SURFACE MORPHOLOGY OF BRAZILIAN MORACEAE POLLEN GRAINS

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RESUMO

Superfícies dos grãos de pólen de 39 excicatas de 19 gêneros e 30 espécies foram examinadas por meio da microscopia eletrônica de varredura, a fim de obter esclarecimentos sobre o desenvolvimento da morfologia polínica nesta família. Foi possível reconhecer os seguintes grupos polínicos: o de *Ficus*, o de *Cecropia* e o *Dorstenia*; todos os demais gêneros examinados pertencem a um mesmo grupo polínico que compreende diferentes subgrupos, correspondentes a uma variação do número e da disposição de espículos sobre a superfície dos grãos. Somente através de uma correlação entre os aspectos das superfícies e a textura das exinas poderão ser obtidas afinidades de parentesco para este grupo de espécies.

ABSTRACT

Pollen grain surfaces from 39 samples of 19 genera and 30 species were examined by scanning electron microscopy, in order to elucidate the development of pollen grain morphologies in this family. It was possible to recognize the *Ficus, Cecropia* and *Dorstenia* pollen groups. All other examined genera belong to the same pollen group, with several sub-groups, based upon variation in the number and arrangement of surface spinules or scabrae. Only through correlation of surface aspects with exine textures may one ascertain the affinities of this group of species.

INTRODUCTION

Pollen grains from Moraceae of different phytogeographic regions have been studied in several papers; older references are to be found in ERDTMAN (1952). Since then, in Brazil, MELHEM (1966) and SALGADO-LABOURIAU (1973) examined species from the Brazilian "cerrado" and BARTH *et al.* (1975) from anemophilous species; pollen grains of *Dorstenia* were examined by BARTH in CARAUTA *et al.* (1979) and of *Cecropia* by BARTH (1974).

Papers on pollen grains of Moraceae from other regions were elaborated as catalogues: NAIR & SHARMA (1965) for India; STRAKA (1966) for Madagascar and the Mascarene Islands, TARNAVSCHI *et al.* (1967) for Romania; PALACIOS (1968) and RAMOS-ZAMORRA (1977) for Mexico; and HAMILTON (1976) for East Africa. Anemophilous species from Great Britain were studied by HYDE & ADAMS (1958). Taxonomically, pollen grains of some genera were analysed by NIEZGODA & NOWACZYK (1976) and PUNT & EETGERINK (1982).

The grains commonly present a primitive exine structure, but it is possible to establish evolutionary relationships on the basis of variations among genera. BARTH (1976a) has made some observations on this matter using light microscopy (LM) with an optical resolution of ca. 0.1 μm. Yet some questions remain which require analysis of submicroscopic structures by electron microscopy. HAMILTON (1976), NIEZGODA & NOWACZYK (1976), PUNT (1978) and PUNT & EETGERINK (1982), for example, have examined pollen grains from numerous genera by scanning electron microscopy (SEM).

Our immediate purpose here is to examine by SEM the pollen grain surfaces of more species and genera, some of which have been previously studied by LM (BARTH, 1976a, b), so that the relationships among the genera and species may be revised and amplified.

MATERIAL AND METHODS

Material from the following Herbaria was used: Botanial Garden, Rio de Janeiro (RB); the Brazilian National Museum, Rio de Janeiro (R);

