

MORPHOLOGY AND ULTRASTRUCTURE OF *SCHIZAEA FISTULOSA* (SCHIZAEACEAE) SPORES FROM CHILE

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Summary: The spores of *Schizaea fistulosa* from Chile were studied using light microscopy (LM), scanning (SEM) and transmission electron microscopy (TEM). The spores are monolete and elliptic in polar view. The major equatorial diameter is 71-85 μm and the polar diameter is 54-61 μm . The laesurae are 50-60 μm long and, in some cases, bifurcated. The sporoderm ultrastructure is first mentioned and described here. The exospore is two-layered in section, verrucate-tuberculate with single or fused elements forming short ridges. The perispore is single-layered, 10-30 nm thick and it is only visible under transmission electron microscopy. On the spore surface, numerous, single or fused, spheroids and nanospheroids of different sizes were observed attached to the perispore surface. The results are discussed and compared with previous studies in *Schizaea*.

Key words: Schizaeaceae, *Schizaea fistulosa*, spores, morphology, ultrastructure.

Resumen: Morfología y ultraestructura de las esporas de *Schizaea fistulosa* (Schizaeaceae) de Chile. Se estudiaron las esporas de *Schizaea fistulosa* de Chile con microscopía óptica (MO), electrónica de barrido (MEB) y transmisión (MET). Las esporas son monoletes y elípticas en vista polar. El diámetro ecuatorial mayor es de 71-85 μm y el diámetro polar de 54-61 μm . Las lesuras son de 50-60 μm de longitud y en algunos casos están bifurcadas. La ultraestructura de la esporodermis se describe por primera vez. El exosporio en sección posee dos capas y es verrucoso-tuberculado con elementos aislados o fusionados formando lomos cortos. El perisporio posee una sola capa, de 10-30 nm de espesor y solamente es visible con microscopía electrónica de transmisión. Sobre la superficie de la espora, se observan numerosos esferoides y nanoesferoides de diferentes tamaños, adheridos a la superficie. Se discuten los resultados y se los compara con estudios previos en *Schizaea*.

Palabras clave: Schizaeaceae, *Schizaea fistulosa*, esporas, morfología, ultraestructura.

INTRODUCTION

The spores of *Schizaea fistulosa* Labill were analyzed as part of the study of the Schizaeales spores from the southern South America (Ramos Giacosa *et al.*, 2012; Ramos Giacosa *et al.*, 2013; Ramos Giacosa 2014).

The genus *Schizaea* Sm. is part of the Schizaeaceae. Previously, this family included the genera *Anemia* Sw., *Mohria* Sw., *Lygodium*

Sw., *Schizaea* and *Actinostachys* Wall. However, according to recent phylogenetic studies (Smith *et al.*, 2006), the Schizaeaceae is a monophyletic family that comprises only two genera: *Schizaea* and *Actinostachys*.

Schizaea includes about 30 species distributed in tropical and south warm-temperate areas of the New and Old World (Wikstrom *et al.*, 2002). It is distinct by its erect, apically annular sporangia on flag-like projections of the blade and grass-like to flabellate lamina (Mickel & Smith, 2004).

Schizaea fistulosa was reported as growing in Argentina (Malvinas Islands), Chile, New Zealand, Auckland Islands, Australia, Madagascar, New Caledonia and Fiji (Moore, 1968; Large & Braggins, 1991; de la Sota *et al.*, 1998; Zuloaga *et al.*, 2008). Nevertheless, this species has not been

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seen in Malvinas Islands since it was reported by Gaudichaud-Beaupré in 1825 (as *Schizaea australis*) (Gaudichaud-Beaupré, 1825). Thus, this species could be considered extinct for the Argentinean flora (de la Sota *et al.*, 1998).

Concerning palynological contributions, Heusser (1971) studied with light microscope (LM) the spores of *Schizaea fistulosa* from Chile and described the spore ornamentation as gemmate with gemmae of irregular size. When Morbelli (1980) analyzed material from Chile with LM, she described the ornamentation as scabrate with gemmae, verrucae and granules. This author asks some questions about the presence or absence of perispore and wonders if the granules are part of the exospore or the perispore.

