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## Spore morphology and ultrastructure of *Cyathea* (Cyatheaceae, Pteridophyta) species from southern South America

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### Abstract

Spore sculpture and wall structure of eight *Cyathea* (Cyatheaceae) species from southern South America were studied using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques. Two layers, i.e. an inner and an outer layer, were observed in the perispore. The inner layer has two strata: the inner stratum is attached to the exospore and composed of rodlets tangentially oriented to the spore surface and randomly inter-mixed; the outer stratum consists of a three-dimensional network of rodlets with either free or fused distal edges forming spinules. The outer layer is thin, darkly contrasted and covers the rodlets. In most cases, the exospore has two layers and a pitted surface. In *Cyathea atrovirens*, the exospore surface is smooth, while in *C. delgadii* and *C. myriotricha* it is verrucate. The homogeneity of perispore features within the genus *Cyathea* is evident, while exospore features are heterogeneous. The exospore has different kinds of surface-structures that are of potential interest for assessing evolutionary trends within the group.

**Keywords:** Spores, morphology, ultrastructure, *Cyathea*, southern South America, Cyatheaceae

### Introduction

The tree fern genus *Cyathea* includes about 150 species that are mainly distributed in the New World and islands across the Pacific Ocean (Korall et al., 2007). The environments of greatest diversification are the humid montane forests and the cloud forests (Tryon & Tryon, 1982). The species have an arborescent habit, abaxial leptosporangiate sori, and marginate scales without an apical seta on the petiole (see Tryon, 1970, p. 7).

The first modern classification of the Cyatheaceae was based on features of the petiole scales, and only one genus, i.e. *Cyathea s. l.*, including all the scaly tree ferns, was included (Holttum & Sen, 1961). In later studies, Tryon (1970) and Tryon and Tryon (1982) identified six genera of scaly tree ferns: *Alsophila*, *Cnemidaria*, *Cyathea*, *Nephelea*, *Sphaeropteris* and *Trichipteris*. Lellinger (1987) redefined the circumscription of *Cyathea* based on the scaly features

and spores, and included *Cyathea sensu* Tryon, *Trichopteris* and most American species of *Sphaeropteris* within this genus, but excluding the *S. horrida* group.

Phylogenetic analyses over the last two decades (Conant et al., 1994, 1995, 1996; Korall & Taylor, 2006; Korall et al., 2007; Schuettpelz & Pryer, 2007) have identified three evolutionary lineages within the scaly tree ferns: *Alsophila*, *Cyathea* and *Sphaeropteris*. In this paper, we adapt the genus concept *Cyathea sensu* Lellinger (1987), with the addition of the genus *Hymenophyllopsis* (Christenhusz, 2009).

Several authors have described the spores of *Cyathea* (under different circumscriptions) as trilete with a smooth, verrucate or perforated exospore and a pilose perispore (Harris, 1955; Holttum & Sen, 1961; Erdtman & Sorsa, 1971; Murillo & Bless, 1974; Barth, 1975; Gastony & Tryon, 1976; Tryon, 1976; Barrington, 1978; Tryon & Tryon, 1982; Esteves & Felipe, 1985; Lellinger, 1987; Simabukuro

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et al., 1998). Recently, Moran et al. (2008) transferred *Polypodium myriotrichum* to *Cyathea myriotricha* and described its spores as verrucose, covered with rodlets.

Scanning electron microscopy (SEM) studies of *Trichipteris* spores by Gastony (1979) revealed a perispore of intermixed slender filaments and great morphological variation in exospore morphology. Similarly, Tryon and Lugardon (1991) showed that in spores of *Cyathea*, *Trichipteris*, *Sphaeropteris* subg. *Sclephropteris* and in some species of *Alsophila* and *Nephelea* the perispore is formed by rods. It is now clear that there is close similarity in spore morphology and structure among species of the *Trichipteris*, *Cyathea* and the *Sphaeropteris aterrima* group. Analyses of the pteridophytes spores of Rio Grande do Sul, Brazil, by Lorscheitter et al. (1999) evidenced that the spores of *C. atrovirens*, *C. corcovadensis*, *C. delgadii* and *C. phalerata* have a thin perispore with dense rodlets attached to it.

