



Palynological analysis of Dennstaedtiaceae taxa from the Paranaense Phytogeographic Province that produce monolet spores and its systematic implications (I): *Blotiella lindeniana*, *Histiopteris incisa* and *Paesia glandulosa*

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ABSTRACT

The genera of Dennstaedtiaceae have sporophytes with very different morphological characteristics between each other, and this feature has made difficult the systematic circumscription of the family. This reason makes necessary the study of new characters that allow a better understanding of the relations within the group. The spore morphology and wall structure of *Blotiella lindeniana*, *Histiopteris incisa* and *Paesia glandulosa* from the Paranaense Phytogeographic Province were studied using light microscope, and scanning and transmission electron microscope. The exospore has two layers and, according to the species, the exospore surface bears pila, echinae, verrucae, bacula and tubercles. The perispore has two or three layers and its surface is psilate, baculate or rugulate. The variability found in the sculpture of the spores and their stratification and ultrastructure of perispore reflects the morphological differences observed in the sporophyte of the species studied. Additionally, while the stratification and ultrastructure of the exospore is shared by the Dennstaedtiaceae species, their ornamentation could be a character to distinguish species into the clade “hypolepidoide”. The finding of spores with similar characteristics in phylogenetically unrelated families allows us to suggest that palynological features do not have an evolutionary value to establish relationships between groups above the genus level.

Key words: ferns, morphology, South America, sporoderm, ultrastructure.

INTRODUCTION

This work is part of a project in which the morphology and ultrastructure of the spores of the family Dennstaedtiaceae in the Paranaense phytogeographic Province, within the Neotropical region (Cabrera and Willink 1973, Morrone 2001,

2014) are being studied. The Paranaense Province, together with Atlantic Province, include the Atlantic Forests which is considered a “hotspot” for its high number of species (Myers et al. 2000), it represents an area with hypothetical endemism centers (Prance 1987, Thomas et al. 1998), and it is one of the most important areas of evolution and speciation of Ferns and lycophytes (Prado and Hirai 2014). The analysis of Dennstaedtiaceae taxa with

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trilete spores: *Dennstaedtia* Bernh., *Microlepia* C. Presl and *Pteridium* Gled. ex Scop., was carried out (Yañez et al. 2016a, b).

The family Dennstaedtiaceae includes 10 genera with about 265 pantropical and, occasionally, boreal or temperate regions species (PPG I 2016). The taxonomic delimitation of this family has been traditionally highly controversial and the subordinated taxa were grouped under different schemes into subfamilies (Holttum 1947 1949, Crabbe et al. 1975, Lovis 1977, Kramer and Green 1990), tribes (Tryon and Tryon 1982) or in three independent families: Dennstaedtiaceae, Hypolepidaceae and Lindsaeaceae (Ching 1940, Nayar 1970, Pichi Sermolli 1970, 1977).

Recent molecular studies have identified as Dennstaedtiaceae *sensu stricto* a monophyletic group made up of Dennstaedtiaceae and Hypolepidaceae *sensu* Pichi Sermolli (1970) (Hasebe et al. 1994, Wolf et al. 1994, Pryer et al. 2004, Schuettpelz and Pryer 2007, Christenhusz et al. 2011, Perrie et al. 2015). On the bases of morphological and molecular analysis, Smith et al. (2006) also recognized the Dennstaedtiaceae *s.s.* (except the genus *Saccoloma* Kaulf.), and this work followed the classification suggested by these authors.

The difficulty on classifying the Dennstaedtiaceae is because the genera assigned to this family have sporophytes with very different morphological characteristics between each other. This reason makes it necessary the study of new characteristics about the relationships within the group to a better understanding.

From the palynological point of view, the family includes species that produce spores with different ornamentation, and different kind of apertures both triletes and monoletes. The latter group includes the following genera: *Blotiella* R. M. Tryon, with one neotropical species and 14 species from Africa; *Paesia* A. St-Hill, with 14 neotropical and Asiatic species; and *Histiopteris* (J. Agardh) J. Sm., with

about 7 pantropical species. In all the cases, the taxa grow in wet forests, mountain forests or cloud forests, and in clearings and natural openings along the borders.

Mickel (1973) suggested that the Dennstaedtiaceae spores characteristics would be useful for the genera and subgenera classification and the author considered necessary to carry out comparative studies using electron microscopic techniques. Tryon (1986) arrived to the same conclusion. However, the palynological study of the above genera has been scarce and those were restricted to local palynological floras.

The first observations were made with Light Microscope (LM) by Harris (1955) in New Zealand, who described the perispore of the spores of *Paesia scaberula* Kuhn as striate or scabrate and those of *Histiopteris incisa* (Thunb.) J. Sm. as verrucate to rugulo-verrucate with coalescent projections. Later, Nayar et al. (1968a) and Nayar (1964) concluded that the exospore surface forms the tubercles of ornamentation in *Histiopteris incisa*, and the tubercles sometimes coalesce in rugulae. Additionally, the latter author observed a finely ornamental perispore that easily detached in species of *Paesia* and absence of perispore in *Blotiella*. In contrast, Welman (1970), in his work on fern spores from South Africa, observed a fine and echinate perispore in *B. glabra* (Bory) R. M. Tryon and *B. natalensis* (Hook.) R. M. Tryon. The author also noticed that the spores of *H. incisa* had verrucate-rugulate spores with a psilate laesurae.

The presence of exospore with granules or echinae and the absence of perispore in *Paesia glandulosa* (Sw.) Kuhn were observed by Erdtman and Sorsa (1971). These authors did not observe a perispore in *H. incisa* and they described “spores probably with perine” in *Blotiella pubescens* R. M. Tryon (under genus *Lonchitis* L.). In the palynological work about Dennstaedtiaceae *sensu* Holttum from Malaysia, Soepadmo and Khoo (1977) agreed about the absence of perispore in

Histiopteris and they identified an exospore densely verrucate-tuberculate in *Histiopteris stipulacea*.

The first studies with Scanning Electron Microscope (SEM) were carried out by Tryon and Tryon (1982). The authors confirmed the presence of a coarsely echinate perispore often with slender strands connecting the echinate elements in *Blotiella* species. Additionally, the authors recognized an outer rugose perispore overlaying a papillate exospore in *Paesia glandulosa* and a coarsely tuberculate surface apparently formed by perispore in *Histiopteris*. In contrast, Large and Braggins (1991) recognized that the elements of ornamentation of *Histiopteris incisa* were originated by exospore while those of the perispore were thin and papillate surface coat following the exospore ornamentation. Spores of *Paesia scaberula* were also described by these authors as provided with a rugulate or scabrate perispore covering a scabrate or papillate exospore.

The studies with Transmission Electron Microscope (TEM) in Dennstaedtiaceae were carried out by Tryon and Lugardon (1991). In *Paesia* the authors confirmed the presence of a tuberculate exospore and perispore with two layers. The authors also observed that in *Histiopteris* the exospore constitutes the sculpture and the perispore is a thin and diffuse layer. Up to the present, it was not found ultrastructural studies on spores of *Blotiella* species.

Recent works about the taxa mentioned deal with descriptions based on observations performed at LM and SEM of *Histiopteris* species from Asia (Dai et al. 2005, Faridah-Hanum et al. 2008) and South America (Lorscheitter et al. 2002, Contreras-Duarte and Giovanni Bogotá-Ángel 2006, Estevez and Coelho 2007).