Van Konijnenburg-van Cittert (1991), in a comparative study of fossil and extant spores of Schizaeaceae, illustrated with scanning electron microscope (SEM) the spores of *Schizaea elegans* (Vahl) Sw. and *S. pectinata* (L.) Sw.

Tryon & Lugardon (1991) analyzed with SEM and transmission electron microscope (TEM) the spores of a few species of *Schizaea*. The authors illustrated with SEM, material of *Schizaea fistulosa* from Australia and described the spores as spheroidal, with a proximal face papillate and large spherules.

Large & Braggins (1991) studied with LM and SEM the spores of *Schizaea* species from New Zealand. *Schizaea fistulosa* was one of the species studied by these authors and they described the spore ornamentation under SEM as granulate with large, rounded globular structures, irregularly distributed and frequently more common and larger in size around the laesura. They also mentioned that apparently the perispore was absent.

Later, Parkinson (1994, 1995 a, b) studied the spore wall development in *Schizaea pectinata* from South Africa. The author described the species sporoderm as having a two-layered exospore and a two-layered perispore.

No studies on the spores of *Schizaea fistulosa* with TEM were carried out. The sporoderm ultrastructure of this species is still unknown.

The aim of this work is to analyze the spore morphology and wall ultrastructure of *Schizaea fistulosa* from Chile in order to get a better knowledge of the spores within the genus.

MATERIAL AND METHODS

Spores were obtained from herbarium specimens from the following institutions (abbreviations according to Holmgren *et al.*, 1990): División Plantas Vasculares, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (LP) and Instituto de Botánica Darwinion (SI).

The spores were studied using light (LM), scanning (SEM) and transmission (TEM) electron microscopy. For LM, the spores were treated with hot 3% sodium carbonate for two minutes in order to preserve the perispore (Morbelli, 1980) and acetolyzed according to the method of Erdtman (1960).

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.

For studies with TEM, dry material from herbarium specimens was hydrated following the technique by Rowley & Nilsson (1972), by use phosphate buffer and alcian blue (AB). Then, the material was fixed with Glutaraldehyde + 1% Alcian Blue in phosphate buffer for 12 h and post-fixed with 1% OsO₄ in water plus 1% Alcian Blue. The spores were dehydrated in an acetone series and then embedded in Spurr soft mixture. Three µm thick 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 three min.

The observations were made with an Olympus BH2, a JEOL JSMT-100 scanning electron microscope, and a Zeiss T-109 transmission electron microscope.

Studied material: CHILE: Prov. Chiloé, III-1924, Werdermann 298 (SI). Prov. Magallanes, Dpto. Ultima Esperanza, Isla Virtudes, 2-XII-1977, Roig y Méndez s/n° TBPA-FIT 2613 (LP).

RESULTS

Morphology

The spores are monolete and elliptic in polar view. In equatorial view, the proximal face is plane to convex and the distal face is convex. The major equatorial diameter is 71-85 µm and the polar diameter is 54-

61 μm . The laesurae are 50-60 μm long and in some cases, they are bifurcated near their apices.

Analyzed with SEM, the exospore is verrucate-tuberculate. The verrucae and tubercles observed are single or partially fused forming short ridges, which are distributed in several directions (Fig. 1E-F). Among the ornamental elements some perforations are also visible (Fig. 1E).

The perispore is not evident under SEM.

On the spore surface, numerous single or fused spheroids are evident. In some spores the high density of spheroids fused to the spore make its surface visualization difficult (Fig. 1A-D).

Ultrastructure

Analyzed with TEM, the exospore is 1.2-3 μm thick and two-layered: having an inner layer of 100-150 nm thick and an outer layer of 1.2-3 μm thick. The outer exospore layer forms the elements of the ornamentation which is composed of irregular, partially fused verrucae and tubercles (Fig. 2A, B).

Radial channels with dark content are observed in the outer layer of the exospore (Fig. 2E, F).

The perispore is 10-30 nm thick and more contrasted than the exospore. This wall is adhered to the exospore and covers the outer surface. Nanospheroids of 55-120 nm of diameter are attached to the perispore surface (Fig. 2E, F).

On the spore surface, there are spheroids of 0.4-3.5 μm in diameter. The spheroids are single, fused to one another or to the perispore. Exceptionally, they are fused to the exospore. They have a structure similar to the exospore but with parallel lines which traverse the spheroids all throughout. They are covered by a perispore layer with abundant nanospheroids (Fig. 2A-D).

