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Palynological analysis of Dennstaedtiaceae taxa from the Paranaense Phytogeographic Province that produce Trilete spores II: *Microlepia speluncae* and *Pteridium arachnoideum*

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ABSTRACT

The spore morphology and wall ultrastructure of *Microlepia speluncae* and *Pteridium arachnoideum* from the Paranaense Province were analyzed with LM, SEM and TEM and a comparative analysis was carried out. In both species the spores are covered by a three-dimensional network of threads branched and fused, tangentially arranged to the surface, and some free-end threads are also seen. The species were differentiated by morphology and the frequency of threads fusion and the networks distribution on the surface of the spores. In both species the exospore is two-layered in section, both layers are traversed by single or branched channels. The perispore is three-layered in section: the inner layer is adhered to the exospore, the middle layer is formed of a three-dimensional network of threads and the outer layer is discontinuous. The perispore ultrastructure of *Microlepia speluncae* was interpreted as formed of helical subunits displayed around a central channel. The spore morphology and perispore ultrastructure allow differentiating *Microlepia* from *Pteridium* but also to the other Dennstaedtiaceae genera that grow in the Paranaense Province. The results obtained allow establishing relationships that let us recognize different groups and gave a new reference to get a better knowledge of the family.

Key words: Spores, ultrastructure, Dennstaedtiaceae, Paranaense Province, *Microlepia speluncae*, *Pteridium arachnoideum*.

INTRODUCTION

This paper is part of the palynological study for Dennstaedtiaceae family growing in the Paranaense Phytogeographic Province (Cabrera and Willink 1973), also known as Paraná Forest Province and Araucaria Forest Province (Morrone 2001a, b, 2014). Up to the present, have been published the results of *Dennstaedtia* genus (Yañez et al. 2011);

and genus *Blotiella*, *Histiopteris* and *Paesia* are currently under study.

Dennstaedtiaceae Bernh. family has about 170 species growing in pantropical, boreal or temperate regions (Smith et al. 2006). The genera assigned to the family, show wide morphological variability in the sporophyte and spores, as there are species producing spores monolete and the other trilete ones. *Microlepia* C. Presland *Pteridium* Gled ex Scop. are among the last ones, these being two broadly distributed taxa.

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Within the Paranaense Phytogeographic Province, *Microlepia speluncae* (L.) T. Moore is distributed mainly in Eastern Paraguay and SE Brazil, in the Brazilian states of Minas Gerais, Rio de Janeiro, São Paulo and Santa Catarina. *Pteridium arachnoideum* (Kaulf.) Maxon is also present in Paraná, Rio Grande do Sul, and NE Argentina (Ponce et al. 2008, Schwartzburd 2010, Anton and Zuloaga 2014).

Microlepia is a genus with 46 taxa, most of its species growing in tropical and warm-temperate Asian Pacific Ocean areas. Only one species, *Microlepia speluncae*, is present both in the Old World and in America, being broadly distributed and therefore, Tryon and Tryon (1982) suggested the taxon's adventitious nature and claimed that the species shows local variations. It has sorus with half cup-shaped indusium, basal and laterally attached to the lamina, slightly withdrawn from the margin (Kramer and Green 1990).

Pteridium is distinguished from *Microlepia* by having continuous marginal sorus, protected by an abaxial membranaceous indusium and by the recurved foliar margin. It is a cosmopolitan taxon, broadly distributed in tropical and temperate areas worldwide (Tryon and Tryon 1982). This distribution rank is due to the fact that most *Pteridium* species are considered weeds and colonize open spaces, generally as a consequence of anthropic activities, due to the fast growth of its extensive and resistant branched rhizomes (Den-Ouden 2000), as well as the great spore production, dispersing easily with the wind and germinating in short periods of time (Conway 1949, 1957). As a consequence of its broad distribution, poorly stable diagnostic features, population phenotype plasticity, and the existence of intermediate morphotype, there are several views on the taxa classification within *Pteridium*. While some authors describe it as a monotypic genus with several varieties (Tryon 1941, Cooper-Driver 1976, Van Cotthem 1970, Taylor 1990), others have acknowledged several species (Thomson

and Alonso-Amelot 2002, Thomson 2004, 2012, Thomson et al. 2005, 2008). In the present work we follow the classification scheme used by Der et al. (2009), based on phylogenetic and biogeographical studies. The authors acknowledge two species in South America: *Pteridium caudatum* (L.) Maxon and *Pteridium arachnoideum*, and only the latter is present in the Paranaense Province (Sehnem 1972, Mickel and Smith 2004, Prado 2004, Assis and Salino 2011).

Pteridium and *Microlepia* palynology have been scarcely studied and most of the available research is restricted to optical microscopy analysis. The first authors to analyze the spores *Microlepia* taxa with LM described their ornamentations as psilate, granular, tubercled, banded (Knox 1938, Erdtman 1957), verrucate (Tardieu-Blot 1966) or echinulate (Selling 1946). Furthermore, the last author observed granular sculptured spores in *Pteridium* while Harris (1955) distinguished a scabrated or irregularly subgranular exospore.

Later, Nayar and Devi (1968a, b) studied the spores of the Pteridaceae family *sensu* Copeland (1947) and analyzed *Pteridium aquilinum* and 13 *Microlepia* species. In the first case, the authors defined a granular exine with rugulated coalescent granules, and the presence of an occasionally cracking fine outer layer. In *Microlepia*, they observed a smooth or densely granular exospore coinciding with that described by Devi (1977), Soepadmo and Khoo (1977), and Welman (1970). This last author indicated the presence of a granular perispore both in *Pteridium aquilinum* as well as in *Microlepia speluncae*, while Erdtman and Sorsa (1971), and Large and Braggins (1991) only informed by *Pteridium*. On the contrary, Huang (1981) identified the presence of perispore in *Microlepia* which he described as striate, reticulate, granular, psilate or scabrata.

