*Siparuna*) from a 100 km² area, Reserva Florestal Adolpho Ducke (03°00'00''S – 59°52'40''W) in the municipality of Manaus, Amazonas, aiming to supply taxonomic studies in Laurales. Pollen material was included in the Amazon Palynological Database as a reference for other studies. The pollen analysis in light microscopy (LM) and Scanning Electron Microscopy (SEM) include the species *Sparattanthelium acreanum* Pilg., *Mollinedia ovata* Ruiz & Pav., *Siparuna cristata* (Poeppl. & 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.

Authors:
Scherer C¹, Absy ML¹ and Lorscheitter ML².

Institution:
1. Laboratório de Palinologia, Coordenação de Pesquisas em Botânica, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil.
2. Laboratório de Palinologia, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
3. Departamento de Botânica, Instituto de Biologia, Universidade Federal de Pelotas, Caixa Postal 354, Pelotas, RS, Brazil.

Corresponding author:
Caroline Scherer

Email:
cacabio@yahoo.com.br

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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; Renner, 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 Adolfo 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 Adolfo Ducke, with an area of 100 km², in the municipality of Manaus, Amazonas (03°00'00''S – 59°52'40''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 (alie 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 Adolfo Ducke, Manaus, AM.

For analysis by light microscopy (LM), the pollen was prepared according to the standard acetolysis method (Erdtmann, 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...
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 Erdtmann (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:

**Magnoliidae**

**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.


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*)


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 ekteexine and endexine, while the rest of the pollen wall is made up entirely of endexine. Thus, although the bandlike area must be designated the
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).


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.


Ecological data: Tree or shrubs, chance in plateau aquifer forests soil of large areas from Amazonian region (Ribeiro et al., 1999).

*Siparuna depressa* Jangoux (Figures 3B–E)

In SEM: Scattered echinae in a dense irregular microverrucate surface.

Size: 13-25 µm (Table 1).

Exine thickness: 1µm.


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)

In SEM: Echinae of two distinct sizes, projected from a plane surface.

Size: 13-27 µm (Table 1).

Exine thickness: 1µm.


Ecological data: tree, until 20 m high, in clayed soils of forest or brushwood. Occasional in Brazilian Amazon (Ribeiro et al., 1999).

*Siparuna guianensis* Aubl. (Figures 3M–S)

In SEM: Echinae with more or less uniform size.

Size: 11-32 µm (Table 1).

Exine thickness: 1 µm.


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

<table>
<thead>
<tr>
<th>Species</th>
<th>Axis interval (n = 25) (µm)</th>
<th>Mean (x ± s_x) (µm)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sparattanthelium acreanum</em></td>
<td>20-26</td>
<td>23.16±1.77</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Mollinedia ovata</em> (Ema)</td>
<td>22-27</td>
<td>23.16±1.77</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Mollinedia ovata</em> (Esa)</td>
<td>16-20</td>
<td>18.55±3.59</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Siparuna cristata</em></td>
<td>13-35</td>
<td>21.88±3.07</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Siparuna depressa</em></td>
<td>13-25</td>
<td>21.28±4.7</td>
<td>0.92</td>
</tr>
<tr>
<td><em>Siparuna ficoides</em></td>
<td>13-27</td>
<td>20.01±5.21</td>
<td>1.02</td>
</tr>
<tr>
<td><em>Siparuna guianensis</em></td>
<td>11-32</td>
<td>15.76±1.54</td>
<td>0.3</td>
</tr>
</tbody>
</table>

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

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

*Siparuna poeppigii* (Tul.) A. DC. (*Figure 3T–W*)


In SEM: Echinae with uniform size.

Size: 13-19 µm (Table 1).

Exine thickness: about 1 µm.


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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