The main goal of this work is to extend the palynological knowledge through the study of the morphology and ultrastructure of the spore wall and its correlation with spore ornamentation of *Blotiella*, *Paesia* and *Histiopteris* species that grow

in the Phytogeographical Paranaense Province. The results will allow us to make more accurate interpretations about the spores morphology of the mentioned species and the results will also provide useful information for genera circumscription. In addition, this information will allow comparisons with other taxa of the Dennstaedtiaceae and it will contribute to improving the palaeobotanic assignments.

MATERIALS AND METHODS

The species under study *Blotiella lindeniana* (Hook.) R. M. Tryon, *Histiopteris incisa* and *Paesia glandulosa* were identified in the field with the aid of floristic and taxonomic works (Prado 2004, Costa Assis and Salino 2011, Gasper 2012).

Spores were obtained from herbarium specimens from the following institutions: BHCH (Universidade Federal de Minas Gerais, Minas Gerais, Brasil), CTES (Instituto de Botánica del Nordeste, Corrientes, Argentina), HB (Herbarium Bradeanum, Rio de Janeiro, Brasil), NY (New York Botanical Garden, New York, USA), PACA (Instituto Anchietano de Pesquisas, Rio Grande do Sul, Brasil), SI (Instituto de Botánica Darwinion, San Isidro, Argentina), SP (Instituto de Botânica, São Paulo, Brasil) (Appendix).

The spores were studied using Light Microscope (LM), Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM).

For LM spores were studied without previous chemical treatment since the perispore does not resist acetolysis treatment (Erdtman 1960), and they were mounted in gelatin glycerin jelly. For each specimen, 25 spores were selected and polar diameter, major and minor equatorial diameters (Ramos Giacosa et al. 2009; figure 1), perispore and exospore thickness were measured (Nayar 1964). The observations were performed with Olympus

BH2 LM and photographs were taken with a Nikon Coolpix S10 digital camera.

For SEM the spores were treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates (Morbelli 1980). After drying, they were coated with gold. To fracture the sporoderm, some spores underwent ultrasound for 10'. The observations were performed with a Jeol JSMT-100 from the Microscopy Service of Facultad de Ciencias Naturales y Museo de La Plata (La Plata).

For TEM, dry material from herbarium specimens was hydrated following the technique proposed by Rowley and Nilsson (1972) that consist of the use of a buffer plus alcian blue (AB), then the material was fixed with 2% glutaraldehyde (GA) + 1% alcian blue in phosphate buffer for 12 h, rinsed with phosphate buffer + AB, and post-fixed with 1% OsO₄ in water plus 1% AB.

The spores were dehydrated in an acetone series and then embedded in Spurr medium mixture. Three µm thick sections were stained with toluidine blue and observed with LM. Ultra-thin sections were stained with 1% uranyl acetate for 15 min followed by lead citrate for 1 min.

The observations were performed with Zeiss M-10 from microscopy service of Laboratorio Nacional de Investigación y Servicios de Microscopía Electrónica, LANAIS-MIE (Ciudad Autónoma de Buenos Aires).

The letters MP, in the specimens investigated list indicate the reference number of each palynological sample filed in the Laboratorio de Palinología, Facultad de Ciencias Naturales y Museo de La Plata (La Plata, Argentina).

The works of the following authors Nayar (1964), Kremp (1965), Viane and Cotthem (1977), Huang (1981), Tryon and Lugardon (1991), Punt et al. (1994, 2007), Lellinger (2002) and Sáez-Láin (2004) were used in the descriptions the spore morphology and their wall ultrastructure.

RESULTS

GENERAL MORPHOLOGY

The spores studied are monoete, bilateral, oblong to elliptic in polar view (Fig. 1a, b; Fig. 2a, b; Fig. 3a, b). In equatorial view the spores are plane-convex to concave-convex (Fig. 1c; Fig. 2c; Fig. 3c) and sometimes they are reniform-shaped (*Histiopteris incisa*). The morphological data of the spores of *Blotiella lindeniana*, *Paesia glandulosa*, and *Histiopteris incisa* are summarized in Table I.

Blotiella lindeniana

The sculpture is baculate. Baculae are 1.5-7 µm in high, elliptical in section. A kind of thickening at the base of each baculae forming mamelons (Fig. 1e-f) sometimes is fused (Fig. 1d-e). Occasionally, echinae are observed above the surface (Fig. 1d, f) distributed at random. Laesurae are straight and generally covered by thickenings of the perispore, which can have baculae on the surface (Fig. 1d).

The spore surface is microverrucate, and threads connect the elements of the ornamentation (Fig. 1 f-g).

Paesia glandulosa

The sculpture is rugulate, formed by sinuous ridges of 1.4-3.6 µm in width, with narrow spaces of 0.2-2.1 µm and arranged in a laberhentic pattern. Occasionally, some ridges are fused forming isolated open lumina (Fig. 2e-f). Isolated ridges were observed in few spores (Fig. 2g).

On the proximal face, short ridges of 5-6 µm length are fused between them and form a border near to laesurae (Fig. 2d). The lesura is straight.

The ridges surface is granulose and spheroidal bodies are also present (Fig. 2f).

Spores without perispore were observed. In those cases, the exospore are covered with tubercles (Fig. 2h-j) distributed at random.

TABLE I
 Characteristics of *Blotiella lindeniana*, *Histiopteris incisa* and *Paesia glandulosa* spores.

	Diameters			Lesura (μm)			Perispore			Exospore		
	DE > (μm)	DE < (μm)	PD (μm)	Color	Thickness (μm)	Ornamentation	Color	Thickness (μm)	Ornamentation	Color	Thickness (μm)	Ornamentation
<i>Blotiella lindeniana</i>	37-54	22-38	22-37	Hyaline	0.3-0.9 (7.6 including bacula)	Baculate	Hyaline - yellowish	0.8-1.5 (4.4 in lesurae)	Psilate			
<i>Histiopteris incisa</i>	36-52	22-37	22-37	Brown	0.1-0.4	Psilate	Light Brown - yellowish	1.4-3.5 (12 in lesurae)	Tuberculate / Reticulate.			Margin with bacula, verrucae and echinae.
<i>Paesia glandulosa</i>	50-67	29-42	34-50	Hyaline (immature) - dark-gray (mature)	0.09-0.2 (4 including ridges)	Rugulate	Hyaline - yellowish	1.8-3.8 (4.5 in lesurae)	Tuberculate. Margin of tubercles, psilate or granulose.			

DE: Equatorial diameter; PD: polar diameter; >: Mayor; <: Minor. The color of Perispore and Exospore refers to the color observed with MO.

Histiopteris incisa

The spores sculpture is tuberculate-reticulate. In the proximal face, the reticule has small lumina, and generally is laevigate next to the lesura (Fig. 3e). The laesura is straight, crassimarginate and partially covered at both ends (Fig. 3e).

On the equator, tubercles are presents in the spaces between reticules (Fig. 3e, g).

On the distal face tubercles of 2-5 μm in diameter are fused between them forming reticules with muri and lumina variable in size (Fig. 3f).

The surface of the tubercles is microverrucose (Fig. 3h) and spheroidal bodies are also present (Fig. 3f).