There are few studies with electronic microscopy in both genera. In *Pteridium aquilinum*, Lugardon (1971, 1974) observed a "blechnoide"

exospore type with attenuated ornamentation and a perispore with granular or lump-textured vertical expansions, without any defined shape. Later, Tryon and Tryon (1982) described the perispore of this species as granular with mass fused or short rod-like particles. The last authors described the *M. speluncae* perispore with thin rods or threads conforming an echinulate pattern and indicated the presence of a rod or thread grid under the perispore ornamented layer in both species. On the contrary, Tryon and Lugardon (1991) distinguished an inner granulated layer only for *Microlepidium*.

Up to the present *Microlepidium speluncae* spores have been scarcely studied for South America (Tryon and Tryon 1982, Tryon and Lugardon 1991, Graf-Meier 1985). Graf-Meier (1985) studied *Pteridium arachnoideum* spores (under *P. aquilinum*) with LM. Furthermore, South American species of *Pteridium* were studied with SEM in some local palynological flora (Lorscheitter et al. 2002, Contreras-Duarte et al. 2006, Giudice et al. 2006) as well as in morphologic studies on gametophyte (Ramírez-Trejo et al. 2013).

The purpose of this work is to make a comparative analysis of the *Microlepidium speluncae* and *Pteridium arachnoideum* spore morphology of the Paranaense Province with LM, SEM, and TEM, in order to determine the ornamentation types present in these taxa and to establishing their relation respect to with stratification and wall ultrastructure. The results obtained will allow to compare these species with other Dennstaedtiaceae taxa and to establish relationships that would allow to circumscribe the family better. Finally, the intention is to contribute with the analysis of the reproductive structures present in the *Pteridium* species in order to generate controlling tools to this species.

MATERIALS AND METHODS

Spores from herbarium specimens were obtained from Museo Argentino de Ciencias Naturales

Bernardino Rivadavia, Buenos Aires, Argentina (BA); Instituto de Botánica del Nordeste, Corrientes, Argentina (CTES); Herbarium Bradeanum, Rio de Janeiro, Brazil (HB); Museo de Ciencias Naturales de La Plata, La Plata, Argentina (LP); Missouri Botanical Garden. Missouri, Saint Louis, USA (MO); Instituto de Botanica Darwinion, San Isidro, Argentina (SI); Instituto de Botânica, São Paulo, Brazil (SP) and Universidade Federal do Paraná, Curitiba, Brazil (UPCB). As the material from the Phytogeographical Paranaense Province was not enough, additional material from the Atlantic Province was studied (Cabrera and Willink 1973).

The spores were studied by using Light Microscope (LM), Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). For LM the spores were embedded in glycerine-jelly without any chemical treatment, since the perispore does not resist acetolysis treatment (Erdtman 1960). On the basis of measurements (25 spores for each sample) we obtained values of polar diameter, mayor equatorial diameter, minor equatorial diameter, and exospore and perispore thickness. Photographs were taken with 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.

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

The spores were dehydrated in an acetone series and, then, embedded in Spurr soft mixture. 3 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 5 min. In *Pteridium arachnoideum*, some ultra-thin sections were stained with 1% uranyl acetate for 15 min, followed by lead citrate for 2.3 min.

The observations with LM were performed with an Olympus BH2 LM and those of the spore surface with a JEOL JSMT-100 SEM. The observations were made with a Zeiss M-10 TEM.

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

To morphological and ultrastructural descriptions we used the work of Kremp (1965), Nayar and Devi (1966), Tryon and Lugardon (1991), Punt et al. (1994, 2007) and Lellinger (2002).

STUDIED MATERIAL

Microlepiea speluncae

Brazil: Rio de Janeiro; Estrada de la Covonca, 30/09/1959, *De la Sota* 2298 (LP); Teresópolis, Parque Nacional da Serra dos Órgãos, 14/01/2008, *Labiak et al.* 4522 (UPCB). São Paulo, Tiete, 02/12/1906, Gerdes 94 (HB); Mun. Teodoro Sampaio, Pontal do Paranapanema, Parque Estadual Morro Do Diabo, 30/01/1995, *Silva* 1680 (MO); Santa Catarina, Hammonia, 07/1910, Luederwaldt s/n (SP). **Paraguay:** Guairá, Cerro de Villa Rica, 04/1924, *Rojas* 4878 (MO).

Pteridium arachnoideum

Argentina: Corrientes, Ituzaingó. Isla Apipé Grande, Panco cué, 05-Oct-1978, *Schinini & Vanni* 15792 (MO). Misiones, Dpto. 2 de Mayo, Colonia 2 de Mayo, 22-May-1951, *Cabrera et al.* 335 (LP); Dpto. San Pedro, ruta 14 entre San Pedro y Fracran, km 327, 16-Dic-1970, *De la Sota et al.* 6167 (LP). **Brazil:** Paraná, Mun. Tijucas do Sul, Capina 46 km S de Curitiba, 14-Feb-1978, *Krapovickas & Cristóbal* 33652 (LP); Mun. São Mateus do Sul,

BR-476, km 152, 26-Jan-1985, *Krapovickas & Cristóbal* 39667 (LP). Rio Grande do Sul, Rio Pardo, 1906, *Rosenstock* 267^a (SI). São Paulo, estrada entre São Paulo e São Roque, 15-July-1972, *Siegel* 25 (HB). **Paraguay:** Caazapá, Tavai, 2 km del destacamento, 21-Dic-1988, *Soria* 3020 (MO); Tavai, Cerro Tupasy, 29-Oct-1988, *Degen* 906 (MO).

