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Morphology and ultrastructure of megaspores and microspores of *Isoetes sehnemii* Fuchs (Lycophyta)

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ABSTRACT

The morphology and wall ultrastructure of megaspores and microspores of *Isoetes sehnemii* that grows in Brazil were analyzed as part of the study of the Isoetaceae present in Southern South America. The observations were performed with light, scanning and transmission electron microscopes. The megaspores are trilete, 350–450 µm in equatorial diameter. The surface is reticulate. In section, the sporoderm is 100 µm thick including the ornamentation. The wall is composed of a siliceous perispore, which consists of short fused flattened elements forming a three-dimensional mesh. The exospore has two zones of different structure. The endospore is fibrillar. The microspores are monolet, 21–27 µm in equatorial diameter. The sporoderm is composed of a sporopollinic rugulate perispore. A space between the perispore and the exospore is evident. The exospore is compact. The endospore is fibrillar. The ultrastructural analysis makes homologies evident concerning structure and organization of the layers below the perispore in both spore types. A possible similarity and stability in the ultrastructure of the present spores and fossils could be also inferred. In addition, there would be a correlation among the plant habitat, the spore ornamentation and the geographic distribution.

Key words: Lycophyta, *Isoetes*, Brazil, spores, ultrastructure, sculpture, Palynology.

INTRODUCTION

The Isoetaceae constitute a family with a wide distribution. They live from mild to warm regions of all the continents, from sea level to near 4200 m. In southern South America, they are present in Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay. As part of the palynological study of the *Isoetes* that grow in this region, the morphology and ultrastructure of the spores of *Isoetes sehnemii* that grows in the state of Rio Grande do Sul, Brazil, are analyzed. Fuchs-Eckert (1982) carried out an updated study of the South American species of *Isoetes* and found a list of 64 taxa, among which the

Eckert described and typified the *Isoetes sehnemii* in the Flora Ilustrada Catarinense (1986).

Systematic (Pastore 1936, Capurro 1966) and floristic (de la Sota et al. 1998) aspects of *Isoetes* in southern South America have received some attention, but little information can be found in those contributions regarding palynological characteristics. In Argentina, Musselman mainly studied the microspores of *Isoetes* species with scanning electron microscopy (SEM). The microspores of some species of *Isoetes* in South America have been analyzed with SEM and transmission electron microscopy (TEM) by MacLuf (2004). In 2006a, b, MacLuf et al. analyzed with



(1986) is included. The joint study of microspores and megaspores in taxa of *Isoetes* with electronic microscopy has only been carried out for *Isoetes savatieri* Franchet by Morbelli (1980), Hickey (1985, 1986), Hickey et al. (2003) and MacLuf et al. (2003b). The spores of other species of *Isoetes* were examined with TEM by Lugardon (1973, 1986), Robert et al. (1973), Prada Moral and Saenz de Rivas (1978), Tryon and Lugardon (1991), Taylor (1992, 1993) and Uehara et al. (1991).

In megaspores, a siliceous cover, a sporopolleninous exospore and a well-developed endospore can be distinguished in the sections of mature megaspores, from the outside to the inside, respectively. In microspores, the sporoderm comprises the perispore forming the ornamentation, the para-exospore, a compact exospore and a fibrillar endospore. The term para-exospore (Lugardon 1972) is used to refer to the group of several large superimposed elements located between the exospore and the perispore (MacLuf et al. 2006b).

The aim of this work was to study the morphological characteristics of megaspores and microspores of *Isoetes sehnemii* using light (LM), scanning electron (SEM) and transmission electron (TEM) microscopy, in order to determine whether those sculptural and structural characteristics are reliable enough to be used for systematic purposes, for being regarded as microspore and megaspore characters, whether they are coherent or not, and also to determine if the organization of the sporoderm, the structure and the surface of both types of spores respond to the general characteristics of *Isoetes*.

MATERIALS AND METHODS

Spores were obtained from herbaria specimens housed at the Herbario Anchieta, Instituto Anchieta de Pesquisas, São Leopoldo, Rio Grande do Sul, Brazil (PACA).

For LM, the material was mounted in glycerine-jelly without any chemical treatment. Dimensions were estimated for 25 spores per specimen. The minimum and maximum values in micrometers are given in the text.