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PLATE I

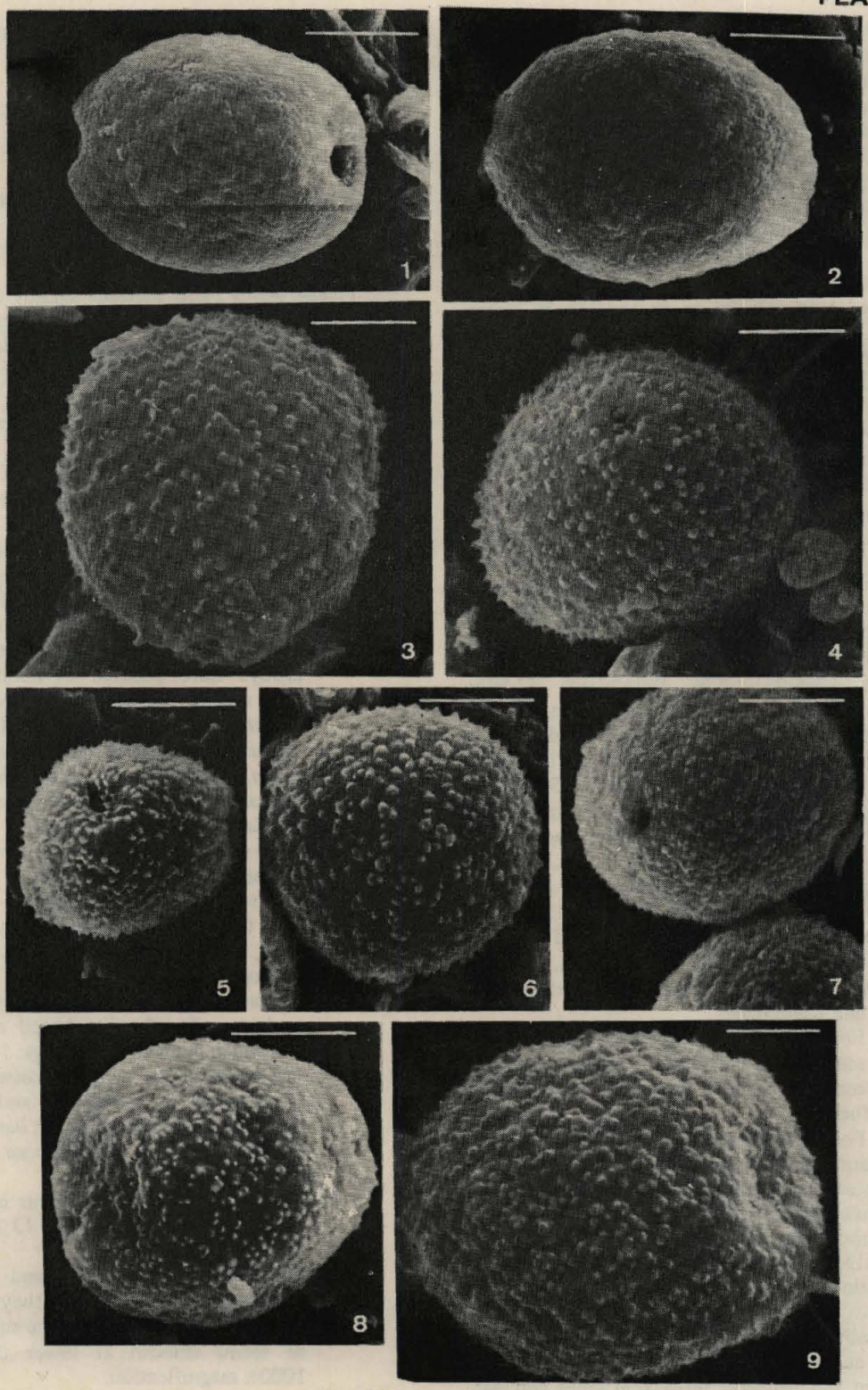

Plate I (bar scale = 5μm)

Fig. 1 – *Ficus insipida*, finely undulate tectum. Fig. 2 – *Ficus pulchella*, finely undulate tectum. Figs. 3, 4 – *Artocarpus heterophyllus*, spinules and aperture. Fig. 5 – *Batocarpus amazonicus*. Fig. 6 – *Castilla ulei*. Fig. 7 – *Chlorophora tinctoria*. Fig. 8 – *Helicostylis tomentosa*. Fig. 9 – *Pourouma acutiflora*.