RESULTS

The spores of *Microlepiea speluncae* (Fig. 1) and *Pteridium arachnoideum* (Fig. 2) from Paranaense Province are trilete, triangular to globose in polar view, with straight to concave or convex sides and rounded corners (Figs. 1a-b, 2a-b). In equatorial view the spores are plane-hemispheric, sometimes convex-subconic in *P. arachnoideum* (Figs. 1c, 2c).

The laesurae are straight extending 3/4 of the way to the equator, and generally are covered by ornamentation (Figs. 1d-e, 2d). With LM the exospore is light brown-yellowish and the perispore is brown. The morphological data of the spores of *M. speluncae* and *P. arachnoideum* are summarized in Table I.

Spore morphology

The spore surface bears a three-dimensional network of threads in both species. In *Microlepiea speluncae* the basal threads are cylindrical, circular in section, with smooth surface, uniformly distributed, and they are tangentially oriented to the spore surface (Fig. 1d-f). Some of these threads of about are 0.5-2.6 µm long and extend perpendicularly from the surface and may be fused at their tips to form spinules (Fig. 1g). Sometimes, a granular layer covers the threads background and only the tangential threads can be observed (Fig. 1h-i). In *Pteridium arachnoideum* the threads are shorter than those in *Microlepiea speluncae*, flattened, with sharp tips like spinules, and with a microverrucate surface (Fig. 2d-g).

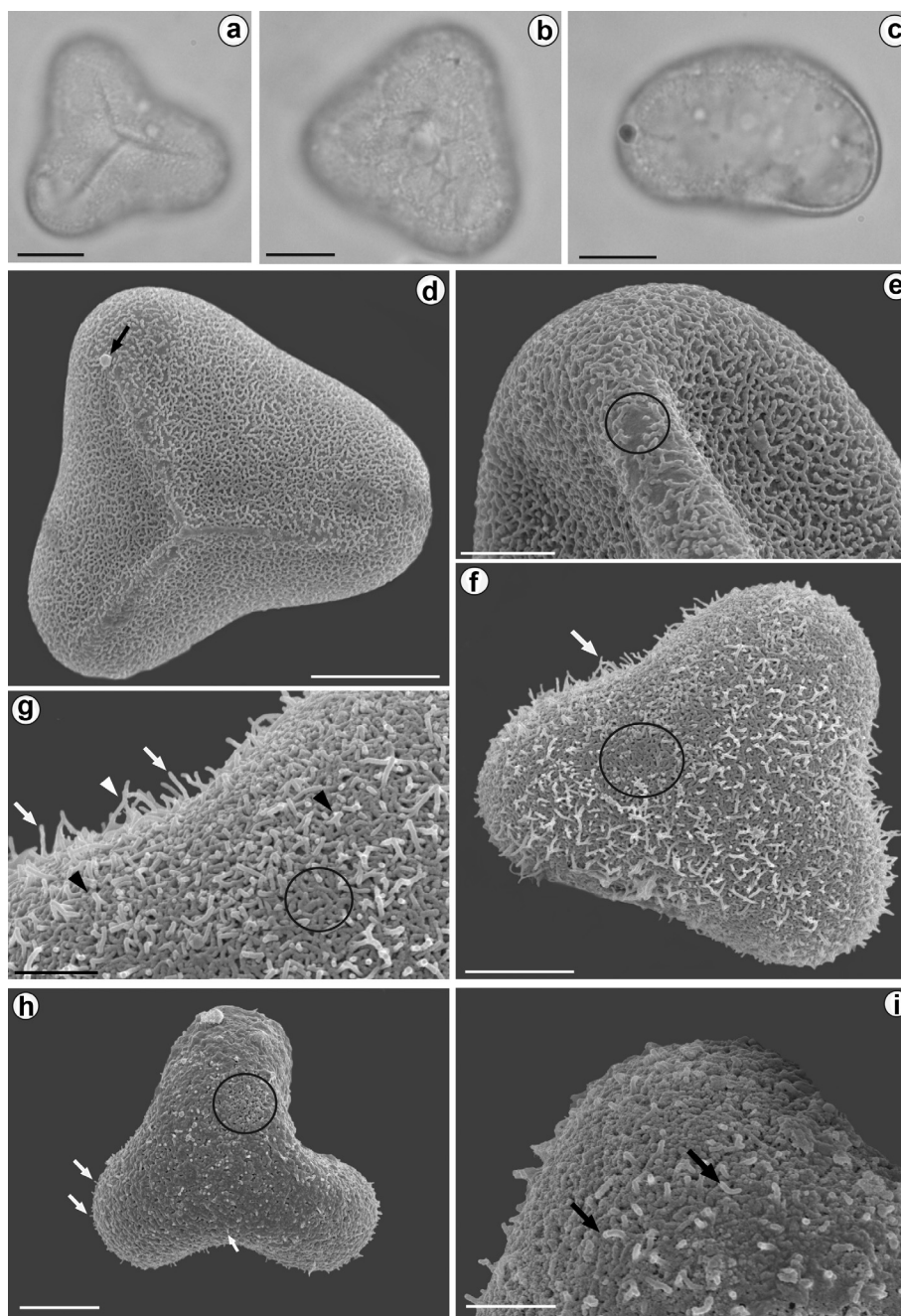


Figure 1 - Spores of *Microlepiea speluncae* with LM (a-c) and SEM (d-i). **a.** Spore in proximal view. Scale bar = 10 μm . **b.** Spore in distal view. Scale bar = 10 μm . **c.** Spore in equatorial view. Scale bar = 10 μm . **d.** Spore in proximal view. The spore surface bears a three-dimensional network of branching threads. Spheroids are observed (arrow). Scale bar = 10 μm . **e.** Detail of the proximal view. The laesurae are partially covered of threads. An irregular surface below the threads network is observed (circle). Scale bar = 6 μm . **f.** Spore in distal view. The ornamentation is formed of a three-dimensional network of fused and ramified thread (circle). Some threads, oriented perpendicular to the surface are evident, which are higher on the spore sides (arrows). Scale bar = 10 μm . **g.** Detail of the distal surface, formed of threads tangentially (circle) and perpendicular (arrow) oriented. Some of the last ones are fused at the top to form spinules (arrowhead). Scale bar = 6 μm . **h.** Spore in distal view. A granular layer covers the threads background, and only the perpendicular threads can be observed (arrow). Threads tangentially oriented to the spore surface are only visible where the granular layer is not present (circle). **i.** Detail of the distal surface. The tips of the threads tangentially oriented are evident (arrow). Scale bar = 5 μm .