For studies with SEM, the spores were handled with moist brushes without any chemical treatment and placed on double-stick tape on bronze stubs. The sam-

plated by following the technique of Rowley and Nilsson (1972) using phosphate buffer and alcian blue (AB), and then the material was fixed with glutaraldehyde + 1% AB in phosphate buffer and postfixed with 1% osmium tetroxide (OsO_4) in water plus 1% AB. The material was washed with phosphate buffer with AB, and then the spores were dehydrated an increasing concentration of acetone and embedded in Spurr soft mixture. Semithin sections were stained with toluidine blue and observed with LM. Ultrathin sections were stained with 1% uranyl acetate for 15 min, followed by lead citrate for 3 min. The samples were examined under a Zeiss T-109 microscope.

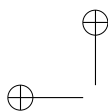
MATERIAL STUDIED

Isoetes sehnemii. Brazil. Rio Grande do Sul, Faz do Cerro, Vacaria, Rio dos Refugiados. Sehnem 14629 (PACA); Rio dos Refugiados, Fazenda Cedro, Vacaria. Sehnem 17094 (PACA); Rio dos Refugiados, afl. Antas, Vacaria. Sehnem 14987 (PACA).

RESULTS

MEGASPORES

The megaspores are triletes (Plate I, 1-8), 350-450 μm in equatorial diameter and subtriangular in polar view. The proximal face is convex and the distal one is hemispheric in equatorial view (Plate I, 2). Each laesure is fused to the equatorial zone, which is low, slightly differentiated from the ornamentation. Adjacent to the area, in the distal face, the girdle (*cf.* Tryon and Lugardon 1991, Taylor et al. 1993) shows the same ornamentation as the rest of the spore (Plate I, 2). The surface is reticulate heterobrochate in both polar faces. The proximal muri are lower compared to those of the distal face (Plate I, 5). In the lumens, the background consists of anastomosed bars that form a three-dimensional mesh with irregular spaces (Plate I, 7). The terminal ends of some elements are joined and form echinulae. In the muri, these echinulae are arranged on both sides of the major axis of each wall, with its ends oriented towards the lumens (Plate I, 8).



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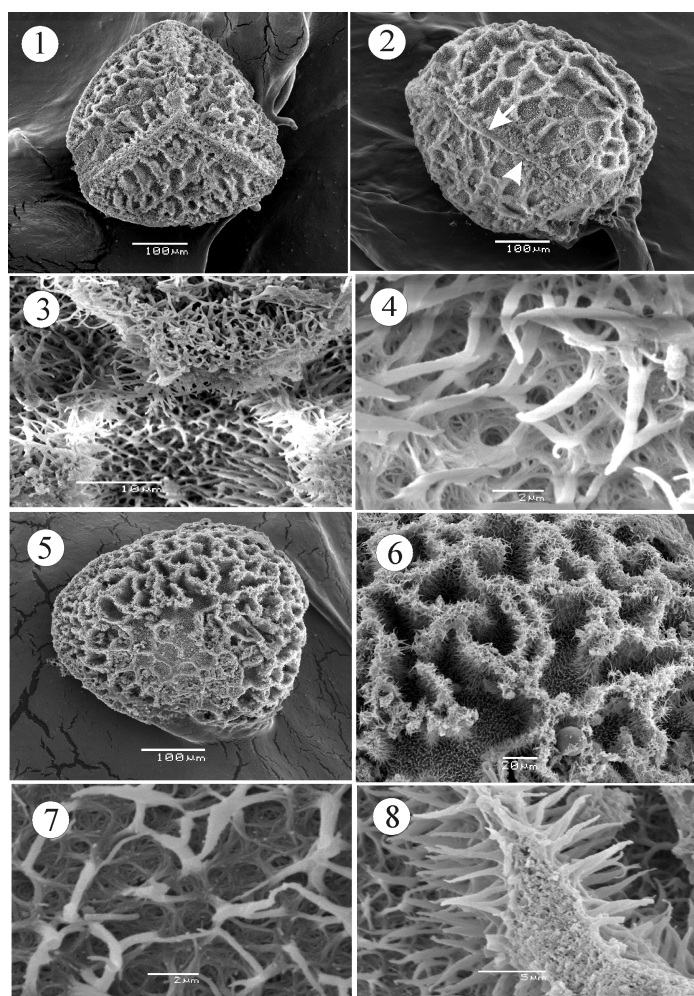