"Barbosa Rodrigues" Herbarium, Itajaí, Santa Catarina (HBR); and "Alberto Castellanos" Herbarium, Rio de Janeiro (GUA). The following specimens were observed by SEM: *Acanthinophyllum ilicifolia* (Spreng.) Burger, Sucre 5.287; *Artocarpus heterophyllus* Lamarck, Souza s.n.; *Cannabis sativa* L., Reitz and Klein 8.880; *Bagassa tiliaefolia* (Ham.) Benoist., Ducke on 01.08.1926; *Batocarpus amazonicus* (Ducke) Fosberg, Ducke 947; *Brosimum discolor* Schott ex Sprengel, Klein 1.807; *B. guaianense* (Aubl.) Hub., Ducke on 30.01.1933; *B. lactescens* (S. Moore) Berg, Reitz and Klein 212; *Castilla ulei* Warb., Ducke on 23.02.1932; *Cecropia catarinensis* Cuatrecasas, Hatschbach 5.103; *C. glazioui* Snethlage, Klein and Souza 7.329; *C. obtusa* Trécul, Reitz C-467; *C. palmata* Willdenow, Carauta 554 and 969A; *Chlorophora tinctoria* (L.) Gaud., Veloso 160, P. da Silva s/n, October 1938, Rambo 38.604 and Reitz C-177; *Clarisia racemosa* R. et Pav., Duarte 5.969; *Coussapoa schottii* Miguel, Reitz 4.176; *Dorstenia asaroides* Gardner, Prance s/n, October 1973; *D. brasiliensis* Laur., Rambo 38.432; *D. cayapia* Vell., Carauta 1.408, Duarte 3.725, Sucre 2.026; *D. morifolia* Fisch. and Meyer, Carauta 1.878, Hatschbach 32.898; *D. tenuis* Bonpland, Smith and Klein 13.155, Smith and Klein 14.111; *D.* sp., Emygdio 2.911 and Emmerich 3.457; *Ficus insipida* Willdenow, Reitz and Klein 2.290; *F. pulchella* Schott ex Sprengel, Klein 1.807; *Helicostylis tomentosa* (P. et E.) Rusby, det.: W. Buerger; *Maquira calophylla* (P. et E.) Berg, Ducke on 08.11.1932; *Pereba tessmannii* Macby, Ducke on 19.09.1931; *Pourouma acutiflora* Trécul, Klein 1.110; *Pseudolmedia hietulla* Kuhlm., Occhioni s/n; *P. laevis* (R. et P.) Macby, Ducke on 12.10.1913; *Sorocea bonplandii* (Baillon) Burger *et al.*, Reitz and Klein 8.761.

For LM observations the pollen grains were acetolysed (ERDTMAN, 1952) and kept in glycerol; sometimes they were stained with basic fuchsin.

For SEM observations, acetolysed pollen sediment stored in glycerol or samples directly separated from the herbaria material, always suspended in 50% or 70% ethanol, were used. To remove the glycerol in the first case and the anther material in the second from the pollen grain surfaces a Bransonic B-12 ultra-sound apparatus or similar gave best results by a total application during 15 minutes, first in distilled water and then in ethanol; the pollen grain suspension was dropped onto SEM stubs and left to air-dry overnight at 37°C, prior to sputtering with a 200 Å-thick gold layer in a Balzers-Union apparatus. The preparations were observed in a JEOL – 25S – II SEM at magnifications up to 10,000 X.