In this contribution, the spore morphology and wall ultrastructure of eight *Cyathea* species were analysed as part of the palynological studies on Cyatheaceae from southern South America (Márquez, 2009; Márquez et al., 2007, 2008, 2009). Analyses were performed using light microscopy (LM), SEM and transmission electron microscopy (TEM) techniques. The aim of this paper is to characterise each species according to its palynological features and evaluate the systematic, phylogenetic, developmental, and functional significance of these features.

## Materials and methods

### Material

Spores were obtained from herbarium specimens stored at the following institutions: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina), Instituto de Botánica del Nordeste (Corrientes, Argentina), Museo de Ciencias Naturales de La Plata (La Plata, Argentina), Instituto Anchieta de Pesquisas (São Leopoldo, Brasil), Instituto de Botánica Darwinion (San Isidro, Argentina), Universidade de São Paulo (São

Paulo, Brasil), the Smithsonian Institution (Washington, USA), and Universidade Federal do Paraná (Curitiba, Brasil).

Terms suggested by Tryon and Lugardon (1991) were used to describe spore surface and structure, while terms proposed by Nayar and Devi (1966) were applied to the description of spore shape in equatorial view. The letters MP on the list of specimens investigated indicate the reference number of each sample filed in the Laboratorio de Palinología, Facultad de Ciencias Naturales y Museo de La Plata.