DISCUSSION AND CONCLUSIONS

In the present work, we defined the spore ornamentation of *Schizaea fistulosa* as verrucate-tuberculate. These two elements are single or partially fused and form short ridges distributed in several directions.

These results are similar to the descriptions of spores from Chile mentioned by Heusser (1971) and Morbelli (1980) and they are also in accordance with the observations made by Large & Braggins (1991) of spores from New Zealand.

In some spores, the laesurae is bifurcated near their apices. This characteristic was also mentioned by Heusser (1971).

Tryon & Lugardon (1991) observed with SEM a spore of *Schizaea fistulosa* from Australia and mentioned the presence of spherules. The authors defined the spherules as small bodies with a concentric arrangement of the different perispore materials.

However, based on the information provided here, the ultrastructural analysis of *Schizaea fistulosa* performed with TEM has demonstrated that the abundant spheroids on the spore surface have in their centre a similar structure to that of the exospore but with parallel lines that traverse the spheroids all throughout. They are covered by a perispore layer which is more contrasted than the exospore.

These spheroids were resistant to the acetolysis method and, according to the characteristics mentioned above, they would be "globules" as defined by Lugardon (1981). These globules may be homologous to the Ubisch bodies of the Spermatophytes.

Large & Braggins (1991) mentioned that, in the analyzed specimens, the globules were frequently more common and larger in size around the laesura. These characteristics were not observed in the material analyzed in the present study. However, when tetrads were observed, the globules were especially abundant in the contact area with the adjacent spores.

The sporoderm ultrastructure for the species is defined here for the first time.

The ultrastructural analysis has demonstrated the presence of a single-layered perispore in this species that could only be visible under TEM.

The two-layered exospore is the thickest wall of the sporoderm and the one that bears the spore ornamentation. The thin perispore covers the outer surface of the exospore.

On the other hand, spore development studies in other species of this genus as *Schizaea pectinata* from South Africa (Parkinson, 1994, 1995a), described a two-layered exospore and also a two-layered perispore.

In the present work, abundant nanospheroids attached to the surfaces of the perispore and of the globules were observed. The presence of nanospheroids on the perispore and the globules could mean that they transport material from the tapetum to complete the development of the perispore.

Although the type of tapetum is unknown for the species studied here, previous work on *Schizaea*