There are variations in the fusion degree of the threads and in the complexity of the network, so, occasionally, some masses of threads can be observed. The variation in the perispore thickness is the result of the heterogeneous threads distribution

(Fig. 2d-f). Spheroids are also seen distributed on the spore surface (Figs. 1d, 2g).

An irregular surface below the three-dimensional network of threads is observed in both species (Fig. 1e).

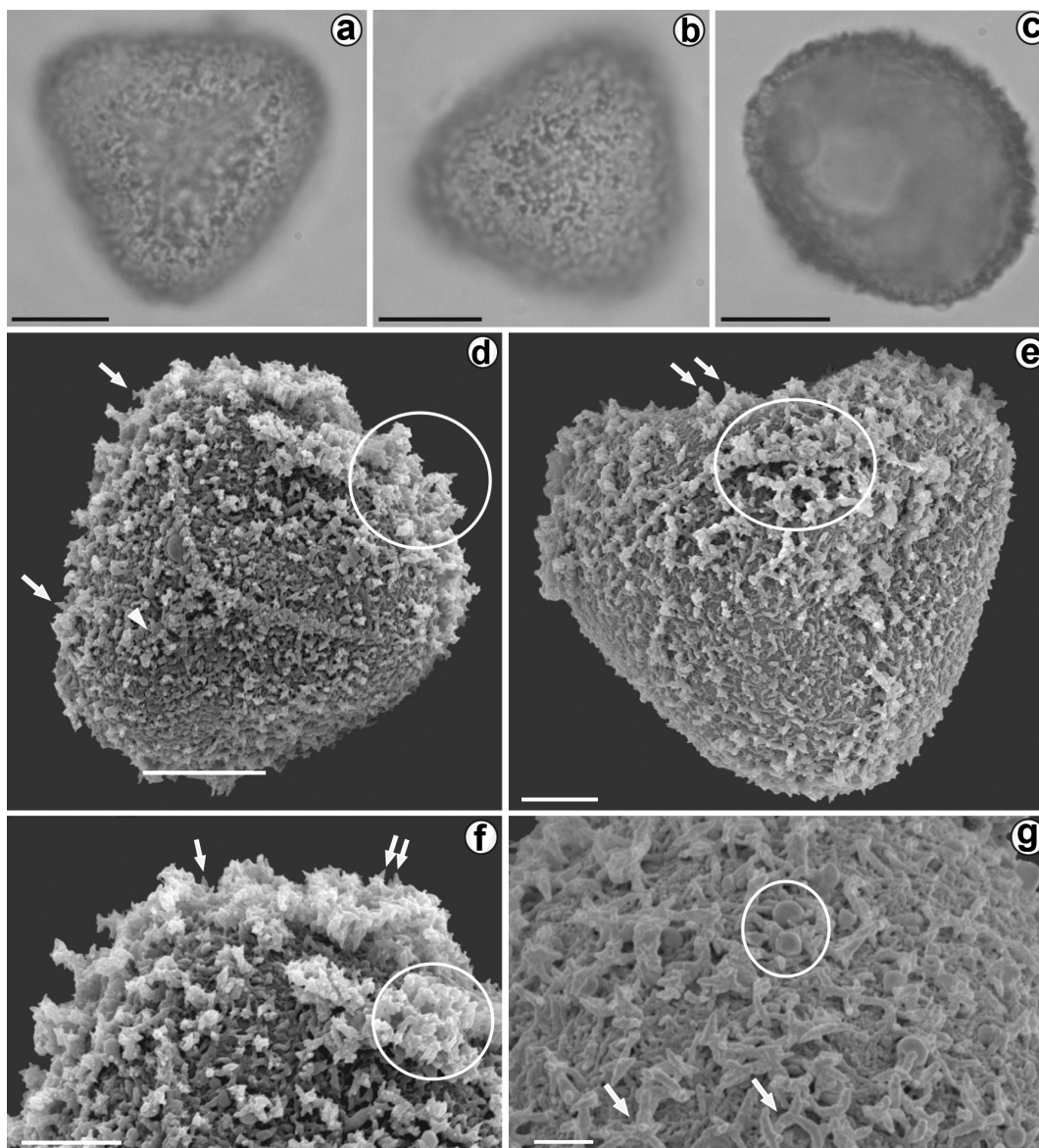


Figure 2 - Spores of *Pteridium arachnoideum* with LM (a-c) and SEM (d-g). **a.** Spore in proximal view. Scale bar = 10 μ m. **b.** Spore in distal view. Scale bar = 10 μ m. **c.** Spore in equatorial view. Scale bar = 10 μ m. **d.** Spore in proximal view. Short threads with spinulate tips (arrow) are fused to form agglomerations (circle). The ornamentation covers the laesura (arrowhead). Scale bar = 10 μ m. **e.** Spore in distal view. The threads (arrows) are distributed at random on the spore surface and areas with big agglomerations of them are observed (circle). Scale bar = 5 μ m. **f.** Surface detail in proximal view. Fused threads (arrow) form agglomerations (circle). Scale bar = 5 μ m. **g.** Detail of the surface in distal view. The threads are ramified and fused to form a massive network (arrow). Dispersed spheroids are observed (circle) Scale bar = 2 μ m.