SPORODERM ULTRASTRUCTURE

Exospore

With TEM, the exospore is two-layered in the studied species. The inner layer (iE) is 20-70 nm thick, with low electrodensity and homogeneous structure, and it is thicker at the laesurae bases (Figs. 4b, e; 5b; 6c). The outer layer (oE) is 0.2-3.8 μm thick and formed of two strata: in the inner stratum (oE₂) there are channels and cavities filled with a contrasted content (Figs. 4b; 5b; 6c), and occasionally the channels are ramified forming a network with continuity with those of the outer stratum (Fig. 5b; Fig. 6c).

The outer stratum (oE₁) is thicker than inner stratum. In *Blotiella lindeniana* the oE₁ does not have variations of thickness as in *Histiopteris incisa* and *Paesia glandulosa*.

With TEM, the exospore margin is ornamented in all the species: *Blotiella lindeniana* has a micrornamentation composed for pila of 30-60 nm in diameter, wich are densely arranged (Fig. 4d-f); *P. glandulosa* and *H. incisa* have tubercles (Fig. 5c-f; Fig. 6d-f).

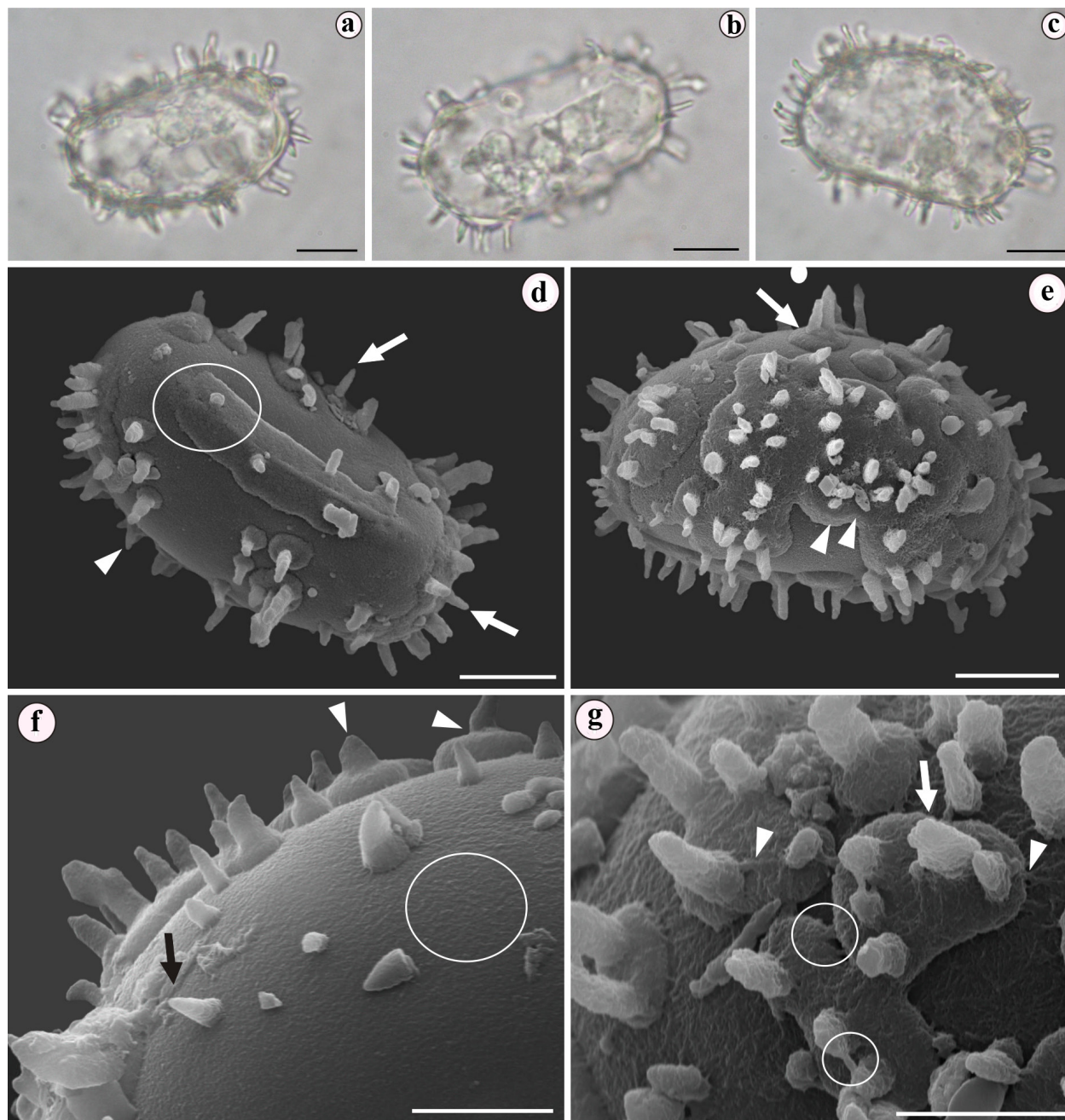


Figure 1 - Spores of *Blotiella lindeniana* with LM and SEM. Baculate sculpture.

Figs. a-c. Spores with LM. **a.** Proximal view. **b.** Distal view. **c.** Equatorial view. **Figs. d-g.** Spores with SEM. **d.** Spore in proximal view. The sculpture is baculate, with bacula distributed at random on the surface (arrows). Echinae occasionally are observed (arrowhead). Lesurae are covered by thickenings of the perispore (circle). **e.** Spore in distal view. A thickening at the base of each baculae is present forming mamelons (arrow). Those thickenings sometimes are fused (arrowheads). **f.** Detail of the equatorial surface. Few echinae are observed (arrow). A thickening at the base of each baculae can be seen, which forming mamelons (arrowhead). The spores surface is microverrucose (circle). **g.** Detail of equatorial surface. The bacula are elliptical in section (arrow). There are threads on the spore surface (arrowheads), which connect the ornamentation elements (circles). Scale bars: **a, b, c, d** and **f** = 10 μm ; **e** and **g** = 5 μm .

