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Palynological analysis of *Sphaeropteris gardneri* (Cyatheaceae, Pteridophyta)

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ABSTRACT

The spore morphology and wall ultrastructure of *Sphaeropteris gardneri* (Hook.) R.M. Tryon from Brazil were analyz with LM, SEM and TEM. The spores are trilete with an ornamentation formed of short low ridges with spines in th margins. The exospore is 2.5μm thick, two- layered in section and single or branched channels are present. T perispore is 1.2μm thick and two-layered. The inner layer has three strata: the inner stratum is formed of a netwo of branched and fused threads, the middle stratum has threads with a radial orientation and in the outer stratum th dark fibres are immersed in a less dense contrasted matrix. The outer layer of the perispore is the one that forms t echinate-ridges and is constituted of threads arranged in a compact way. Globules of different sizes are observed on t surface. The differences found in the perispore ornamentation and ultrastructure in *Alsophila*, which was previous studied, and those of *Sphaeropteris*, show a tendency to wall complexity.

Key words: *Sphaeropteris*, spores, morphology, ultrastructure.

INTRODUCTION

The *Sphaeropteris* genus includes about 120 species (Tryon and Tryon 1982), most of them are distributed in Asia and Oceania. Six species (Tryon 1971, Windisch 1977) were reported in America: *S. Brunei* (Christ) Tryon, *S. cuatrecasasii* Tryon, *S. Gardneri* (Hook.) Tryon, *S. horrida* (Liebm.) Tryon, *S. Insignis* (D.C. Eaton) Tryon and *S. quinduiensis* (Karst.) Tryon. The distinctive characteristics to differentiate this genus from the rest of the Cyatheaceae is the presence of conform scales at the bases of the petioles (Tryon 1970) and spores with echinate perispore (Korall et al. 2007).

The former classification of the Cyatheaceae was proposed by Holttum (1963). This author takes the kind of scale at the base of the petioles as a diagnostic charac- the species that have settiferous scales. Tryon built later a new classification of the Cyatheace proposed the *Sphaeropteris* genus to gather the with conform scales (= settiferous) at the petiole

In 1987, Lellinger differentiated four ge the Cyatheaceae: *Cyathea*, *Alsophila*, *Cnemida Sphaeropteris*. Within *Sphaeropteris*, the author ed the species of the Old World, and the six spec constitute the "neotropical group *S. horrida*".

Recent works about phylogeny of the Cyath including morphologic, molecular and paleon data, have supported Lellinger criteria regarding gin of *Sphaeropteris* genus (Conant et al. 1994 1996, Korall et al. 2006, 2007). In these works, et al. (1996) and Korall et al. (2007) used the spo

of this genus in Southern South America. It is characterized by having sphaeropteroids indusia, the abaxial surface of the costulae covered with abundant simple or ramified trichomes, and small scales and scamules on the abaxial surfaces of pinnae (Tryon 1971, Fernandes 1997). Its distribution area comprises Rio de Janeiro, Minas Gerais, São Paulo and Santa Catarina States.

Regarding the palynological antecedents on the genus, Erdtman and Sorsa (1971) described with LM *Sphaeropteris cooperi* (under *Cyathea*) spores. Gastony and Tryon (1976) analyzed *Sphaeropteris* spores with SEM and noticed a significant variability in their ornamentation.

In 1976, Liew and Wuang worked with SEM on Cyatheaceae spores from Taiwan and gave a key for species determination, taking into account the palynological characteristics. The authors characterized the spores of *Sphaeropteris* and those of some *Alsophila* species as echinulate and established relationships based on the spore morphology.

Tryon and Tryon (1982) made a distinction of the species of *Sphaeropteris* from America and considered three spore types based on the perispore characteristics. Based on observations with LM and SEM, Braggins and Large (1990) described the *Sphaeropteris medullaris* spores (under *Cyathea medullaris*) as having narrow, rare or absent proximally and abundant distally echinae.

The works of Lugardon (1971, 1974) were the first to describe the wall ultrastructure in these spores. This author illustrated with TEM the *Sphaeropteris cooperi* spores, native species from Australia, and *S. medullaris*, from New Zealand and Fiji. The author described a blechnoide exospore and a two-layered perispore, with a deep layer composed of three strata. In 1991, Tryon and Lugardon described and illustrated, with scanning and transmission electron microscopes, the spores of the Cyatheaceae. The spores of *Sphaeropteris* subgenus *Sphaeropteris* were characterized by having echinae on low ridges and with TEM, showed a complex three-layered perispore.

The aim of this work is to analyze the spore morphology and wall ultrastructure in *Sphaeropteris gardneri* from the study area. In this way we hope to complete the knowledge on this species, contribute to works about conservation in the area and give new data to phylogenetic studies that other authors have carried out.