Plate I – Megaspores of *I. sehnemii* as seen with SEM – Figure 1 – Proximal view. The laesurae are low and are fused to the equatorial area. The distal face is slightly developed. The sculpture of the silica cover is reticulate in both faces. Scale bar: 100 μ m. Figure 2 – Equatorial view. The distal face is slightly developed at the top. The zone (arrow) marks the equator. The surface is reticulate in both polar faces. The girdle (arrowhead) has the same sculpture as the rest of the distal face. Scale bar: 100 μ m. Figure 3 – Detail of the silica surface proximally among the laesurae. The background is composed of a three-dimensional network of fused rodlets forming irregular spaces. Scale bar: 10 μ m. Figure 4 – Detail of the proximal surface showing a three-dimensional network of fused rodlets forming irregular spaces. Some of the bars join and form echinulae. Scale bar: 2 μ m. Figure 5 – Distal view. The sculpture of the silica cover (Si) is reticulate. The muri are higher in this face than in the proximal face. Scale bar: 100 μ m. Figure 6 – Detail of the distal surface showing an irregular reticule. The muri are high. Scale bar: 20 μ m. Figure 7 – Detail of the distal surface. Background of a lumen consists of a three-dimensional mesh of bars. Some of them are fused and form echinulae. Scale bar: 2 μ m. Figure 8 – Detail of the distal surface showing echinulae on both sides of the muri, with their ends towards the lumens. Scale bar: 5 μ m.

siliceous cover (Si), a sporopolleninuous exospore and an endospore; (Plate III, 12).

The sporoderm is 100 μ m thick, including the muri

40 μ m thick, formed by short fused flattened elements forming a three-dimensional mesh and leaving thin spaces between the ends at the surface. This layer is thicker in the



(Si) is strongly contrasted when fixed and stained for TEM. It is formed by structural elements that are rectangular to lenticulate, flattened and fused by their ends. (Plate III, 12, 13, 14). The chains formed by the link of these elements take articulated forms (Plate III, 13) and also form an open network that communicates with the outside. A connection with the exospore is also observed in some points (Plate III, 14). The exospore is 6 to 10 μm thick, and less contrasted than the siliceous cover. The structural elements show a more contrasted margin. They are tangentially elongated, arranged in several levels and fused with others of different levels, forming elongated spaces and lacunae. Due to the presence of a great amount of spaces, the thickness is variable. Silica is present in a variable amount in the outermost exospore spaces. The presence of silica decreases towards the inside and is absent in the internal area near the endospore (Plate IV, 15, 16). The nature and contrast to TEM of silica in the exospore spaces is seen identical to that of the perispore. The exospore area with silica impregnation is 1.5 to 4 μm thick (Plate IV, 16). Among the exospore elements, a space or “gap” is observed, which varies in amplitude distally and equatorially in different spores (Plate IV, 15). The endospore is 1 to 2 μm thick, has a fibrillar type structure (Plate IV, 15) and can be deposited in two stages probably marked by a discontinuity.

MICROSPORES

The microspores are monolet (Plate V, 17-22), 21-27 μm long and 18-20 μm wide, elliptic in polar view, and biconvex in equatorial view. A swelling (*cf.* Muselman 2003) perpendicular to the laesura was observed (Plate V, 17). A supra-laesural expansion is present. An equatorial 4.5 μm high projection is evident (Plate V, 20). The perispore is rugulate on the whole surface. The background is composed of perforations and granules.

Microspore wall structure and ultrastructure

The microspore wall in section from the outside to the inside is composed of perispore, para-exospore, exospore and endospore (Plate V, 23); (Plate VII, 28). The sporo-

structure but with different characteristics of their elements (Plate VII, 26). The outer stratum (Po) has thin, fused, laxly distributed bars (Plate VII, 26). The outside of this stratum forms the ornamentation. The inner perispore stratum (Pi) consists of thicker bars, circular in section, fused in different directions. This stratum is in contact with the elements of the para-exospore (Plate VII, 26, 27).

The para-exospore is 0.3 to 0.6 μm thick and formed by large structural elements tangentially arranged, which are fused to others at different levels (Plate VI, 23); (Plate VII, 26, 27). The center of each element is seen with less contrast than the margins (Plate VII, 26, 27). The elements of the internal para-exospore are generally thinner. An equatorial-distal separation or gap between the para-exospore and the exospore is present (Plate VI, 23, 24).

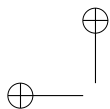
The exospore is 0.25 μm thick and compact, with an irregular margin. The endospore is 0.2 μm thick, with a fibrillar structure (Plate VII, 26, 27, 28).

In a transverse section of a microspore along its major polar axis, a supra-laesural expansion is observed, which forms a kind of vestibule of variable height that is higher at the central part and diminishes towards both ends of the laesurae (Plate V, 23, 24). All the thickness of the perispore and the more external structural elements of the para-exospore participate in the expansion (Plate VI, 25). The structure and arrangement of the external para-exospore in the supra-laesural expansion takes the form of a zip (Plate VI, 25).