RESULTS

Thirty-nine samples representing 19 genera and 30 species were observed by SEM. The following pollen surface patterns were distinguished:

1. The genus *Ficus* presents slightly irregular surfaces (Figs. 1 and 2) because of numerous very straight puncta in the tecta; there are no spinules or scabrae.
2. Another configuration is represented by spinulous (scabrate) surfaces; the form, number and distribution of the spinules (scabrae) vary among the genera:
   a) They are of irregular sizes, not so numerous and irregularly dispersed over the pollen grain surfaces (Figs. 3 – 5); their basal diameters may be as large as they are high and they always have pointed extremities. This surface pattern is observed in *Acanthinophyllum ilicifolia, Artocarpus heterophyllus, Bagassa tiliaefolia, Batocarpus amazonicus, Coussapoa schottii, Maquiva calophylla* and *Pereba tessmannii.*
   b) The spinules are more numerous and smaller, but still irregularly distributed or clustered (Figs. 6 – 9) as in *Castilla ulei, Chlorophora tinctoria, Helicostylis tomentosa* and *Pourouma acutiflora.*
   c) Numerous very fine spinules, more or less uniformly distributed over the pollen grain surfaces (Figs. 10 – 12) occur in the genera *Brosimum* (*B. discolor, B. guaianense* and *B. lactescens*), *Pseudolmedia* (*P. hietulla* and *P. laevis*) and *Sorocea* (*S. bonplandii*).
   d) Nearly verrucate grains occur only in *Clarisia racemosa* (Figs. 13 and 14).
   e) *Cannabis sativa* (Figs. 15 and 16) has such fine spinules that they seem to be merely granula; the surface seems smooth at lower than 1000x magnification.
3. A especialized configuration is presented by the genus *Cecropia*. The spinules occur only at the equatorial regions