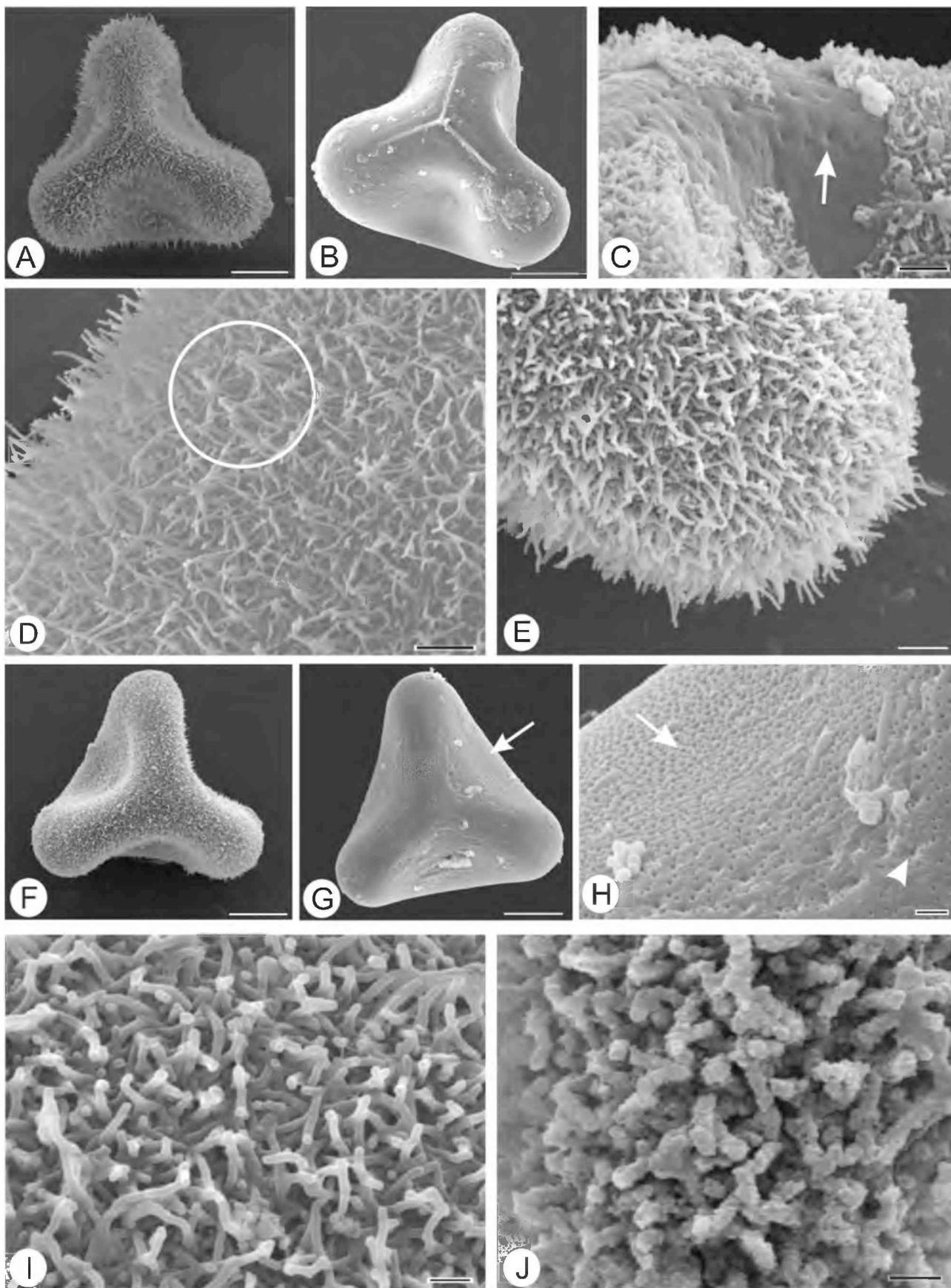
### Techniques

For LM investigations, the spores were studied without any chemical treatment. Polar and equatorial diameter as well as exospore and perispore thickness were measured (25 spores for each specimen). For SEM investigations, the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates. After drying, the material was coated with gold. For TEM investigations, dry material from herbarium specimens of *Cyathea atrovirens*, *C. corcovadensis* and *C. delgadii* was hydrated with buffer + alcian blue (AB). The material was subsequently fixed with 1% glutaraldehyde (GA) + 1% AB in phosphate buffer for 12 hours, rinsed with phosphate buffer + AB, and post-fixed with 1% OsO<sub>4</sub> in water plus 1% AB (Rowley & Nilsson, 1972).

Living material of *Cyathea atrovirens* was fixed with 1% GA + 0.0025% ruthenium red (RR) in phosphate buffer, washed in phosphate buffer + RR, then post-fixed with 1% OsO<sub>4</sub> in water + 0.0025% RR in phosphate buffer. The spores were dehydrated in an alcohol series and then embedded in Spurr medium mixture. Sections (3 µm thick) were stained with toluidine blue and studied under LM. Ultra-thin sections were stained with 1% uranyl acetate for 15 minutes followed by lead citrate for three minutes. For the investigations an Olympus BH2 light microscope, a Jeol JSMT-100 scanning electron microscope, and a Zeiss M-10 transmission electron microscope were used.

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Figure 1. Spores of *Cyathea atrovirens* and *C. corcovadensis* by means of SEM. **A–D.** *C. atrovirens* spores: **A.** Proximal view, showing the surface covered by rodlets; **B.** Proximal view of a spore devoid of perispore; the exospore surface is smooth, the perforations are evident near the laesurae; **C.** Fracture through the perispore in the place where the laesurae join; the arrow indicates the perforations next to the laesurae; **D.** Surface in detail, where interwoven rods have their tips either free or fused forming spinules (circle). **E–J.** *C. corcovadensis* spores: **E.** Detail of the distal surface with perispore; large interwoven rodlets, with their tips free are visible; **F.** Proximal view, with superficial rods; **G.** Proximal view of a spore devoid of perispore that shows the perforated surface of the exospore (arrow); **H.** Detail of the spore in (G), where superficial perforations (arrow) and those close to the laesurae (arrowhead) are visible; **I.** Detail of the perispore rodlets with a smooth surface; **J.** Detail of perispore rods with irregular surfaces. Scale bars – 10 µm (A, B, F, G), 2 µm (C, D, E), 1 µm (H, I, J).