DISCUSSION AND CONCLUSIONS

The organization of the sporoderm, the number and ultrastructure of the layers respond to the general characteristics of both types of *Isoetes* spores. Homologies have been found as regards the organization and structure of the internal layer to the perispore, i.e. exospore of megaspores and para-exospore of microspores. In both, the structural elements are long, tangentially arranged and fused in different levels. They also determine spaces that may or may not be filled with silica.

MacLuf et al. (2003b, 2006a) have found in differ-



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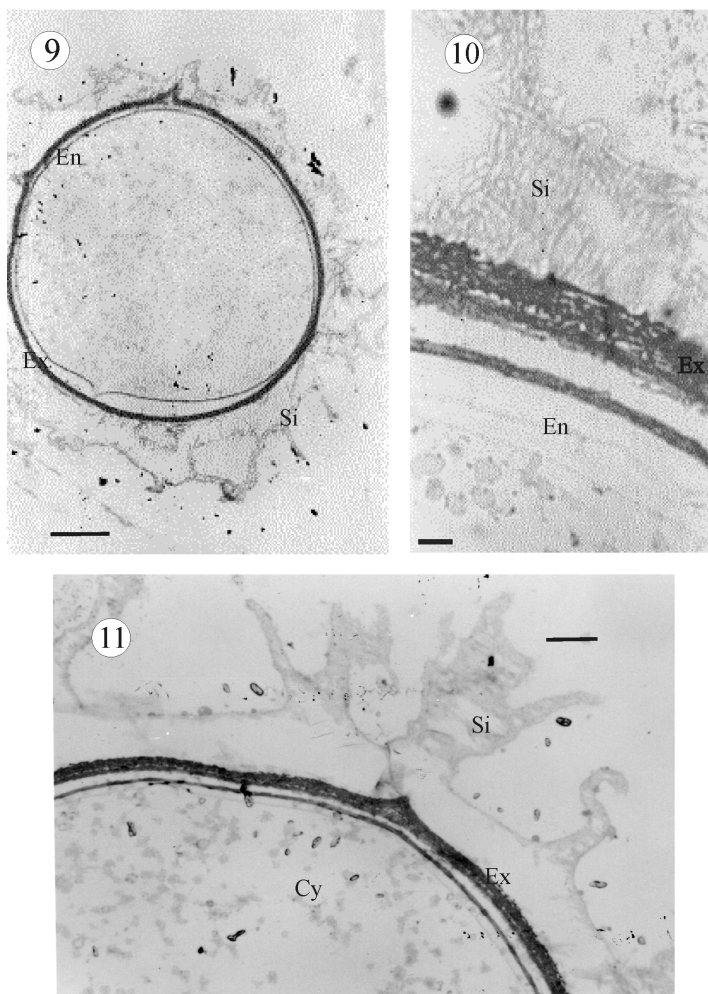


Plate II – Megaspores of *I. sehnemii* as seen with LM – Figure 9 – Section of the megaspore. The distal ornamentation (lower part of the phloem) consists of the highest structural elements of the silica cover (Si), which are ramified and with different shapes. Below, the sporopollenin (Ex) and the fibrillar endospore (En). Scale bar: 40 μ m. Figure 10 – Detail of the sporoderm in section. The silica cover (Si) consists of flattened and fused elements. Low contrasted with toluidine blue. The exospore (Ex) consists of tangentially elongated and anastomosed cells with an external area impregnated with silica. The endospore (En) is shown as fibrillar. Scale bar: 40 μ m. Figure 11 – Detail of the sporoderm in section. Most of the thickness of the silica cover (Si) forms the ornamentation. Below, the exospore (Ex), endospore (En) and cytoplasm (Cy). Scale bar: 20 μ m.

crospores, which may be used with systematic purposes. Likewise, the ornamentation of megaspores is a very useful diagnostic for the infrageneric classification. In virtue of this, megaspores and microspores of *Isoetes sehnemii* show superficial characters that, in an isolated or com-

the thickness forms the structural elements, whereas in the microspores only the outside stratum participates.

In megaspores, the sporoderm shows space throughout its thickness. From the exospore towards the endospore, a system of interconnected spores forms the