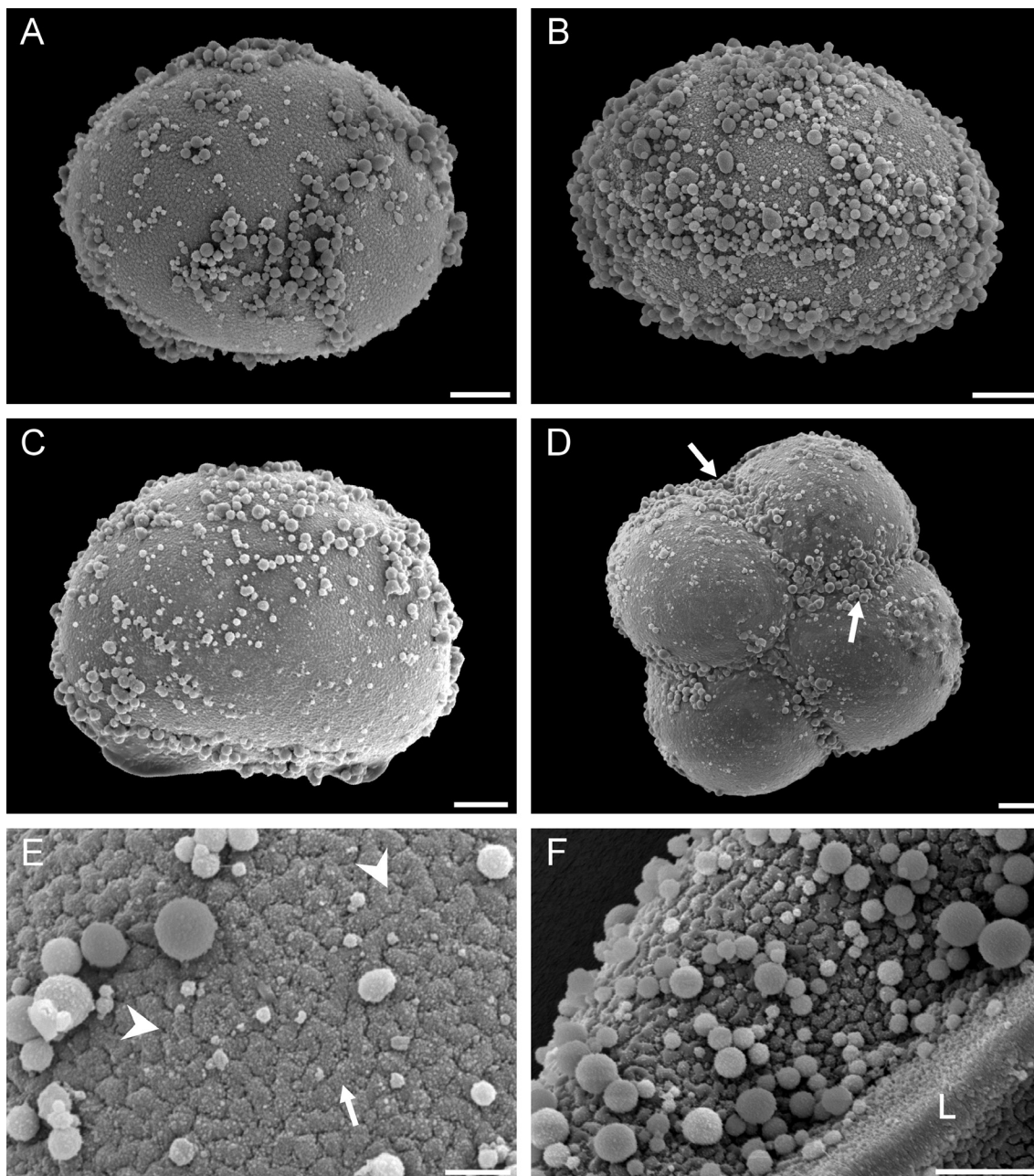


Fig. 1. Spores of *Schizaea fistulosa* with SEM. **A:** Distal view of an elliptic spore. **B:** The high density of spheroids, makes the visualization of the spore surface difficult in some areas. **C:** Equatorial view of a plane-convex spore. **D:** Tetrad of spores. The globules are especially abundant in the area of contact between adjacent spores (arrows). **E, F:** Detail of the spore surface. The exospore is verrucate-tuberculate. The verrucae and tubercles are single or partially fused forming short ridges (arrow). Some perforations are also visible (arrowheads). Numerous single spheroids or spheroids fused to others are observed. The laesurae (L) is evident. Scale bars: **A-D:** 10 μm . **E:** 2 μm . **F:** 5 μm .