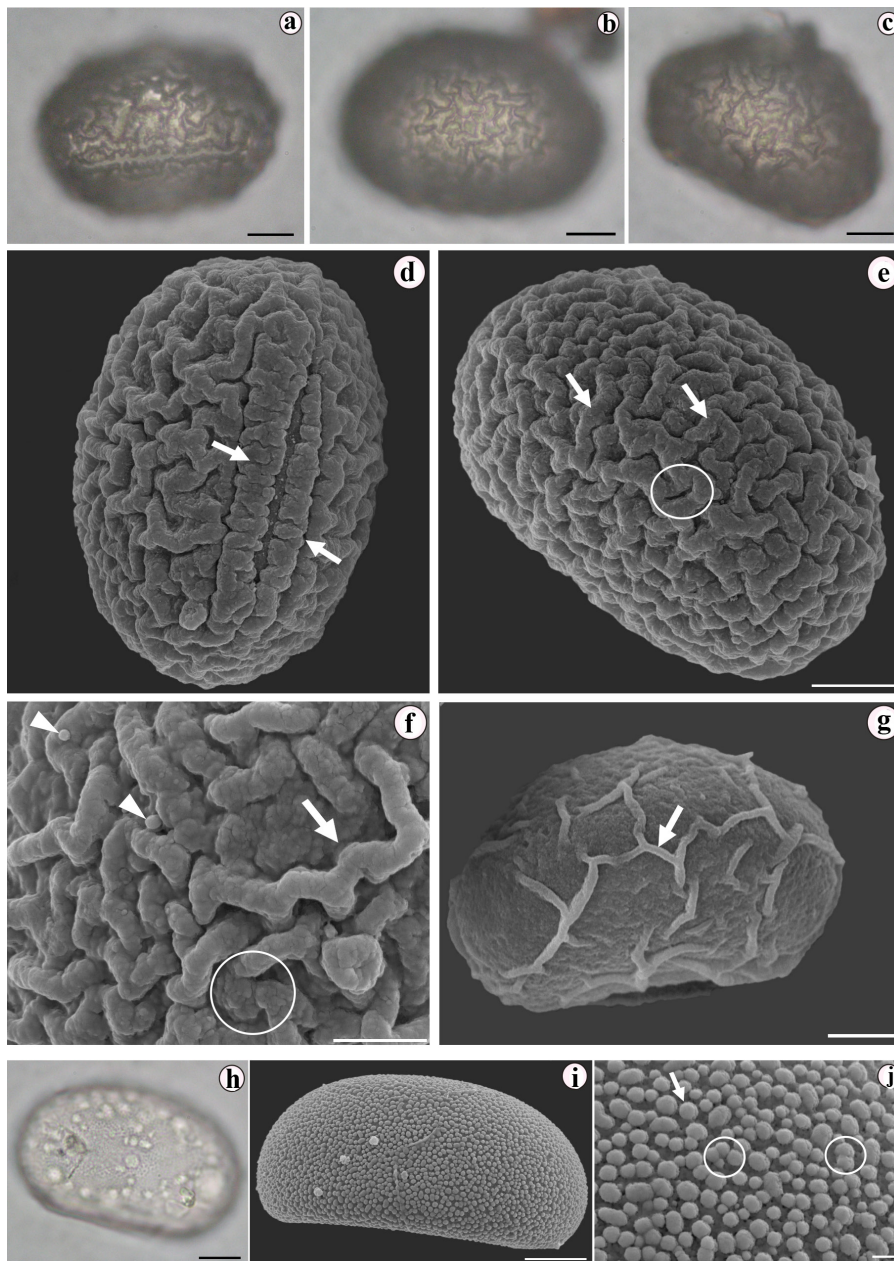


Figure 2 - Spores of *Paesia glandulosa* with LM and SEM. Rugulate sculpture. **Figs. a-c.** Mature spores with LM. **a.** Proximal view. **b.** Distal view. **c.** Equatorial view. **Figs. d-g.** Spores with MEB. **d.** Spore in proximal view, with short ridges adjacent and perpendicular at the laesurae (arrows). **e.** Spore in distal view. Sinuous ridges that conform an laberhantic arrangement (arrows) cover the surface. Ridges are fused and form open and isolated luminae (circle). **f.** Detail of distal view. Ridges (arrow) are separated by narrow spaces. The surface of ridges is granulose (circle) and spheroid bodies can be seen (arrowheads). **g.** Spore in equatorial view. In few spores analyzed, isolated ridges like crest were observed (arrow). **Figs. h-j.** Spores without perispore. **h.** Spore in distal view with LM. **i.** Detail of distal view with SEM. **j.** Spore in equatorial view with SEM. The exospore is covered with tubercles distributed at random. Tubercles with psilate or granulate surface are distributed at random (arrow) and can be fused in groups (circle). Specimen MP 4226. Scale bars = **a-e, g-i** = 10 μm ; **f** = 5 μm ; **j** = 1 μm .

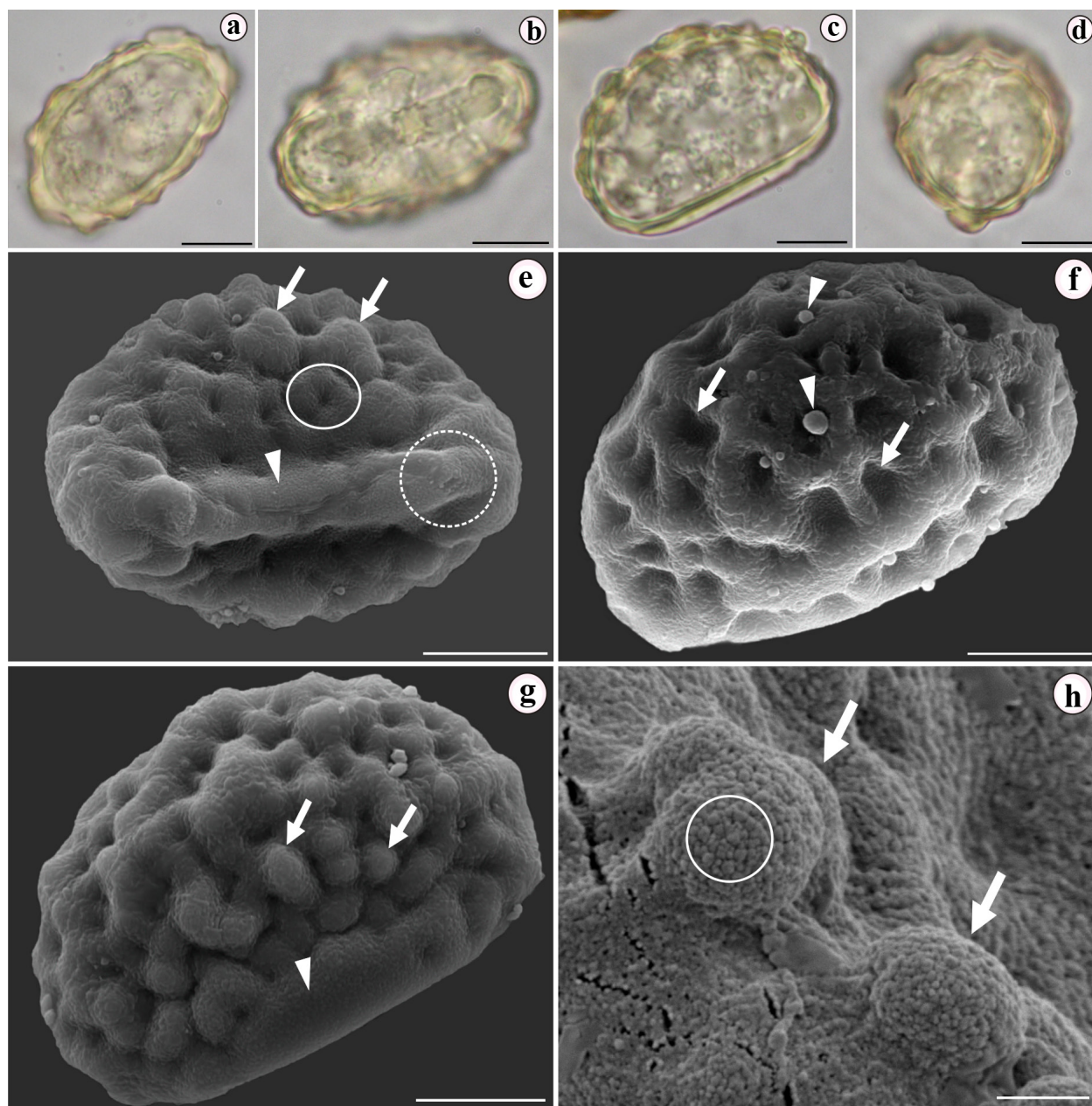


Figure 3 - Spores of *Histiopteris incisa* with LM and SEM. Tuberculate-reticulate sculpture.