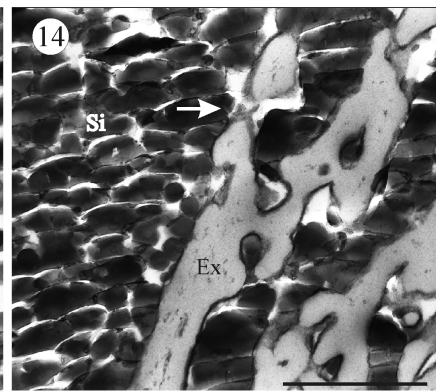
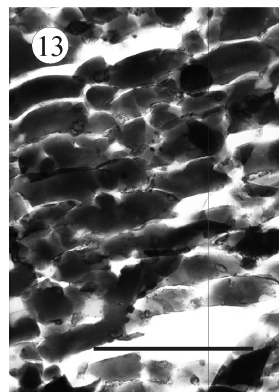
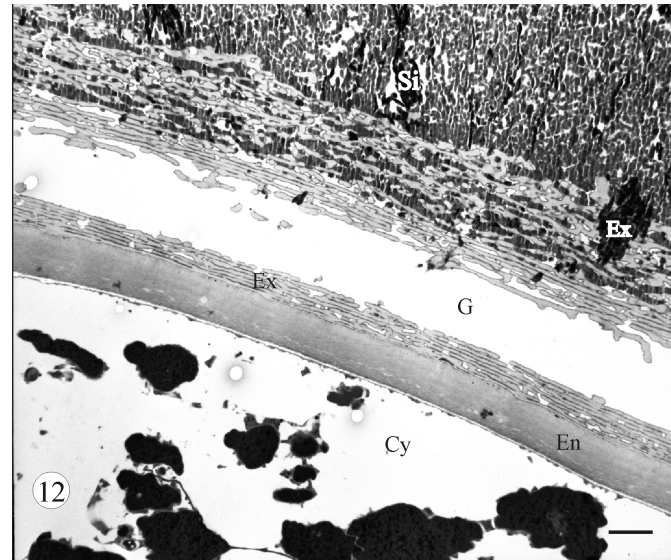
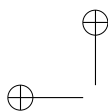


Plate III – Megaspores of *I. sehnemii* as seen with TEM – Figure 12 – Section through the sporoderm. The silica cover (Si) consists of elements fused forming bars that form a lacunose structure. The exospore (Ex) shows silica in the lacunae of the most external layers. There is a space (G) between the elements of the outer and the inner exospore. Below the exospore, the endospore (En) is in contact with the cytoplasm (Cy). Scale bar: $1\mu\text{m}$. Figure 13 – Detail of the silica cover structure. It consists of structural elements that are rectangular to lenticulate and fuse with one another. Scale bar: $1\mu\text{m}$. Figure 14 – Section that shows the sporoderm. The silica cover (Si) consists of short rectangular to lenticulate elements and forms a closed or dense structure. A connection with the exospore (Ex) is observed. Silica impregnates the exospore lacunae. The exospore elements have a less contrasted center and a dark edge. Scale bar: $1\mu\text{m}$.

elements of the siliceous layer of the megaspores fuse forming chains, they are likely to show a certain mobility or flexibility when living. The siliceous wall of the megaspores of *I. pedersenii* (Macluf et al. 2003a) shows a similar structure.

Based on the dominant sculptural element on the

the microspores of the genus of the species of the area. A swelling associated with the laesure described by Musselman (2003) and later by Macluf et al. (2006a) is also found in the microspores of the studied species. It has been observed that the perispore, which has a lacunar structure, is similar to that one observed in the mi-



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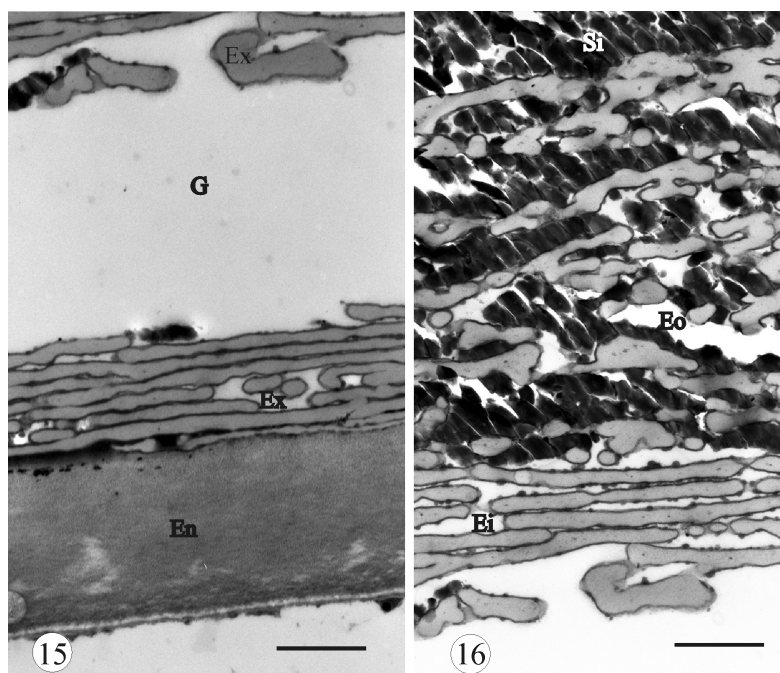