This work is included in the Project about spore morphology and ultrastructure of the Cyatheaceae that grows in Southern South America. Previous contributions related to this project, regarding the spore analysis in the *Alsophila genus*, were those of (Marquez et al. 2005, 2006, 2007, 2009).

METHODOLOGY

Spores were obtained from herbarium specimens from the Instituto Anchietano de Pesquisas (PACA). Spores of different specimens were studied by using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For LM, untreated spores were studied.

On the basis of LM observations and measurements (25 spores for each sample), values of polar diameter, equatorial diameter, exospore and perispore thickness were obtained.

For SEM, the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and, then, transferred to acetate plates. After drying, they were coated with gold.

All the observations were performed with an Olympus BH2 light microscope and a JEOL JSMT-100 SEM.

For studies with TEM, dry material from herbarium specimens was hydrated following the technique proposed by Rowley and Nilsson (1972) that consists 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. 3μm 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 3 min. The observations were made with a Zeiss M-10 TEM.

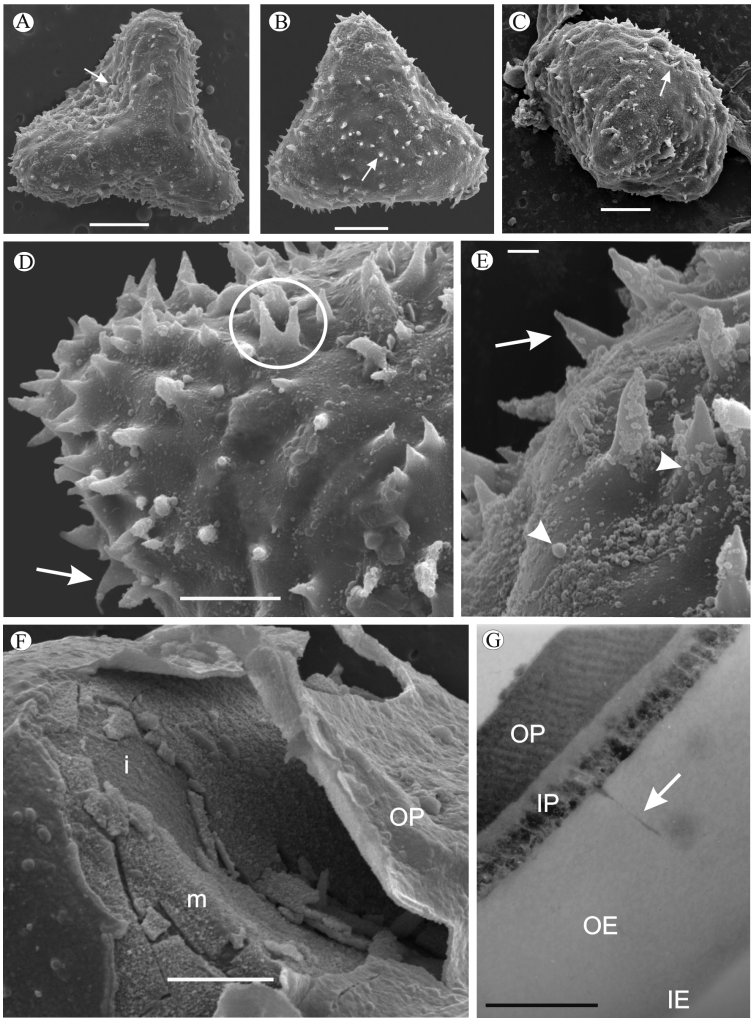

Fig. 1 – Spores of *Sphaeropteris gardneri* as seen with SEM and TEM. **A-F.** SEM micrographs. **A.** Spore in proximal view. The ornamen composed of low-ridges with few spines on their margins (arrow). The laesurae margins are smooth. Scale bar: 10μm. **B.** Spore in dis Low-ridges with scattered spines on their margins are shown (arrow). Scale bar: 10μm. **C.** Spore convex-hemispheric in equatorial v ornamentation is composed of low-ridges with spines on their margins (arrow). Scale bar: 10μm. **D-E.** Details of the distal surface t conic spines (arrow). In some cases, two spines are fused by their bases (circle, in Fig. D). Spheroids of different diameters are seen on th (arrowheads, in Fig. E). Scale Bars: Fig. D, 5μm; Fig. E, 1μm. **F.** Fracture of the sporoderm as seen with SEM. It shows the outer p layer (OP), and the inner (i) and medium (m) stratum of inner perispore. Scale bar: 5μm. **G.** The sporoderm as seen in section with TE figure shows the stratification of the walls and a channel (arrow) within the exospore. IE: inner exospore; OE: outer exospore; IP: inner p OP: outer perispore. Scale bar: 1μm

STUDIED MATERIAL

Brazil: Santa Catarina, Mun. Papanduva, Serra do Es-

RESULTS

The spores of *Sphaeropteris gardneri* are trilete

ameter is 42.3 (45.6) 49μm and the polar diameter is 34 (36.5) 39.8μm. The apertural folds are straight and reach the equator.