Figs. a-d. Mature spores with LM. **a.** Distal view. **b.** Proximal view. **c.** Major equatorial view. **d.** Minor equatorial view. **Figs. e-h.** Spores with SEM. **e.** Spore in proximal view. The sculpture is tuberculate-reticulate. The tubercles are spaced on the equatorial surface (arrows) and are fused forming reticules with small lumina (continue circle) in proximal view. Thickenings are observed at both sides of the laesurae (arrowhead), and can partially cover its ends (incomplete circle). **f.** Spore in distal view. The reticulum is conspicuous and has muri and lumina of variable sizes (arrows). Spheroid bodies can be seen on the surface (arrowhead). **g.** Spore in equatorial view. Tubercles partially fused are observed (arrows). The wall is thickened near of the lesurae (arrowhead). Specimen MP 4229. **h.** Detail of equatorial view. The surface of tubercles (arrows) is microverrucae (circle). Specimen MP 4218. Bars: **a-g** = 10 μ m; **h** = 2 μ m.

The tubercles of *P. glandulosa* are 0.3-1.2 μm in diameter and distributed at random, separated by spaces of 0.04-1.5 μm . Occasionally these elements are partially fused forming groups of two or three (Fig. 5d).

Tubercles of *H. incisa* have a margin with a microrientation formed for bacula, echinae and verruca (Fig. 6e-f). There are channels filled with an electron-dense substance at the base of the microelements (Fig. 6f).

Perispore

The perispore is electron-denser than the exospore in all the studied species. In *Botiella lindeniana* the perispore is three layered. The inner perispore (iP) is 50-400nm thick, covers the pila of exospore and is formed by threads arranged in different positions (Fig. 4c, d-f). The medium layer (mP) is less electron-dense than iP, with variable thickness (100-700nm), and bears the bacula and echinae that constitute the sculpture. The structure of mP is alveolar, and sometimes has cavities filled with an electron-dense substance (Fig. 4c, d-f). Baculae have a verrucate margin and are fused (Fig. 4c). The outer perispore (oP) is 30-200nm thick and cover the mP surface; it is less electron-dense than mP and discontinuous (Fig. 4c, e-f). On the perispore surface threads of 100nm in diameter connect different baculae. These elements are part of oP (Fig. 4c).

Instead, the perispore of *Histiopteris incisa* and *Paesia glandulosa* is two layered. The inner perispore (iP) is thin, 50-150nm thick, with homogeneous structure and lining the exospore ornamentation in both species (Fig. 5d-e; Fig. 6 e-f).

The outer perispore of *Paesia glandulosa* (oP) is 0.1-4 μm thick and is part of the ridges. The structure is alveolar with cavities empty or filled with an electron-dense substance (Fig. 5d-e). In *Histiopteris incisa* the outer perispore (oP) is 80-400

nm thick, discontinuous, with low electron-density and verrucate margin (Fig. 6e-f). Occasionally, this layer is detached from inner perispore (iP).

Spheroids of 0.3-3 μm diameter into the perispore or on the surface are observed. They have a part similar in contrast to the exospore, and surrounded of a substance similar to that of the perispore (Fig. 4d; Fig. 5c; Fig. 6d).

DISCUSSION

Spores of *Botiella lindeniana*, *Paesia glandulosa* and *Histiopteris incisa* studied have three kinds of ornamentation: baculate, rugulate and tuberculate. Additionally, these taxa differ in the origin of the sculptural elements and those of the perispore stratification.

In *Botiella lindeniana*, the spore surface has bacula distributed randomly on the surface, and until now, only Murillo and Bless (1978) described this ornamentation. The present study considers that baculae are part of the perispore, but this conclusion differs from previous authors, who observed with LM and did not identified perispore (Nayar 1964, Devi 1977) or described remains of perispore without a defined ornamentation (Erdtman and Sorsa 1971). Although Welman (1970) with LM, Tryon and Tryon (1982) and Tryon and Lugardon (1991) with SEM indicated the presence of perispore, they described an echinate ornamentation. Even though some isolated echinae were observed in this work, the main ornamentation is formed of elliptical straight elements, with parallel sides and rounded tips, which are coincident with the definition of "bacula" (Punt et al. 1994, 2007, Lellinger 2002). Additionally, threads that connect bacula on the perispore surface were observed as did describe Tryon and Lugardon (1991). These threads did not present a differentiation of electron-density at substructural level unlike the threads found in other species of the family (Yañez et al. 2016b).

This is the first time that the sporoderm ultrastructure of *Blotiella lindeniana* was analyzed with TEM. The perispore is three layered: the inner layer is composed of threads; the middle layer is alveolar and constitutes the bacula; and the outer layer is thin and discontinuous.

Several authors (Hooker and Baker 1867, Smith 1875) included *Blotiella* species in *Lonchitis*. Later, Tryon (1962) separated them by the characteristics of the venation. Our results support the opinion of latter author, since the baculate monoete spores observed in *B. lindeniana* differ from the granulate trilete spores present in most species of *Lonchitis* (Tryon and Lugardon 1991).

The second type of sculpture is rugulate and was observed in *Paesia glandulosa*. The surface is covered of sinuous ridges, which can be fused forming sparsed luminae. Just like in *B. lindeniana*, the elements of ornamentation are constituted by perispore. In *P. glandulosa* the perispore is two layered: the inner layer is thin with homogeneous structure and covered the exospore irregular margin; and the outer layer is thick with alveolar structure that constitute the ridges of the sculpture. Similar layers were observed by Tryon and Lugardon (1991). Our interpretation about of *Paesia glandulosa* ornamentation is in coincidence with the conclusions of Large and Braggins (1991) and Tryon and Lugardon (1991). Our result differs from others authors, who described the perispore as scabrate or rugose (Harris 1955, Erdtman and Sorsa 1971, Tryon and Tryon 1982), which analyzed the material only with LM.

With SEM, few spores with sparsed ridges were observed. However, with TEM we observed that the margin of these ridges is more acute than with SEM. Further studies would be needed in order to understand if the sculpture could be associated with different stages of spore development.

The spores of *Histiopteris incisa* are tuberculated. Tubercles are fused forming reticules with muri and lumina of variable sizes in the equator

and distal face. In the proximal face, the sculpture is reduced or laevigate. These observations are in coincidence with the descriptions made by Nayar (1964), Nayar et al. (1968a, b), Huang (1981) and Lorscheitter et al. (2002). In Harris (1955), Welman (1970), Murillo and Bless (1978) and Faridah-Hanum et al. (2008), the ornamentation of spores was described as verrucose-rugulate, and this descriptions and pictures are quite similar to those shown in the present work.

Unlike to the other two species studied, tubercles are formed due to variations of the outer exospore thickness. These results are partially in coincidence with Erdtman and Sorsa (1971), Soepadmo and Khoo (1977) and Contreras-Duarte and Giovanni Bogotá (2006), who identified a tuberculate exospore without perispore with LM. Tryon and Tryon (1982) used SEM and defined in relation with the tubercles that “The countours appears to be formed by a lower layer of the perispore...”. In our opinion, the different interpretations about the sculpture were due to the difficult to distinguish the perispore from the exospore with LM and SEM.