Plate IV – Megaspores of *I. sehnemii* as seen with TEM – Figure 15 – Section that shows a space (G) that divides the exospore into two areas. The exospore (Ex) elements show a more contrasted margin and are arranged in different directions. The endospore (En) is visible under the exospore. It has a fibrillar structure. Scale bar: 1 μ m. Figure 16 – The silica cover (Si) is in contact with the exospore, which is composed of anastomosing elements. Silica impregnates the most external layers of the exospore (Eo). Scale bar: 1 μ m.

but of the same structural type. The perispore thickness increases towards the equatorial-distal area in both species. Personal observations carried out in microsporangium sections with different degree of development in other species of the area, such as *I. ekmanii* and *I. savatieri* (C. Macluf, unpublished data), would indicate that the amplitude of the “gap” or space between the para-exospore and the exospore in the microspores ranges depending on the stage of development of the spore, and it is observed that it decreases in mature spores. These characteristics were described by Uehara (1991) in his study about the development of the wall in microspores of *I. japonica*.

The “pluriestrata areas” of the apertural area in microspores, described by Lugardon (1972, 1973) and Tryon and Lugardon (1991) for *Isoetes brochoni*, *Isoetes echinospora*, *I. setaceum*, *I. durieui* and for *Selaginella* species, are also present in the apertural area of *I. sehnemii*.

Isoetes sehnemii grows in the south of Brazil in floodable areas of subtropical climate. *I. pedunculata* grows in the northeast of Argentina, in similar climate and habitat. The coincidence in the ecological characteristics could be linked to the similarities found at the ultrastructural level of microspores. Both species produce microspores with open structure sporoderm with spaces. On the other hand, it was compared to *I. didensis*, which grows in the NW of Argentina, in similar climate, and is submerged aquatic, with different wall ultrastructure being observed since it shows a closed structure without the spaces among the elements. Based on these data, a probable correlation between spore ultrastructure and the plant habitat could be inferred. Likewise, Macluf et al. (2006a, b) related the increase of the ploidy level with the increase in the size of the microspores and with the habitat in microspores of *I. sehnemii* and *I. pedunculata*.

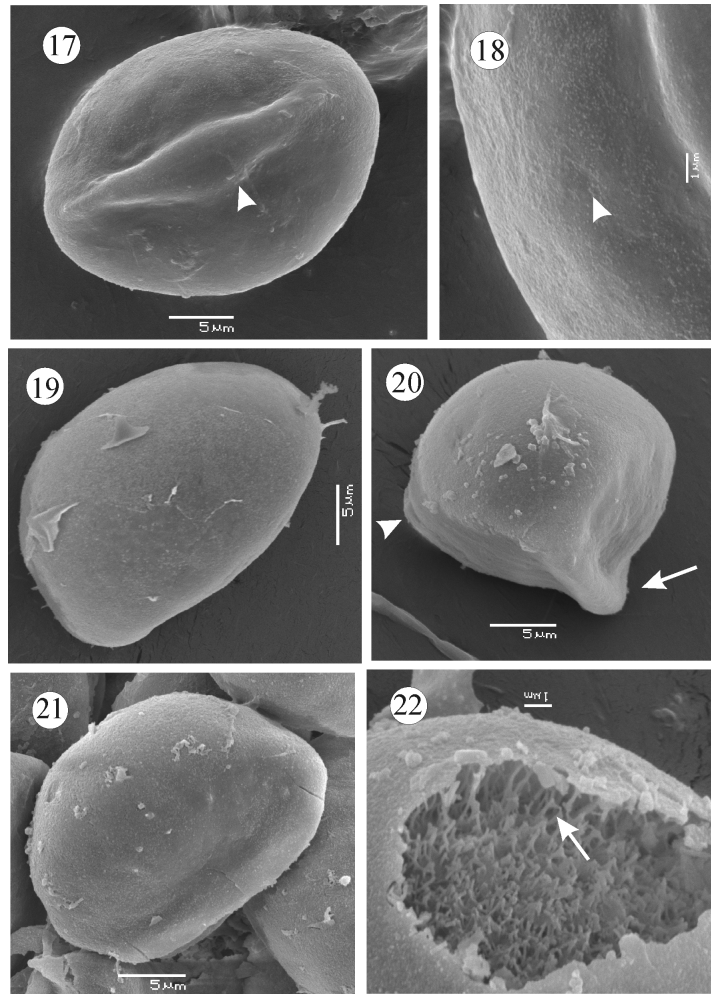
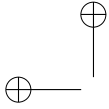
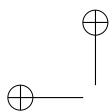