PLATE II

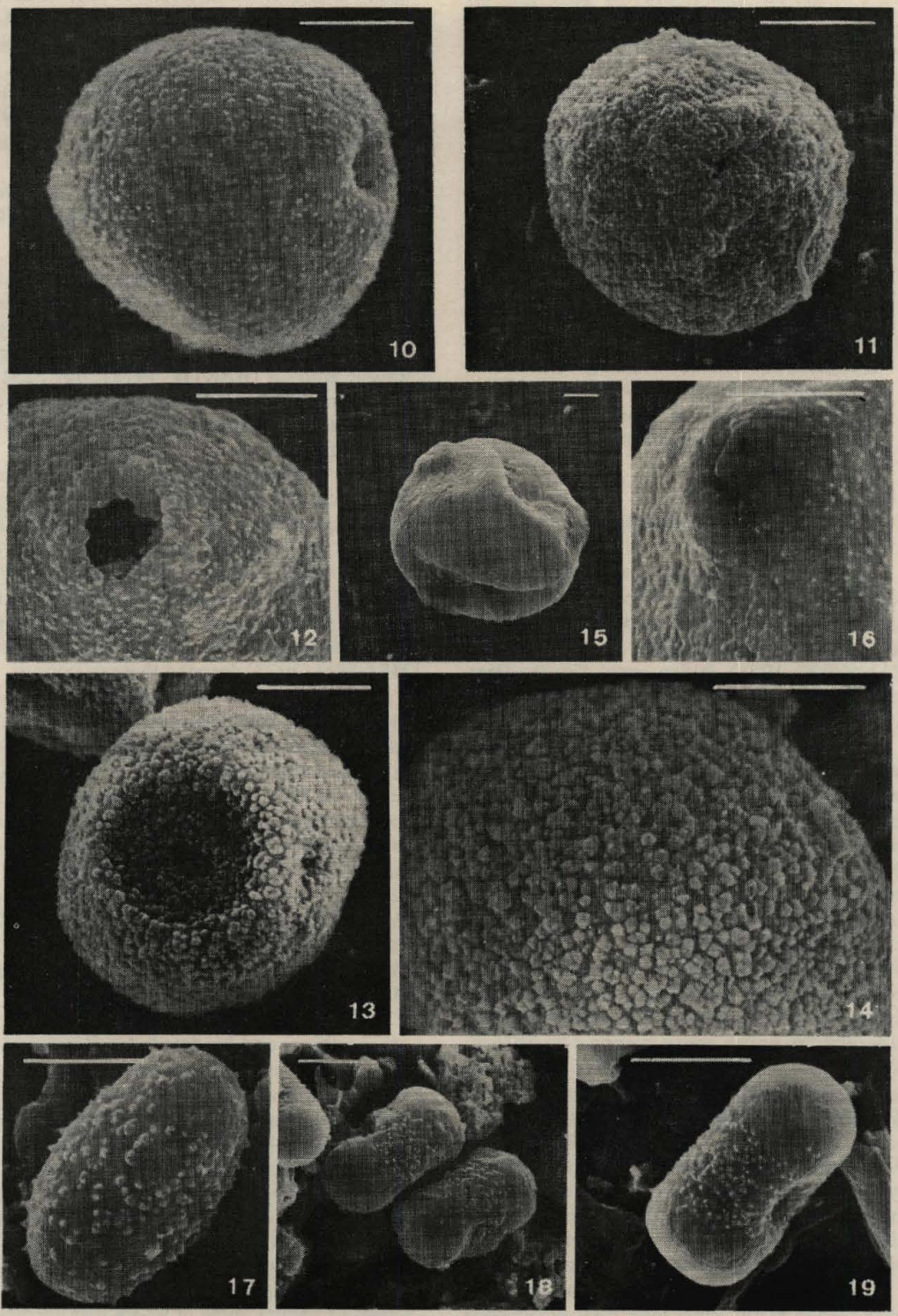

Plate II (bar scale = 5μm)

Fig. 10 – *Brosimum lactescens.* Fig. 11 – *Pseudolmedia laevis.* Fig. 12 – *Sorocea bondplandii*, spinules and aperture. Figs. 13, 14 – *Clarisia racemosa.* Figs. 15, 16 – *Cannabis sativa*, whole grain, spinules and aperture. Fig. 17 – *Cecropia catarinensis.* Fig. 18 – *Cecropia obtusa.* Fig. 19 – *Cecropia palmata.*

of the grains and the apocolpia are smooth. Except for *C. catarinensis* (Fig. 17), with large spinules and reduced, smooth polar areas, the other examined species (*C. glazioui*, *C. obtusa* and *C. palmata*, Figs 18 and 19) have large polar areas and a well delimited spinulous, equatorial band.

4. The most specialized genus is *Dorstenia* (Figs. 20 and 21). The pantoporate surfaces of their grains are aspidote with smooth or spinulate annuli and spinulate or verrucate areas between the annuli and inside the pori. In some species the general surface appearance may be related to the age and the degree of distension of the grains (Figs. 22 and 23), so that the aspides are more or less protuberant.

DISCUSSION

A very fine punctate tectum occurs in the genus *Ficus* and may be considered as a basic structure. The species always seem to be smooth in LM observations (BARTH, 1976a; PALACIOS, 1968). Pollen grains like the *Dorstenia* type were presented as *Ficus carica* by TARNAVSCHI *et al.* (1967).

Another simple pollen grain surface pattern consists of irregularly scattered spinules (scabrae), sometimes with clustered spinules. The spinules are always visible in LM observations (STRAKA, 1966; BARTH *et al.*, 1975; BARTH, 1976a). In order to divide such grains into pollen types, one has to utilize additional exine textures and aperture numbers, which will be commented upon in a future paper. Such a pollen grain surface is found in a great number of genera previously mentioned in this paper (sub-groups 2a and 2b); *Acanthinophyllum ilicifolia* pollen grains observed by SEM by NIEZGODA & NOWACZYK (1976) and *Batocarpus amazonicus* and *Chlorophora tinctoria* (PUNT & EETGERINK, 1982) have the same pollen morphology as that observed here. The Brazilian species *Bagassa guianensis* examined by PUNT & EETGERINK (1982) is also very similar to our investigated specimen; another Brazilian species that they examined, *Maclura brasiliensis*, seems to be close to our group 2b.