We agree with the presence of thin perispore described by Tryon and Lugardon (1991) with TEM and Dai et al. (2005) with SEM. However, we identified a dark layer of perispore with homogeneous structure below that described by these authors to which we named “inner perispore”. The outer layer named “outer perispore” is discontinuous, with heterogeneous structure and verrucose margin. The last one detaches easily with any treatments, and these authors would not have observed it.

Although the elements of the sculpture are constituted of perispore in *Blotiella lindeniana* and *Paesia glandulosa*, the exospore is also ornamented. In *Blotiella lindeniana*, the margin of the exospore is covered by small pila, which can be seen only in TEM sections; whereas in SEM fractures we can see a granular deposit on the exospore surface.

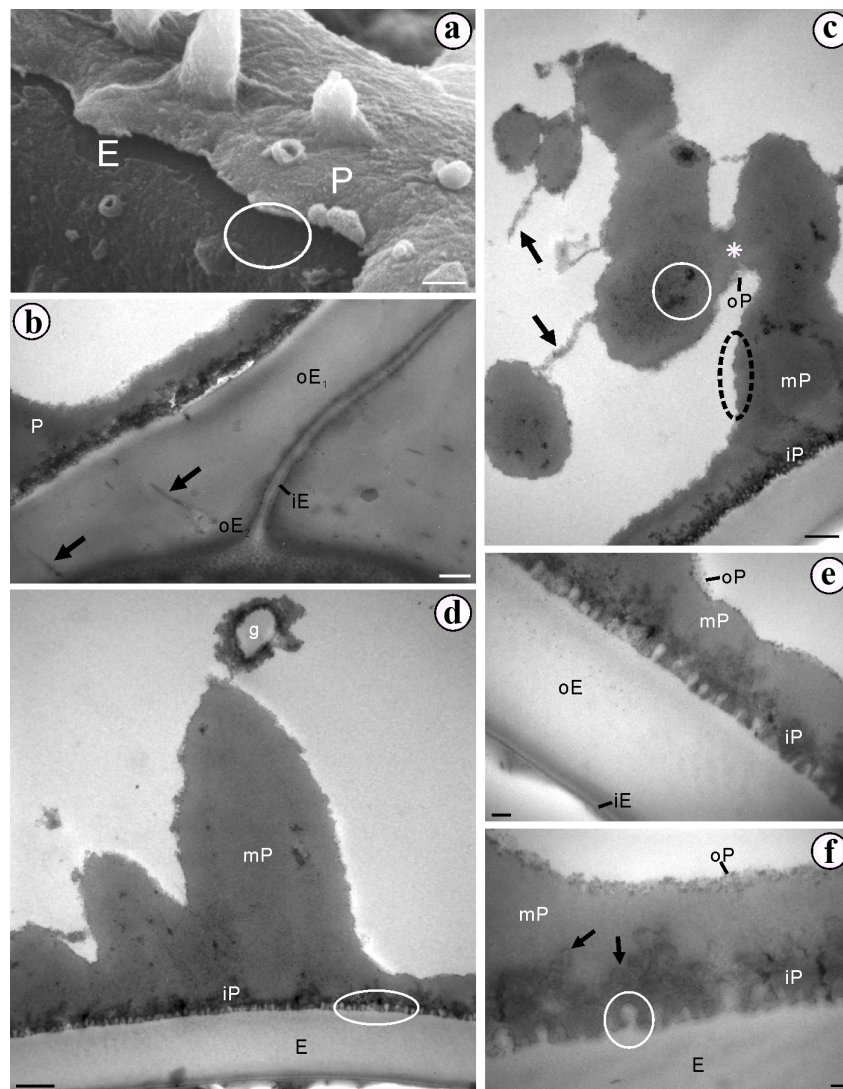


Figure 4 - Spores of *Blotiella lindeniana* with SEM and TEM. Baculate sculpture.

a. Fracture of wall surface with SEM. The exospore has a surface microranmented (circle). P = Perispore. **Figs. b-e.** Sections through the sporoderm with TEM. **b.** Section of the apertural fold. The inner exospore (iE) is thin and homogeneous, with lower electron-density. The inner exospore stratum (oE₂) has cavities and channels filled with an electron-dense substance, which are connected with the ramified channels (arrows) of outer stratum (oE₁). P = Perispore. **c.** The outer perispore is discontinuous (oP). The medium perispore (mP) is alveolar with cavities filled by electron-dense substance (continuous circle) and constitutes bacula of the sculpture. The bacula have verrucate margin (discontinuous ellipse) and are fused (asterisc). oP threads connect bacula between them (arrows). The inner perispore (iP) is more electron-dense than others layers. **d.** Globules are observed on the surface of the perispore (g). The margin of exospore is ornamented (ellipse). mP = medium perispore. iP = inner perispore. **e.** The inner exospore (iE), outer exospore (oE), inner perispore (iP), medium perispore (mP) outer perispore (oP) are observed. **f.** The inner perispore (iP) is formed by threads arranged in different positions (arrows). Exospore margin has densely arranged pila (circle). E = exospore. oP = outer perispore. Specimen MP 4227. Bars: **a** = 1 μ m; **b, c, d** = 0.5 μ m; **e** = 100 nm; **f** = 50 nm.