Short low ridges, parallel to the spore sides, are observed with SEM. The ridge margin bears conic spines 1.2-1.9μm high, which are abundant in the distal hemisphere, and are few or absent on apertural folds. In some cases, the spines could be fused by their bases (Fig. 1D-E).

With SEM, spheroids of different sizes are observed on the surface (Fig. 1E).

As seen with LM, the exospore is light brown, 2.5μm thick. With TEM, two layers are evident: a thin inner layer and a highly contrasted thick outer layer. Single or branched channels are present at the apertural fold sides and base (Figs. 1G, 2B).

The perispore is 0.7-2.1μm thick and dark brown under LM. Using TEM, two layers can be differentiated. The inner layer (IP) is composed of three strata: the outer stratum (**o**) is 150-200 nm thick, with dark fibres immerse in a less dense contrasted matrix. The middle stratum (**m**) is 100-200 nm thick. It is composed of an amorphous substance traversed throughout by threads with channels running along its whole length, with a mainly radial orientation. This complex system is branched, forming a network of threads in the inner stratum (**i**) that is 90-150 nm thick.

The outer layer of the perispore (OP) is 0.8-1.5μm thick and forms the echinate-ridges. In section and at an ultrastructural level, this layer is constituted of interwoven threads in a compact arrangement (Figs. 1F-G, 2A-C).

In TEM sections, spheroids of different sizes are seen on the surface, with a low electro dense core. In some cases, these elements can be fused to one another and to the perispore (Fig. 2A).

DISCUSSION AND CONCLUSIONS

The spores of *Sphaeropteris gardneri* have a surface formed of short low ridges with high spines in their margins. These results are similar to those observed by other authors for other taxa of the genus (Tryon 1971, Erdt-

The spines are higher and more abundant in the distal hemisphere, and few on the apertural folds. Braggins and Large (1990) observed similar characteristics in *Sphaeropteris medullaris*.

The spore surface is smooth and with spheroids of different sizes. When analyzed under TEM, the spheroids have a low electro-dense center similar to that of the exospore, and a contrasted coat with similar structure to that of the perispore. Based on these ultrastructural characteristics, it can be inferred that the spheroids are "globules", according to Lugardon's definition (1981).

At the ultrastructural level, we recognized an exospore with two layers. The outer layer is thicker than the inner layer and has a deep stratum with many channels and cavities. This kind of exospores was defined by Lugardon (1974) as "blechnoid".

The perispore is composed of two layers, an inner layer with three strata and an outer one with a massive structure. This stratification coincides with the observations by Lugardon (1971, 1974) in *Sphaeropteris cooperi* and *S. medullaris*. Our interpretation differs in some aspects referring to the perispore from that offered by Lugardon (*l.c.*). Thus, our observations showed that the inner perispore layer is formed of threads, that are arranged according to different patterns. Based on the spatial distribution and the frequency of fusion of these elements, each stratum has a different aspect. Nerverless, Lugardon (*l.c.*) observed that the strata were formed of a lumpy substance, in which dark lumps are fused.

In the present contribution, the outer perispore layer is interpreted as formed of densely packed threads. This particular arrangement of the elements and their chemical nature, make difficult its contrast with the usual fixatives and stains in electron microscopy. That would probably be the reason why some authors interpreted the outer part of the perispore as homogenous (i.e. Tryon and Lugardon 1991). Recently, Marquez et al. (2009) studied the spores of *Alsophila* species (Cyatheaceae) of Southern South America and found a strong similitude between this structure and that of *Sphaeropteris*. Differences were observed in the perispore ultraestructure and ornamentation, which is echinate-ridged in *Sphaeropte-*