Plate V – Microspores of *I. sehnemii* as seen with SEM – Figure 17 – Proximal view showing rugulate sculpture. The supra-laesural expansion is evident. Swelling perpendicular to the laesura (arrowhead). Scale bar: 5 μm. Figure 18 – Detail of the proximal face. The background is composed of perforations and granules (arrowhead). Scale bar: 1 μm. Figure 19 – Distal view. The ornamentation consists of rugulae. Scale bar: 5 μm. Figure 20 – Lower equatorial view showing the proximal expansion of the laesura (arrow) and the equatorial projection (arrowhead). Scale bar: 5 μm. Figure 21 – Major equatorial view showing a supra-laesural expansion. Scale bar: 5 μm. Figure 22 – Fracture that exposes the two strata of the perispore. The internal lacunar stratum is evident (arrow). Scale bar: 1 μm.

sporoderm of the studied spores were compared with those of other studies, particularly of fossil material of the Cretaceous (Gammero 1977, Archangelsky 1965), a general stability or similarity could be inferred in the morphology and surface of spores. This would show a non-change or “stasis” condition according to Tryon’s

affinity between mesozoic megaspores and present Lycopphyta megaspores, which would also indicate a probable conservation of the sporoderm structure. Lugardon et al. (1999, 2000) and Grauvogel-stamm and Lugardon (2004) compared megaspores and microspores of living species of *Isoetes* with spores of the Triassic period, as-



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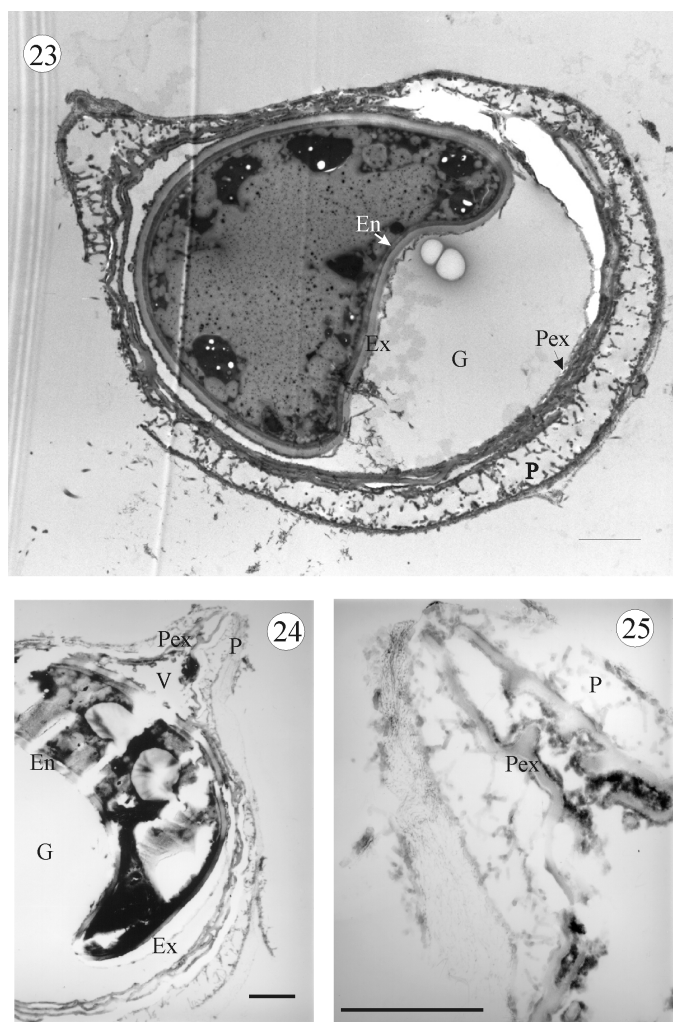


Plate VI – Microspores of *I. sehnemii* as seen with TEM – Figure 23 – Section of the microspore. The proximal pole is at the top left corner. The perispore (P) consists of bars arranged in two strata and form lacunae. The para-exospore (Pex) consists of large tangentially oriented elements. The exospore (Ex) lies on the endospore (En). A gap (G) between the Pex and the exospore is evident distally. Scale bar: 1 μ m. Figure 24 – Section through the polar axis showing the proximal pole of the microspore. The supra-laesural expansion is observed, which forms a kind of vesicle (v). The perispore (P) and the external elements of the para-exospore form the expansion. The exospore (Ex) lies on the endospore (En). The gap (G) between the Pex and the exospore is evident in equatorial and distal regions. Scale bar: 1 μ m. Figure 25 – Section through the aperture. The perispore (P) and the para-exospore (Pex) take part in the formation of the supra-laesural expansion. Due to a lateral growing of the structure of the external para-exospore takes the form of zip (z). Scale bar: 1 μ m.