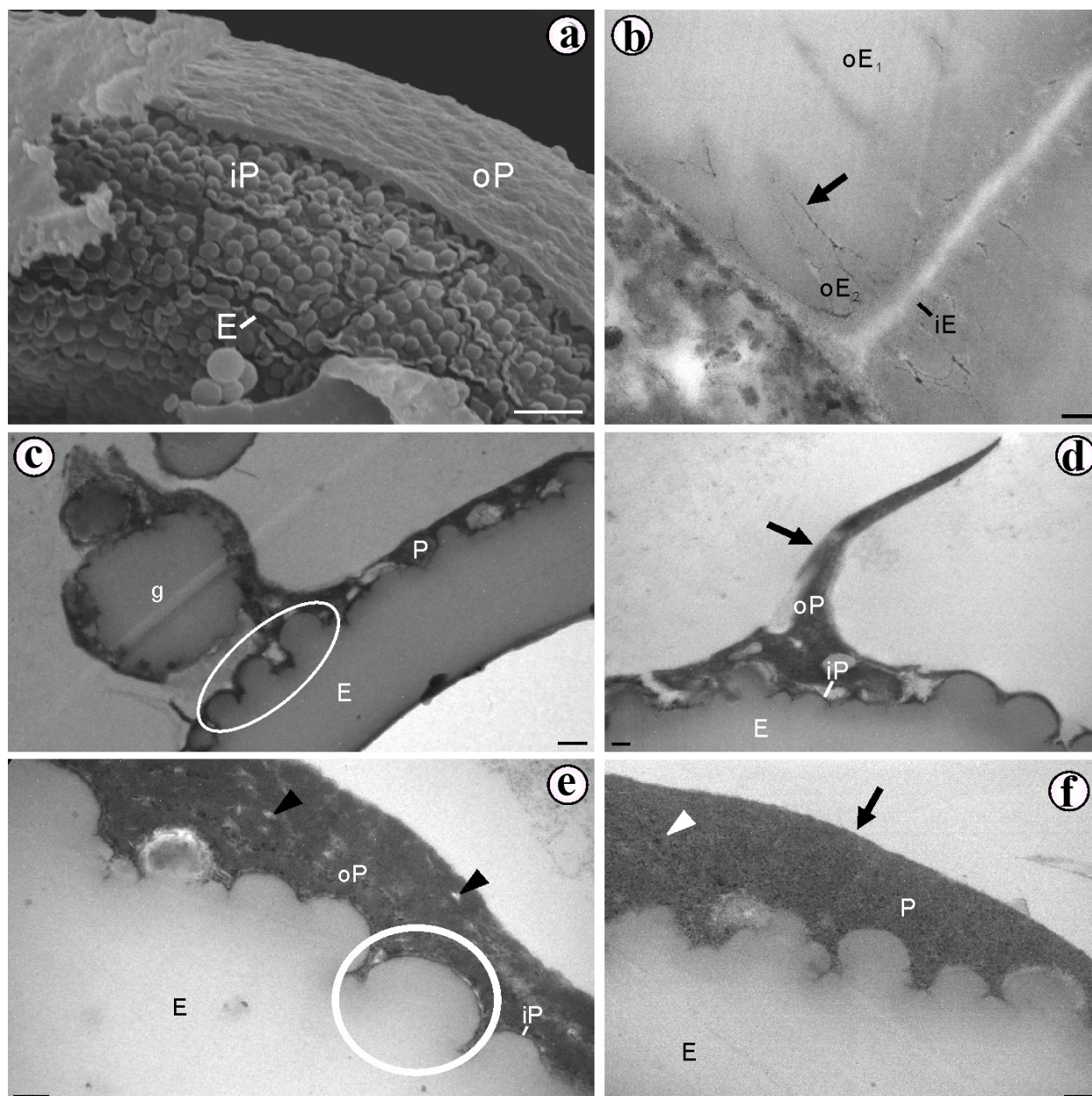


Figure 5 - Spore of *Paesia glandulosa* with SEM and TEM. Rugulate sculpture.

a. Sporoderm fracture with SEM. The exospore is tuberculate (E). The inner perispore (iP) is thin and lining the exospore ornamentation. oP = outer perispore. **Figs. b-e.** Sections through the sporoderm with TEM. **b.** The inner exospore is homogeneous structure and low electron-density (iE). The inner stratum of the outer exospore (oE₂) has ramified channels filled with an electron-dense substance. The channels are connected with the channels present in outer stratum (oE₁) (arrow). **c.** The margin of exospore (E) is ornamented (ellipse). Globules are observed on the surface of perispore (g). P = Perispore. **d.** The outer perispore (oP) is ornamented by high and sharp ridges like crest (arrow). The inner perispore (iP) is thin and high electron-density. E = Exospore. **e.** The outer perispore (oP) is alveolar structure with empty cavities (arrowheads). The inner perispore (iP) is continuous, homogeneous structure and lining the ornamentation of exospore. The exospore has tubercles (circles). **f.** The perispore (P) is ornamented by low and rounded ridges (arrow). The cavities of alveolar substructure are filled with an electron-dense substance (arrowhead). E = Exospore. Specimen MP 4226. Scale bars: **a** = 2 μ m; **b** = 0.1 μ m; **c** = 0.5 μ m; **d**, **e** and **f** = 0.2 μ m.

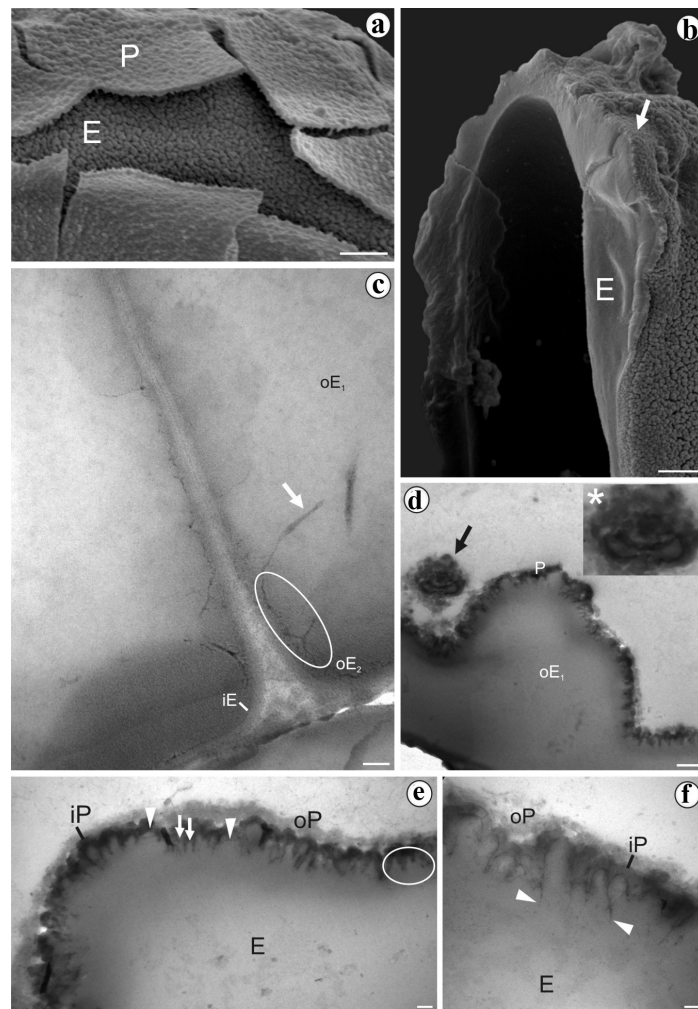


Figure 6 - Spores of *Histiopteris incisa* with SEM and TEM. Tuberculate-reticulate sculpture.

Figs. a-b. Sporoderm fracture with SEM. **a.** The perispore is thin (P). The surface of exospores is microverrucose (E). **b.** thickness variations of exospores (E) constitute tubercles of sculpture (arrow). **Figs. c-f.** Sections through the sporoderm with TEM **c.** Section at the apertural fold. The inner exospore (iE) is homogeneous structure and low electron-density. In the inner stratum of the outer exospore (oE₂) there are cavities and anatomizing channels (ellipse) filled with an electron-dense substance. In the outer exospore stratum (oE₁) there are channels that connect with oE₂ (arrow). **d.** Thickness variations of oE₁ exospore constitute tubercles. On the surface, globules are observed (arrow). * = Detail of globule. P = Perispore. **e.** Tubercles has margin with densely arranged bacula (arrows), echinae (circle) or verrucae (arrowheads). The inner perispore (iP) is thin, continuous, with homogeneous structure and lining the ornamentation of exospore. The outer exospore (oP) has microalveolar structure and microverrucose margin. **f.** Among the elements of the tubercles ornamentation, channels filled with an electron-dense substance were observed extending into the exospore (arrowheads). oP = outer perispore. iP = inner perispore. E = exospore. Specimen MP 4218. Scale bars: **a, b** and **e** = 2 μm ; **c** and **f** = 0.1 μm ; **d** = 0.5 μm .