The number of spinules increases sequentially, while at the same time their dimensions decrease, until reaching a fine, regular spinulous surface as in *Brosimum*, *Pseudolmedia* and *Sorocea*. The spinule dimensions are at the LM resolution limit, so that sometimes the grains apper to be smooth (BARTH, 1976a; MELHEM, 1966; SALGADO–LABOURIAU, 1973; MARTINEZ–HERNANDEZ *et al.*, 1979). For *Sorocea bonplandii*, the spinules are generally not clustered, as they are in *S. guilleminiana* and *S. saxicola* (NIEZGODA & NOWACZYK, 1976), so that specific variations become evident at high magnifications.

The surface configuration of *Clarisia racemosa* is the same as that observed by NIEZGODA & NOWACZYK (1976).

A different surface aspect is found in grains of *Cecropia* which, at the moment, apparently have no similarity with any other genus of this family. In LM, these grains generally appear smooth (BARTH, 1975), but SEM observations (BARTH, 1974) confirmed the equatorial band of spinules (ERDTMAN, 1952) also around the pori.

In *Cannabis sativa* the spinule distribution is less dense at the aperture areas; like the whole grain represented by SEM in HAMILTON (1976), the small scabrae are densely and uniformly distributed over the entire pollen grain surface. TARNAVSCHI *et al.* (1967) recognized by LM the finely granulate surface aspect for some parts of the grain, while MAKINO & MELHEM (1973) describe them as smooth.

The grains of *Dorstenia* present peculiar, more or less spinulous, aspidote surface areas and spinulous or verrucate inter-aspide regions. A species-dependent number of pores is significant for their subdivision into sub-types and for determining their relationships (CARAUTA *et al.*, 1979). A similar surface pattern was described by PUNT (1978) for this genus and for *Scyphocyce*; for *Dorstenia* by MELHEM (1966), SALGADO–LABOURIAU (1973), STRAKA (1966) and BARTH (1976a); for *Dorstenia* and *Craterogyne* by ERDTMAN (1952) and HAMILTON (1976).

As to the pollen types established by other authors and by detailed surface examination, the genus *Ficus* represents one pollen type, as in STRAKA (1966); *Cannabis sativa* occupies a particular position, also in STRAKA (1966) and HAMILTON (1976); *Dorstenia* differs from these, as does *Cecropia* as well; all the other genera may be included within a single pollen surface group having several sub-groups, like those here established under numbers 2a-d. Thus, at the moment, one may recognize four pollen surface groups for Moraceae (excluding *Cannabis sativa*), some of which may be divided into sub-groups.

With respect to the observations made by PUNT & EETGERINK (1982) on pollen morphology of Moreae and from our own observa-