TABLE I
Characteristics of *Microlepidia speluncae* and *Pteridium arachnoideum* spores.

		<i>Microlepidia speluncae</i>	<i>Pteridium arachnoideum</i>
Shape		Tetrahedral	Tetrahedral
Laesura		Trilete 9-14 μm ($\frac{3}{4}$ of ratio)	Trilete 7-14 μm . ($\frac{3}{4}$ of ratio).
PS		Triangular. Rounded corners, straight to concave sides.	Triangular to globose. Rounded corners, straight to convex sides.
ES		Proximal face, plane Distal face, hemispheric	Proximal face, plane to convex Distal face, hemispheric to subconic
PD		22 (36) 31 μm	22 (28) 39 μm
DE >		29 (35) 45 μm	27 (36) 46 μm
DE <		27 (34) 39 μm	22 (34) 44 μm
Exospore	Color (LM)	Light brown-yellowish	Light brown-yellowish
	Wall thickness	0.7-1.5 μm	0.3-0.6 μm
	Color (LM)	Brown	Brown
	Wall thickness	0.1-3 μm	0.2-2.7 μm
Perispore	Ultrastructure	Substructural elements: Threads. Open three-dimensional network	Substructural elements: Threads. Massive three-dimensional network
	Ornamentation	Spinulate Background reticulate	Spinulate Background reticulate

PS: Shape in polar view; ES: Shape in equatorial view; PD: polar diameter; DE: Equatorial diameter; >: Mayor; <: Minor.

Sporoderm ultrastructure

The exospore of *Microlepidia speluncae* and *Pteridium arachnoideum* is two-layered in TEM sections. The inner layer (iE) is thin, with low electron-density and a homogeneous structure (Figs. 3a, 4a-b). The outer layer (oE) is thicker than iE and it is formed of two strata: the inner stratum (oE₂) with short channels, fissures and cavities filled with an electron-dense material; and the outer stratum (oE₁), thicker than oE₂, with margin and a homogeneous structure (Figs. 3a-b, d, 4a-b).

The oE₂ channels are connected with those present in oE₁ (Figs. 3d, 4b). Occasionally, the oE₁ channels are ramified and continuous with those of the perispore (Fig. 4a-b).

The perispore has a higher electron-density than that of the exospore with TEM, and three layers can be seen in both species. The inner layer (iP) is 20-80 nm thick, with irregular margin, homogeneous structure, and it is adhered to the exospore (Figs. 3a, d, 4c, d). The middle layer (mP) is 0.2-2.6 μm thick and it is constituted of a three-

dimensional network of threads. In *M. speluncae* the threads are 0.2-0.3 μm thick in section and are spaced apart, forming an open network. Threads can be fused at the tip (Fig. 3e-f) or through cross elements (Fig. 3g), and the core and the periphery of them are more electron-dense than those in the middle area (Fig. 3d, g).

In *P. arachnoideum* the threads of mP have a uniform electron-density and are close arranged forming a massive network (Fig. 4c-d). Cavities filled with an electron-dense material are observed among the threads of the network (Fig. 4d).

The outer layer (oP) covers the mP threads and it is discontinuous, with low electron-density and a homogeneous structure (Figs. 3e, a, 4c, d).

Two kinds of spheroids of 0.2-1 μm diameter are observed within the perispore. In *M. speluncae*, the spheroids have the same structure and electron-density than that of the perispore (Fig. 3a). However, the *P. arachnoideum* spheroids have a central core like the exospore, and they are covered by a substance similar to that of the perispore (Fig. 4f).