This deposit is in coincidence with the granular layer identified by Tryon and Tryon (1982) under the elements of ornamentation, described as part of the perispore for these authors. It is important to mention that due to we had to expose the spores of this species much longer time to the treatment with ultrasound to achieve the fracture of the perispore (more than 20 minutes, when the habitual is no more than 10 minutes) we interpret that there is a high adhesion between the exospore and the perispore, apprehensively due to the zipper effect of the interface between the two walls.

The exospore of *P. glandulosa* is tuberculate. Tryon and Lugardon (1991) found spores of *Paesia lamiana* with tuberculate surface and explained that “these appear to lack outer perispore”. In the present work, spores with and without perispore were observed in the same sporangium and, although the detachment of perispore was indicated in the genus (Soepadmo and Khoo 1977), it could be related with differences in the spores maturation degree. Analysis of the spore development would allow understanding the different stages of the sporoderm deposition.

In all studied species, the exospore is blechnoid (Lugardon 1971, 1974) two layered. The outer layer has the “strata fissurée”, a network of channels and cavities near to inner exospore. Recent studies carried out in spores of *Dennstaedtia*, *Pteridium*, *Microlepia* and *Hypolepis* Bernh. (Giudice et al. 2006, Yañez et al. 2016a, b, A. Yañez, unpublished data) from South America have the same exospore type.

The phylogenetic affinity among *Blotiella*, *Histiopteris*, *Paesia* and *Hypolepis* genera was indicated as Hypolepidaceae (Ching 1940, Mehra 1961, Pichi Sermolli 1970), Hypolepidae (Christensen 1938), Pteridoideae (Holtum 1949) or Hypolepidoideae (Lovis 1977). Recent molecular studies (Hasebe et al. 1995, Wolf et al. 1994, Schuettpelz and Pryer 2007, Perrie et al. 2015) support this affinity and include them in a derived

position into monophyletic Dennstaedtiaceae clade “Hypolepidoide”. The presence of monolete spores and ornamented exospore in paranaense species of *Blotiella*, *Histiopteris*, *Paesia* and *Hypolepis* (A. Yañez, unpublished data) would support the relationships among these genera, which is broadly consistent with the conclusions of Nayar and Devi (1968b).

We suggest the derived character of the three genera in the evolutionary history of the family, in base of the presence of monolete spores (Wolf 1995) and a thicker and ornamented exospore (Wagner 1974). Also, the presence of a exospore with two overlapping patterns of sculpture, like it was observed in *Histiopteris incisa*, it was indicated by Wagner (1974) as one of the most specialized condition. In Dennstaedtiaceae phylogenies, *Histiopteris* and *Blotiella* are part of the more derived clade of “Hypolepidoide”.

Although some palynological characters can be used to establish relationships into the family, other fern families have similar sporoderm sculpture that species studied here. Thus, bacula formed by perispore observed in *Blotiella lindeniana*, were found in *Drynaria rigidula* (Sw.) Bedd. and *Drynaria quercifolia* (L.) J. Sm. (Polypodiaceae) (Tryon and Lugardon 1991), and fused tubercles on the exospore surface in *Histiopteris incisa*, were also registered in *Pteridoblechnum neglectum* (F. M. Bailey) Hennisman (Blechnaceae) (Hennisman 1966, Tryon and Lugardon 1991). Spores with similar sculpture characteristics in phylogenetically unrelated families suggests that some palynological characters would not have an evolutionary value as to establish relationships among groups above the genus level (Pozhidayev 1998).

Finally, the disperse spheroidal bodies observed with SEM and TEM on surface spores of the three species studied are similar to those described by Lugardon (1974) and Tryon and Lugardon (1991) in diverse species of ferns and lycophytes. These spheroids are characterized by a central structure

similar to the exospore, wrapped in a perispore like layer, and they were defined as “globules” by these authors. Furthermore, globules were also observed in other Dennstaedtiaceae species as *Dennstaedtia bipinnata* (Cav.) Maxon, *Dennstaedtia globulifera* (Poir.) Hieron., *Dennstaedtia dissecta* (Sw.) T. Moore, *Dennstaedtia cornuta* (Kaulf.) Mett., and *Pteridium arachnoideum* (Kaulf.) Maxon (Lugardon 1974, Giudice et al. 2006, Yañez et al. 2016a, b). This resembles structures found on the pollen grains of angiosperms known as Ubisch bodies (Morbelli 1977, Lugardon 1981).

CONCLUSIONS

The variability found in the spore sculpture and ultrastructure of perispore reflects the morphological differences observed in the sporophyte of the species studied. Three different kinds of sculpture in monolete spores of Dennstaedtiaceae were found.

- a. Baculate: bacula constituted by perispore. Perispore with three layers. *Blotiella lindeniana*.
- b. Rugulate: ridges constituted by perispore. Perispore with two layers. *Paesia glandulosa*
- c. Tuberculate-reticulate: tubercles constituted by exospore. Perispore with two layers. *Histiopteris incisa*.

The spores could be an important source of characteristics with systematic value into the family Dennstaedtiaceae. Regardless exospore ornamentation, the stratification and ultrastructure is common in Dennstaedtiaceae species studied from Paranaense Phytogeographic Province until now. In addition, in the clade “hypolepidoide”, species with monolete spores from Paranaense Province share an ornamented exospore.

Palynological studies at low magnifications can lead to different interpretations of the ornamentation (as described in *Blotiella lindeniana*) and stratification of the spore wall (as described in *Histiopteris incisa*). In addition, studies are needed

about the sporoderm to better understanding of the observed morphological diversity.

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APPENDIX

Blotiella lindeniana (Hook.) R. M. Tryon. Brazil, Minas Gerais, Simonésia, RPPN Mata do Sossego, 20/03/2009, A. Salino et al. 14240 (BHCB), MP 4227; Brazil, Santa Catarina, Limoeiro, Sta. Maria, 17/05/1946, A. C. Brade and Apariccio 18285 (NY).

Histiopteris incisa (Thunb.) J. Sm. Brazil, Minas Gerais, Lima Duarte, 26/08/1978, Krieger 15917 (CTES), MP 4229; Brazil, Rio Grande do Sul, São Leopoldo, 15/01/1933, Sehnem s.n. (SP), MP 4219; Brazil, Rio Grande do Sul, São Leopoldo, Dutra 288 (SI); Rio Grande do Sul, Potreiro Novo, 23/02/1978, Sehnem 15886 (PACA); Brazil, Rio de Janeiro, Guanabara, 01/06/1967, Brade 9670 (CTES); Brazil, Santa Catarina, Hammonia, 06/1913, Luederwaldt s.n. (SP); Brazil, Santa Catarina, Foinville, 1905, Rosentstock 124 (SI), MP 4218; Brazil, Santa

Catarina, Represa dos pilaes, Mun. de São José, 26/05/1955, Rohn 3022 (HB); Brazil, Santa Catarina, Campo dos Padres, Bom Retiro, 17/01/1957, Sehnem 6955 (PACA); Brazil, São Paulo, Estac. Alto da Serra, Luederwaldt s.n. (SP).

Paesia glandulosa (Sw.) Kuhn. Brazil, Minas Gerais, Catas Altas, Parque Natural do Caraça, Gruta Nossa Senhora de Lourdes, 11/11/2000, Salino 5813 (BHCB), MP 4226; Brazil, Minas Gerais, Ouro Preto, Itacolomy, 14/02/1884, Glaziou s.n. (NY).