PLATE III

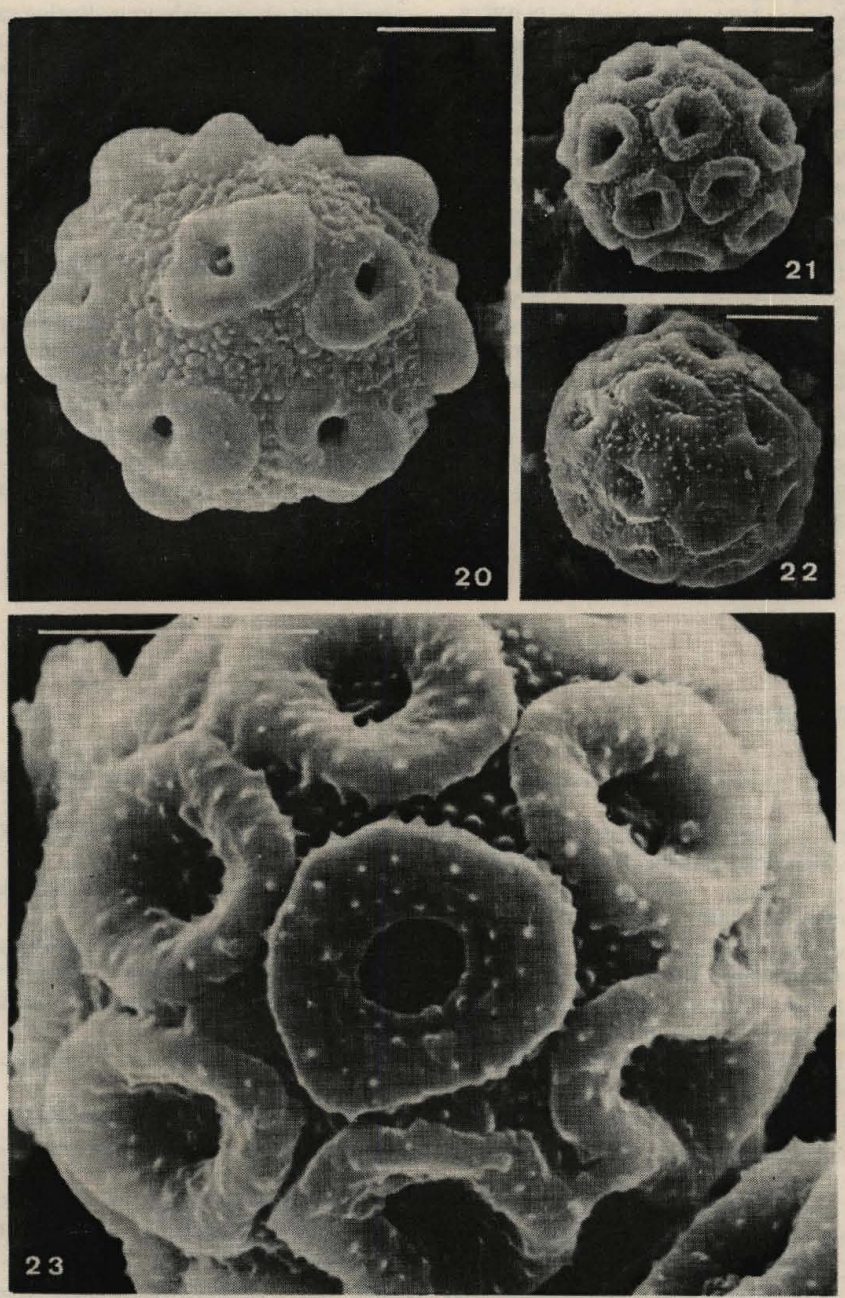

Plate III (bar scale = 5μm)

Fig. 20 – *Dorstenia brasiliensis.* Fig. 21 – *Dorstenia tenuis.* Figs. 22, 23 – *Dorstenia* sp., immature and mature pollen grains, respectively.

tions, three pollen morphology groups occur in this tribe: the *Artocarpus* sub-group (2a) and the *Chlorophora* sub-group (2b), each containing numerous genera. The other is a monospecific pollen sub-group represented by *Maclura pomifera* from Kansas, USA, illustrated in PUNT & EETGERINK (1982), which has some similarities with *Cannabis sativa* pollen grains (Figs. 15 and 16 and HAMILTON, 1976).

It is not possible to establish relationships within the Moraceae on the basis of surface characteristics only (BARTH, 1976b). There are apparent evolutionary trends, but it is difficult to establish their direction (BARTH, 1976a). To better understand the pollen relationships among the genera and/or the species, it is necessary to examine the exine texture. A first attempt using LM was made by BARTH (1976a), who indicated visible bacula for only some genera/species considered more evolved; for others, no bacula were recognized, so that more genera than those illustrated in MARTINEZ–HERNANDES *et al.* (1979) still need to be examined by TEM in the future.

Although the grains of the majority of the genera are small and 2-porate, the exine texture (BARTH, 1976a,b and MARTINEZ–HERNANDEZ *et al.*, 1979) and the detailed surface patterns are so variable that the family may be considered as eurypalynous. Ecological and habitat specializations are indicated by the varied pollen grain morphologies, which, because of the tiny dimensions of their ornamentation, require further high resolution observations.

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