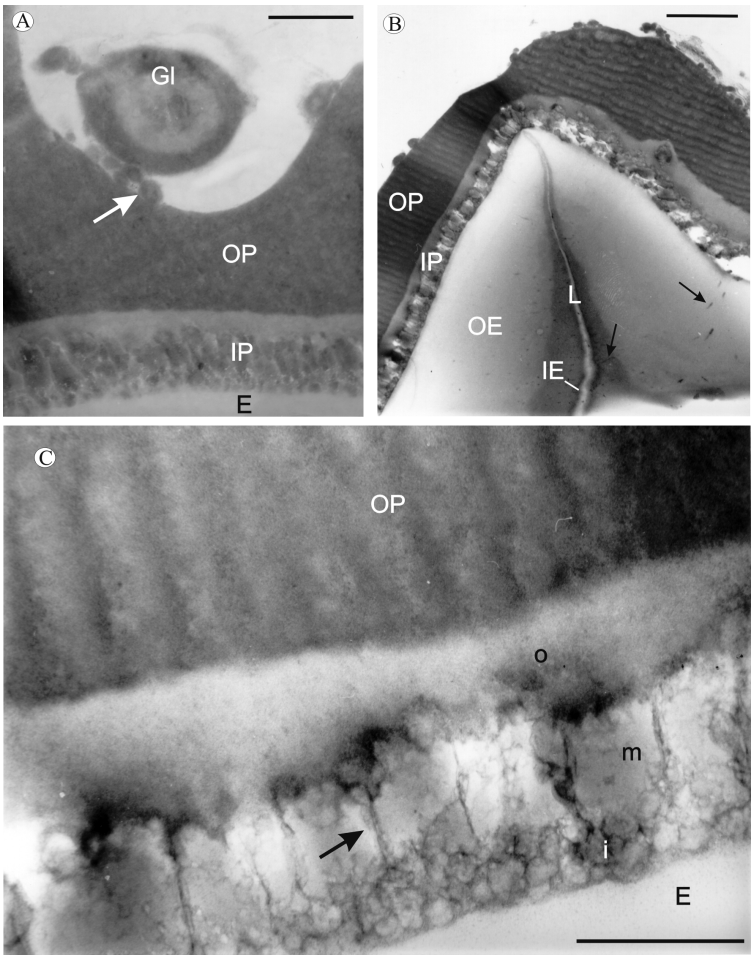

Fig. 2 – Spore wall sections of *Sphaeropteris gardneri* as seen with TEM. **A.** This figure shows globules (Gl) of different sizes, some are fused (arrow) to the perispore surface (OP). IP: inner perispore, E: exospore. Scale bar: 250 nm. **B.** Section throughout the laesura layers of the exospore are visible; the inner exospore (IE) is more contrasted than the outer exospore (OE). The channels associated to th have a dark content (arrows). The inner perispore (IP) is three stratified. The outer perispore (OP) is one stratified and homogeneous. S 1μm. **C.** Section through the perispore. The inner perispore has three strata: the inner stratum (i) is composed of a three-dimensional ne threads and shows an irregular margin. The middle stratum (m) is composed of threads mainly radially oriented (arrow). The outer strat composed of a network of fine highly contrasted fibres immersed in a less contrasted matrix. Within the outer perispore layer (OP), tin are evident. E: exospore. Scale bar: 250 nm.

the threads of the perispore are loosely interwoven and, in some cases, they form bunches.

Thus, it can be suggested that the differences found between the perispore of *Alsophila* and that of *Sphaeropteris* show a tendency to wall complexity. These data support the conclusions of Liew and Wuang (1976) con-

genetic hypotesis that proposes *Sphaeropteris* as group within the Cyatheaceae (Tryon 1970, K al. 2006).

An intermediate structural and morpholog between both genera is observed in *Alsophila ca*

made in this study are related to spore ornamentation and would give information for a better understanding of the identity of *A. capensis* that, according to the recent phylogenetic analysis by Korall et al. (2007), would form a group differentiated from *Alsophila* and *Sphaeropteris*.

These studies make evident the high complexity of the spore wall of some groups of ferns. Further studies would increase our knowledge about the spores in this group and, particularly, about perispore development and the probable function of each of its structural components.

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RESUMO

A morfologia dos esporos e a ultraestrutura da parede de *Sphaeropteris gardneri* (Hook.) R.M. Tryon, Brasil, foram analisadas com MO, MEV e MET. Os esporos são trilete com uma ornamentação formada por cristas curtas e baixas e com espinhos em suas margens. O exosporo possui 2,5μm de espessura, duas camadas em secção e estão presentes canais simples ou ramificados. A camada interna possui três estratos: o estrato interno é formado por uma rede de filamentos ramificados e fusionados, o estrato médio tem fios com uma orientação radial e no estrato externo fino, fibras escuras estão imersas em uma matrix menos densa. A outra camada do perisporo é a que forma as cristas equinatas e é constituída de filamentos dispostos em um arranjo compacto. Glóbulos de diferentes tamanhos são observados na superfície. As diferenças encontradas na ornamentação do perisporo e na ultraestrutura do *Alsophila* estudado previamente e aqueles de *Sphaeropteris* mostram uma tendência à complexidade da parede.

Palavras-chave: *Sphaeropteris*, esporos, morfologia, ultraestrutura.

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