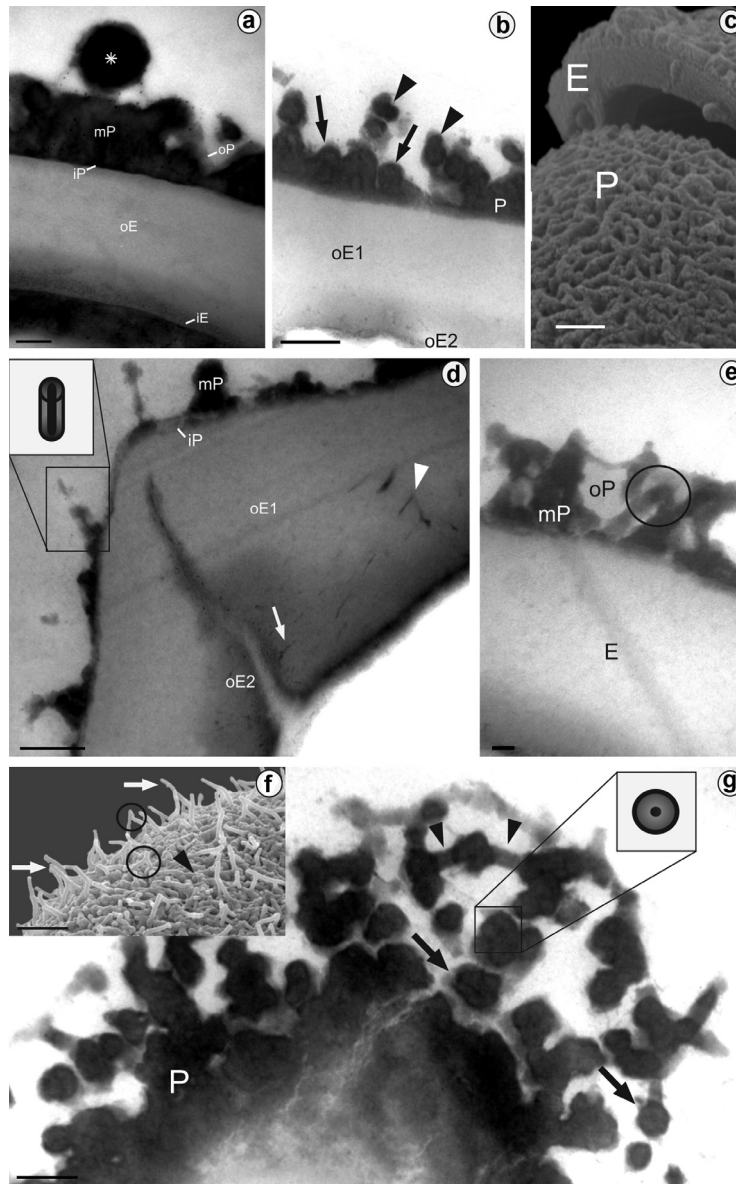


Figure 3 - Spores of *Microlepieia speluncae* with TEM (a-b, d-e, g) and SEM (c, f). **a.** In this picture, the inner exospore (iE) is thin, with a homogeneous structure and low electron-density. Spheroids with the same ultrastructure and electron-density than perispore are observed (asterisk). Outer exospore (oE), inner perispore (iP), medium perispore (mP), outer perispore (oP). Scale bar = 0.4 μ m. **b.** Section through the sporoderm, where: (oE₂) shows short channels, fissures and cavities filled with an electron-dense material; and (oE₁) is thicker than the inner one, with irregular outer margin and homogeneous structure. The perispore (P) is formed of threads tangential (arrows) and perpendicular oriented (arrowheads). Scale bar = 0.4 μ m. **c.** Sporoderm fracture with SEM. The Perispore (P) threads cover the exospore (E). Scale bar = 1 μ m. **d.** Section through the lesurae. In the inner stratum fissures and cavities filled with an electron-dense material (arrow) are present (oE₂). The outer stratum (oE₁) is thicker than oE₂, with an irregular margin, homogeneous structure, and has channels (arrowhead). The inner perispore (iP) is thin; the middle perispore (mP) has threads with a central core and peripheral area more electron-denser than middle area (scheme). Scale bar = 0.4 μ m. **e.** The medium layer (mP) forms a three-dimensional network of threads. The outer layer (oP), is discontinuous; low electron-dense and shows an homogeneous structure. Threads can be fused at the tip (circle). Exospore (E). Scale bar = 0.1 μ m. **f.** The threads of the ornamentation are fused and form a tangential network (arrowhead), with some perpendicular elements (arrows). The perpendicular threads could fuse at the top forming spinules (circles) Scale bar = 2 μ m. **g.** Section through the sporoderm at a spore corner. The threads (arrow) have a central core and a periphery more electron-denser than the medium area (scheme). Some threads are fused (arrowhead). Scale bar = 0.4 μ m. P = Perispore.