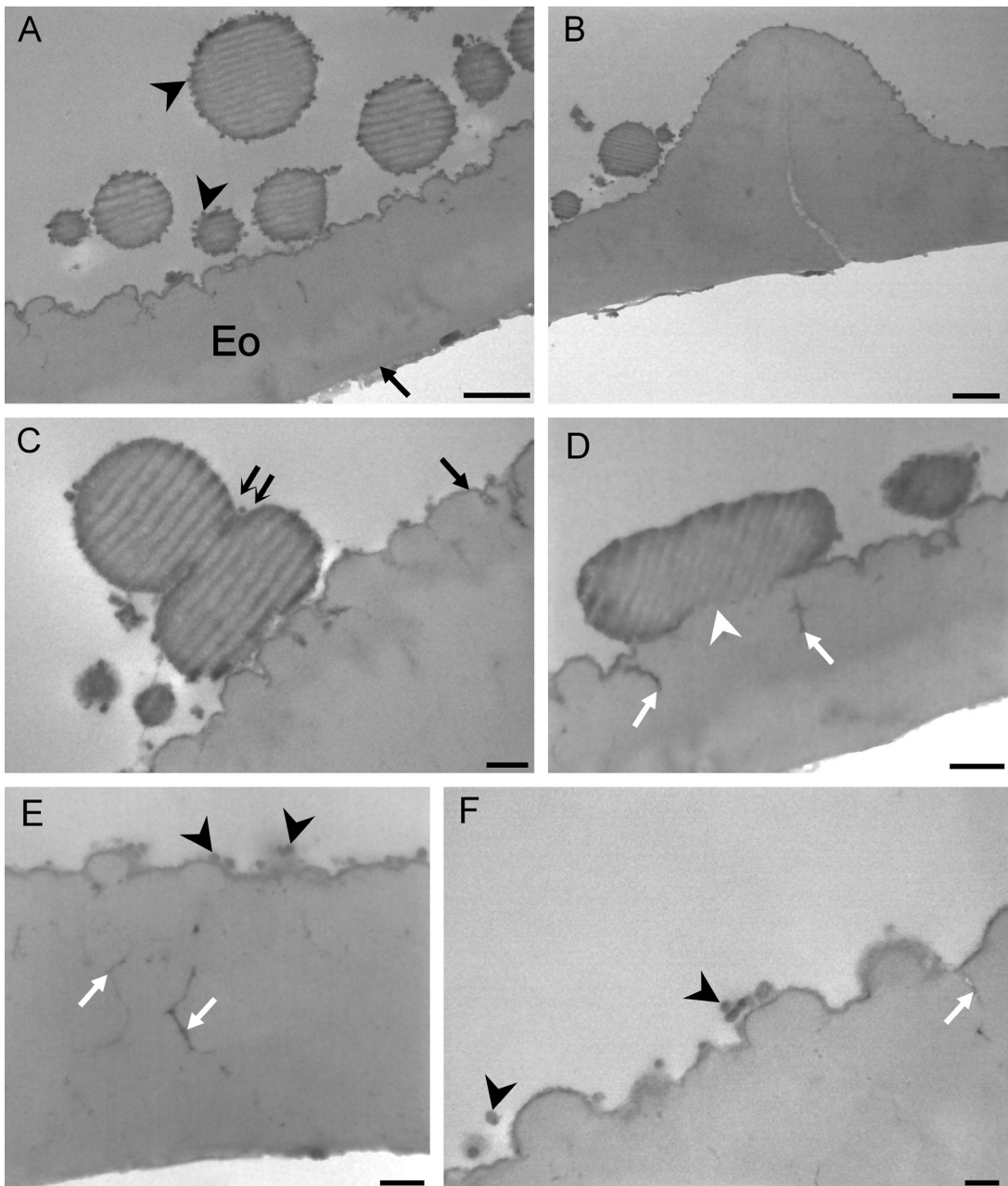


Fig. 2. Spores of *Schizaea fistulosa* with TEM. **A:** Section through the sporoderm. The exospore is two-layered. The inner exospore layer (arrow) is thin and frequently difficult to differentiate. The outer exospore layer (Eo) forms the elements of the ornamentation. Numerous globules are evident on the spore surface. On the globules surfaces, nanospheroids (arrowheads) are seen. **B:** Section through a laesurae. The perispore is thin and continuous in its surface. **C:** The outer exospore forms the verrucae and tubercles of the spore ornamentation. The perispore (arrow) is very thin, it is more contrasted than the exospore and it covers the outer surface. On the spore surface, the globules are fused (double arrow). **D:** Few globules are fused to the exospore surface (arrowhead). Channels with dark content are seen in the exospore (arrows). **E, F:** Detail of the sporoderm. Nanospheroids (arrowheads) are observed on the perispore surface. Channels are also seen (arrows). Scale bars: **A, B:** 1 μ m. **C, D:** 0.4 μ m. **E, F:** 200 nm.

pectinata (Parkinson, 1995 b) reported a combined tapetum consisting of a cellular, parietal and a plasmodial component. This author also suggested that some spherical bodies play a role in the development of the perispore.

Further studies on other species of the genus, which analyze the development of the spore wall, could help to understand if the palynological data given in the present work could be relevant for systematic purposes, and also if these characteristics are representative of the family Schizaeaceae.