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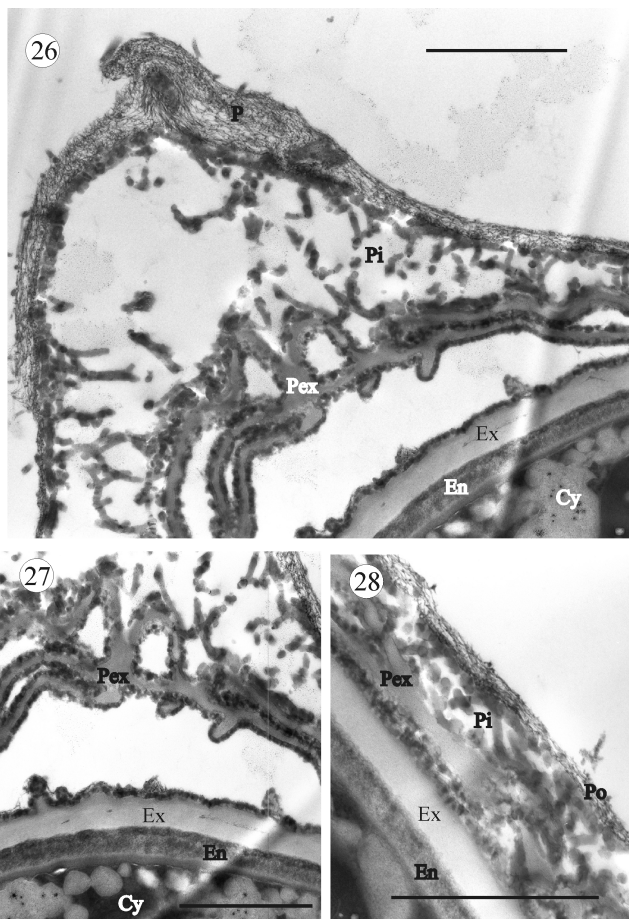
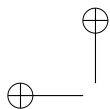


Plate VII – Microspores of *I. sehnemii* as seen with TEM – Figure 26 – Section through the apertural area following the polar axis. The perispore (P) and the para-exspore (Pex) participate in the formation of the supra-laesural expansion. The perispore is lacunar with two strata. The inner stratum (Pi) is attached to the para-exspore (Pex). The structural elements of the para-spore show a center that is less contrasted than the margins. The exspore (Ex) is homogeneous and has a dark-contrasted thick cover with low, blunt elements. The endospore (En), in contact with the cytoplasm (Cy), has a strongly contrasted central zone and shows a fibrillar structure. Scale bar: 1 μ m. Figure 27 – Detail of figure 26 at a higher magnification of the apertural area at the para-exspore elements (Pex). Below, the exspore (Ex) and the endospore (En) in contact with the cytoplasm (Cy). Scale bar: 1 μ m. Figure 28 – Detail of the sporoderm in section in an area without a gap. The perispore has a lacunar structure with two strata. The outer stratum (Po) with thin laxly distributed bars, and the inner stratum (Pi) with thick bars that fuse with one another. The para-exspore (Pex) is formed of tangentially arranged elements. Below, a solid, compact exspore (Ex) with a superficial contrasted layer, which is in contact with the endospore (En) with fibrillar structure. Scale bar: 1 μ m.

RESUMO

A morfologia e a ultraestrutura da parede de megasporos e microsporos de *Isoetes sehnemii* que crescem no Brasil foram analisados como parte do estudo de Isoetaceae presente no sul

rial. A superfície é reticulada. Em secção o esporoderma possui 100 μ m de espessura incluindo ornamentação. A parede é composta de um perispore silicoso que consiste de elementos fusionados curtos e achatados formando uma rede tridimensional. O exspore tem duas zonas com diferentes estruturas. O



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exosporo e o exosporo é evidente. O exosporo é compacto. O endosporo é fibrilar. A análise ultraestrutural torna evidente homologias relativas a estrutura e organização das camadas abaixo do perisporo em ambos os tipos de esporos. Uma possível similaridade e estabilidade na ultraestrutura do presente esporo e fósseis pode também ser inferida. Além disso, haveria uma correlação entre o habitat da planta, a ornamentação do esporo e a distribuição geográfica.

Palavras-chave: Lycophyta, *Isoetes*, Brasil, esporos, ultraestrutura, escultura, Palinologia.

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