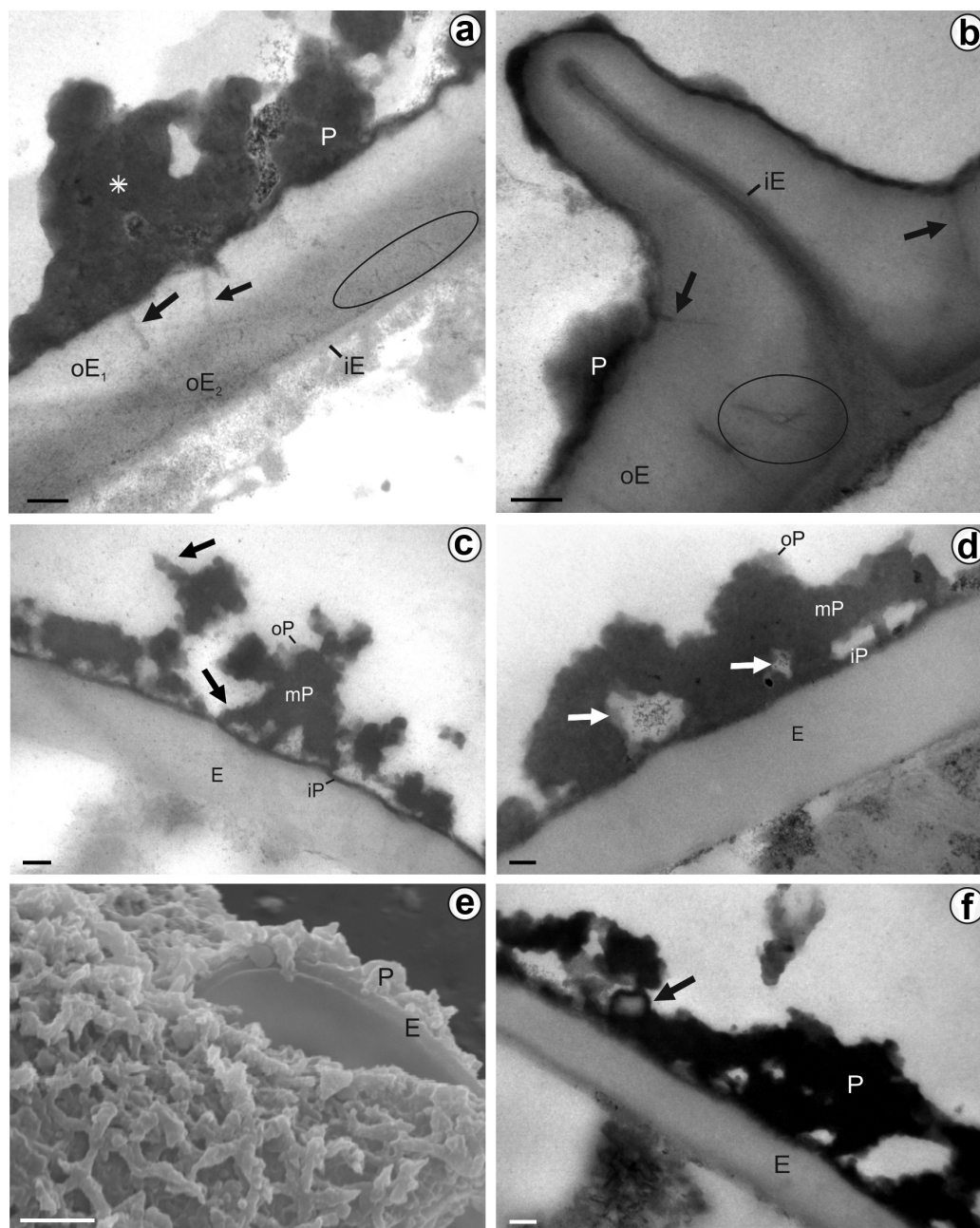


Figure 4 - Spores of *Pteridium arachnoideum* with SEM (**e**) and TEM (**a-d, f**). **a**. The threads of perispore are fused in massive networks (asterisk). The inner exospore (iE) is thin, with homogeneous structure. The inner stratum of the outer exospore (oE₂) has fissures and short, ramified channels (circle). The channels (arrows) of the outer stratum (oE₁) are continuous with the perispore (P). 2.3 min in Pb-c. Scale bar = 0.2 μ m. **b**. Section through the lesurae. The inner exospore (iE) can be seen at the lesura base. The channels of the outer exospore are ramified (circle) and show continuity with that of the perispore (arrow). Perispore (P). 5 min in Pb-c. Scale bar = 0.1 μ m. oE = outer exospore. **c**. The inner perispore (iP) is homogeneous and it is attached to the exospore. The middle perispore (mP) is formed of threads. The threads (arrow) have verrucose margin, narrow and fused tips. The outer perispore (oP) is discontinuous, with low electron-density. Exospore (E). 2.3 min in Pb-c. Scale bar = 0.5 μ m. **d**. Cavities filled with electron-dense material are observed among threads in the network (arrow). Inner perispore (iP); middle perispore (mP); outer perispore (oP); exospore (E). 2.3 min in Pb-c. Scale bar = 0.1 μ m. **e**. Wall fracture with SEM. Threads of perispore (P) cover the exospore (E). Scale bar = 0.2 μ m. **f**. Spheroids are observed in the perispore (arrow). They have a central core like exospore, which is covered by a substance similar to that of the perispore. Perispore (P). Exospore (E). 5 min in Pb-c. Scale bar = 0.2 μ m.

DISCUSSION

Microlepidia spelunca and *Pteridium arachnoideum* spores are covered by a three-dimensional network of branched and fused threads, tangentially arranged to the surface, and some free-end threads, frequently spinule-like, are seen. According to our interpretation the threads are observed under the LM as small spheroidal processes. For the reasons given above, we understood that the spores of both species could be interpreted by previous authors as granular (Selling 1946, Nayar and Devi 1968a, Welman 1970, Lugardon 1971, Soepadmo and Khoo 1977, Devi 1977, Lorscheitter et al. 2002, Esteves and Coelho 2007), fossulate or foveolate (Erdtman and Sorsa 1971), scabrate (Harris 1955, Belling and Heusser 1975, Contreras-Duarte et al. 2006), verrucate (Tardieu-Blot 1966); psilate (Welman 1970, Kremp and Kawasaki 1972). Only Large and Braggins (1991) described granules formed of threads, similar to that observed in this work.

In this sense, the use of scanning electronic microscopy was essential to achieve a better understanding of spore three-dimensional structures.

Although the studied species have the same morphology, ornamentation and fusion degree from the threads and the networks distribution on the surface of the spores varies in both cases. In *Microlepidia spelunca* the threads are cylindrical, circular in section, with smooth surface. These threads are usually fused at tips, they are spaced by wide distances defining an open three-dimensional networks. Additionally, the networks complexity is uniform across the surface of the spores. In contrast, in *Pteridium arachnoideum* the network complexity is highly variable and is related to fusion degree reached by the threads. The threads are separated by narrow spaces and can merge completely to form massive networks or masses. In this species the threads are short, flatt, with sharp tips like spinules and have a microverrucate surface.

Regarding the sporoderm stratification, our observations agree in part with those obtained by Tryon and Tryon (1982) for *M. spelunca* but those authors made different interpretation about perispore layers structure. These authors describe with SEM an inner layer formed of strands, a middle granular layer and an outer layer bearing spinules on the surface. Based in TEM observations, we believe that the perispore inner layer is homogeneous with an irregular margin, the middle layer is formed of threads, and the outer layer is discontinuous and not very apparent. Respect to the middle granular layer described by Tryon and Tryon is for us the granular layer that covers the background of threads. This layer was not identified under TEM analysis and therefore, was not defined by us as forming part of the sporoderm, but could be associated with tapetal remains or material deposited during processing.

Regarding the *P. arachnoideum* sporoderm ultrastructure, Tryon and Tryon (1982) described a granulate perispore with a fused mass or rodlets, in agreement with Lugardon (1971, 1974) and Tryon and Lugardon (1991) observations. In our interpretation, the granular processes covering the spores of this species would be the result of the threads fusion.

Respect to the existence of several opinions about the structure and stratification of the spore wall, may be due to the fact that, for a same specimen, there are spores in different stages of development.

The perispore formed by threads (described as “rodlets”) was also observed in other fern groups such as Matoniaceae, Vittariaceae, Lindsaeaceae and Pteridaceae (Tryon and Lugardon 1991). Furthermore, in genus *Syngamma*, *Ceratopteris* (Tryon and Lugardon 1991), *Cheilanthes* (Morbelli and Michelena 1989) and *Cyathea* (Marquez et al. 2010), the threads form three-dimensional networks.