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BIBLIOGRAPHY

- DE LA SOTA, E. R., M. M. PONCE, M. A. MORBELLI & L. A. CASSÁ DE PAZOS. 1998. Pteridophyta. En: M. N. CORREA (dir.). Flora Patagónica. *Colecc. Ci. Inst. Nac. Tecnol. Agropecu.* 8: 282-369.
- ERDTMAN, G. 1960. The acetolysis method. A revised description. *Svenk Botanisk Tidskrift* 54: 561-564.
- GAUDICHAUD-BEAUPRÉ, C. 1825. Rapport sur la Flore des îles Malouines. *Ann. Sci. Nat. Paris* 5: 89-110.
- HEUSSER, C. J. 1971. Pollen and spores of Chile. Modern types of the Pteridophyta, Gymnospermae and Angiospermae. The University of Arizona Press. 167 p.
- HOLMGREN, P. K., N. H. HOLMGREN & L. C. BARNETT. 1990. Index Herbariorum. Part I: The herbaria of the world. New York Botanical Garden, New York. 120 p.
- LARGE, M. F. & J. E. BRAGGINS. 1991. Spore atlas of New Zealand ferns and fern allies. *N. Z. J. Bot.* (suppl. 29): 1-168.
- LUGARDON, B. 1981. Les globules des Filicinées homologues des corps d'Ubisch des Spermatophytes. *Pollen et Spores* 23: 93-124.
- MICKEL, J. T. & A. R. SMITH. 2004. The Pteridophytes of Mexico. *Mem. New York Bot. Gard.* 88: 48-60.
- MOORE, D. M. 1968. The vascular flora of the Falkland Islands. British Antarctic Survey, *Scientific Report* 60: 49-51.
- MORBELLI, M. A. 1980. Morfología de las esporas de las Pteridophyta presentes en la Región Fuego-Patagónica. República Argentina. *Opera Lilloana* 28: 1-138.
- PARKINSON, B. M. 1994. Morphological and ultrastructural variations in *Schizaea pectinata* (Schizaeaceae: Pteridophyta). *Bothalia* 24: 203-210.
- PARKINSON, B. M. 1995 a. Spore wall development of *Schizaea pectinata* (Schizaeaceae: Pteridophyta). *Grana* 34: 217-228.
- PARKINSON, B. M. 1995 b. The tapetum in *Schizaea pectinata* (Schizaeaceae) and a comparison with the tapetum in *Psilotum nudum* (Psilotaceae). *Plant Syst. Evol.* 196: 161-172.
- RAMOS GIACOSA, J. P., M. A. MORBELLI & G. E. GIUDICE. 2012. Spore morphology and wall ultrastructure of *Anemia Swartz* species (Anemiaceae) from Argentina. *Rev. Palaeobot. Palynol.* 174: 27-38.
- RAMOS GIACOSA, J. P., M. A. MORBELLI & G. E. GIUDICE. 2013. Comparative palynological analysis of *Lygodium venustum* Sw. and *L. volubile* Sw. (Lygodiaceae). *An. Acad. Bras. Cienc.* 85: 699-707.
- RAMOS GIACOSA, J. P. 2014. Abnormal spore morphology and wall ultrastructure in *Anemia tomentosa* var. *anthriscifolia* and *A. tomentosa* var. *tomentosa* (Anemiaceae). *Plant Syst. Evol.* 300: 1571-1578.
- ROWLEY, J. R. & S. NILSSON. 1972. Structural stabilization for electron microscopy of pollen from herbarium specimens. *Grana* 12: 23-30.
- SMITH A. R., K. M. PRYER, E. SCHUETTPELZ, P. KORALL, H. SCHNEIDER & P. WOLF. 2006. A classification for extant ferns. *Taxon* 55: 705-731.
- TRYON A. F. & B. LUGARDON. 1991. Spores of Pteridophyta. Springer-Verlag, New York. 648 pp.
- VAN KONIJNENBURG-VAN CITTERT J. H. A. 1991. Diversification of spores in fossil and extant Schizaeaceae. In: BLACKMORE S. & S. H. BARNES (eds.). Pollen and Spores, Patterns of Diversification. *Publ. Syst. Assoc. spec. vol.* 44: 103-118.
- WIKSTRÖM N., P. KENRICK & J. C. VOGEL. 2002. Schizaeaceae: a phylogenetic approach. *Rev. Palaeobot. Palynol.* 119: 35-50.
- ZULOAGA F. O., O. MORRONE & M. BELGRANO (eds.) 2008. Catálogo de las plantas vasculares del Cono Sur. Vol.1, Pteridophyta, Gymnospermae y Monocotyledonae. *Monogr. Syst. Bot. Missouri Bot. Gard.* 107. 983 p.

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