In all of the above mentioned groups, the threads have a low electron-density core. This

characteristic differ from the substructure of those described in this work for *M. speluncae*. A cross-section view of threads for this species allow to observe the margins and very electron-dense centers, reminiscent of the ones present in some *Dicksonia* species (Tryon and Lugardon 1991). The substructure of the threads observed in *M. speluncae* shows similarities with that mentioned by several authors for lycophytes (Rowley 1995, Wittborn et al. 1998), ferns (Marquez and Morbelli 2015) and angiosperms (Rowley 1981, 1987, Blackmore 1990). In all of these cases, the authors described them as formed by helical subunits displayed around an axis or central channel. Furthermore, Rowley et al. (2003) suggested that the central channel would take part in the transfer of nutrients from the tapetum to the spore cytoplasm during the stages of development.

On the other hand, in *P. arachnoideum* the threads do not show any substructural difference of electron-density like *M. speluncae* and are similar to the coiled rods or coiled threads described both in lycophytes (Morbelli 1995) as well as in ferns (Marquez et al. 2009). In this sense, the microverrucae observed on *P. arachnoideum* threads could be interpreted as spiral loops. Even though in this work no presence of a central channel in threads of this species was found, new studies based on fresh material and other fixations are needed for confirmation. Additionally, in our experience, the perispore threads in *P. arachnoideum* were better observed in ultra-thin sections that were submerged in lead citrate for 2.3 min as compared to ultra-thin sections submerged for 5 min. Similarly, Rowley (1987) concluded that the intensity of staining and the complexity of images of the sporoderm processes were inversely proportional to duration of the sections immersion in lead citrate.

Even though the presence of threads in phylogenetically distant groups shows the limited value of this feature to differentiate families, perispore ornamentation allows to differentiate

the studied species from other genus of the same family present in the area, which have verrucate spores in *Dennstaedtia globulifera*, *D. cicutaria*, *D. obtusifolia* and *D. cornuta* (Yañez et al. 2011), baculate spores in *Blotiella lindeniana*, regulate spores in *Paesia glandulosa*, psilate in *Histiopteris incisa* or cristate-echinulate spores in *Hypolepis stolonifera* *H. repens* and *H. rugosula* (Yañez et al. 2013).

Furthermore, based on morphology, substructure and threads organization we are able to differentiate *Pteridium* and *Microlepia* species from Paranaense Province. In this sense, perispore ultrastructure and ornamentation are useful to characterize genus or subgeneric groups within the same family, a conclusion made also by Tryon (1986), Korall and Taylor (2006), Moran et al. (2007) and Triana-Moreno (2012).

Regarding the exospore, the features observed for the studied species adjust to “the blechnoide type” described by Lugardon (1971, 1974). The channels and cavities network of the innermost stratum observed in this work match the “strata fissurée” described by the French author. The other species of Dennstaedtiaceae from study area also have this type of exospore. However, in *Blotiella*, *Paesia*, *Histiopteris* and *Hypolepis* the exospore margin is ornamented by clavae, tubercle, spines or verrucae.

The disperse spheroidal bodies observed by SEM on spores of both species are similar to those described by Giudice et al. (2006) and Yañez et al. (2011) in *Dennstaedtia*, for the same family. We observed the spheroids with TEM and they have a central structure similar to that of the exospore, wrapped in a perispore-like layer in *P. arachnoideum*, and they were similar to those defined as globules (Lugardon 1974, Tryon and Lugardon 1991). On the contrary, spheroids found in *M. speluncae* do not show electron-density variation and appear to have a substance similar to that of the perispore, whereby we concluded

that they are spherules (Lugardon 1974, Tryon and Lugardon 1991).

CONCLUSIONS

In *Microlepidia aspeluncae* and *Pteridium arachnoideum*, the use of electronic microscopy is necessary in order to achieve a better interpretation of spores morphology due to the threads features conforming their ornamentation.

The ultrastructure observed in the spores of *Microlepidia speluncae* perispore could be due to the helical subunits displayed around an axis or central channel as proposed by Rowley (1981).

The perispore morphology and ultrastructure would allow to differentiate not only *Microlepidia* from *Pteridium* but also the other Dennstaedtiaceae genera of the Paranaense Phytogeographic Province.

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RESUMO

A morfologia dos esporos e a ultraestrutura da parede do *Microlepidia speluncae* e *Pteridium arachnoideum* da Província Paranaense foram analisadas com LM, SEM e TEM e uma análise comparativa foi realizada. Em ambas as espécies, os esporos são cobertos por uma rede tridimensional de cordões ramificados e fundidos, dispostos tangencialmente à superfície, e alguns fios com extremidades livres também são vistos. As espécies foram diferenciadas pela morfologia e a frequência de fusão de fios e redes de distribuição sobre a superfície dos esporos. Em ambas as espécies a exospore é de duas

camadas na seção, ambas as camadas são atravessadas por canais únicos ou ramificados. O perispore é de três camadas na seção: a camada interna é aderida à exospore, a camada do meio é formada por uma rede tridimensional de cordões e a camada exterior é descontínua. A ultraestrutura do perispore de *Microlepidia speluncae* foi interpretada como formada por subunidades helicoidais exibidas em torno de um canal central. A morfologia dos esporos e ultraestrutura do perispore permitem diferenciar *Microlepidia* de *Pteridium*, mas também para os outros gêneros Dennstaedtiaceae que crescem na Província Paranaense. Os resultados obtidos permitem estabelecer relações que nos permitem reconhecer diferentes grupos e nos deu uma nova referência para obter um melhor conhecimento da família.

Palavras-chave: Esporos, ultraestrutura, Dennstaedtiaceae, Província Paranaense, *Microlepidia speluncae*, *Pteridium arachnoideum*.

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