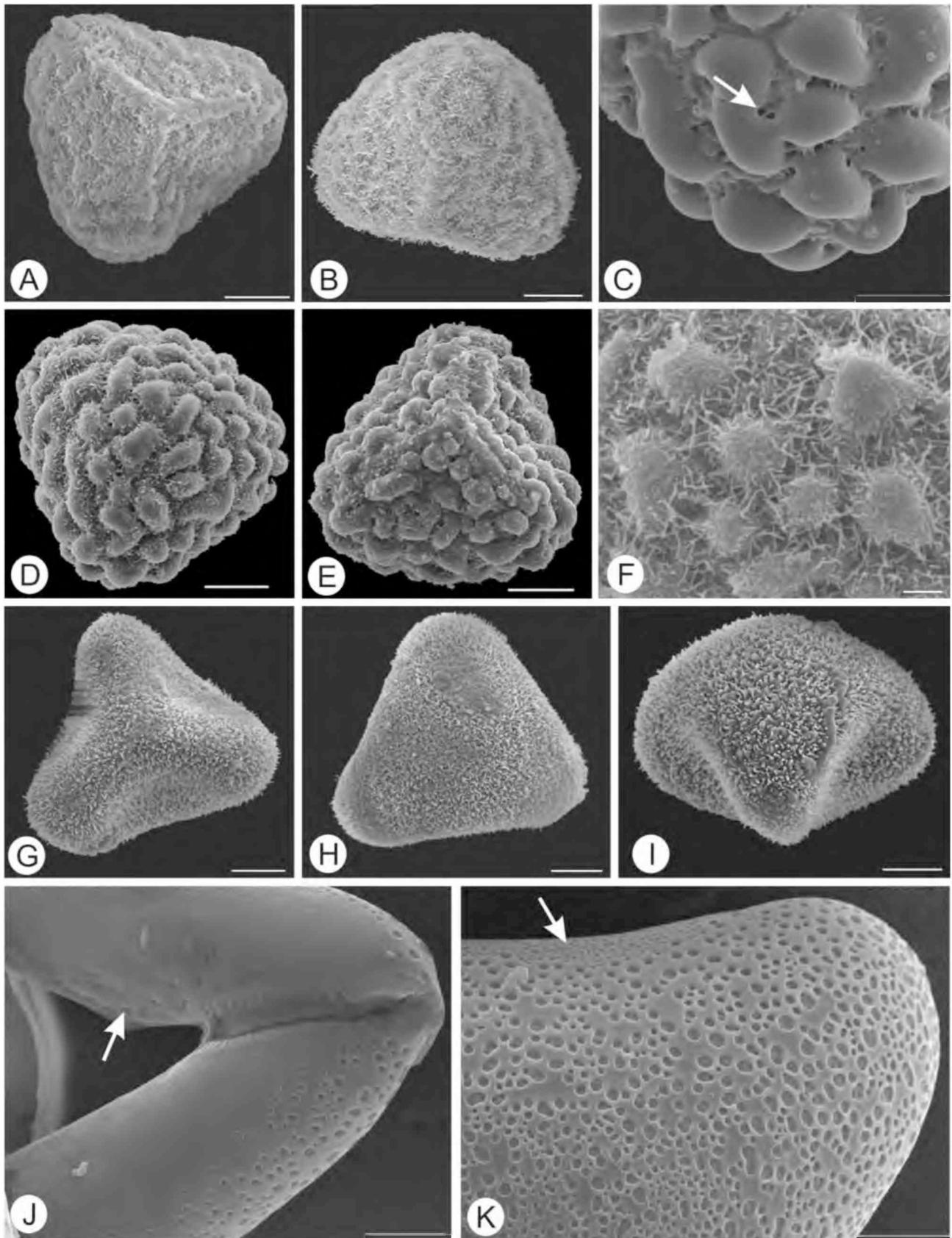


Figure 2. Spores of *Cyathea delgadii*, *C. myriotricha* and *C. phalerata* by means of SEM. **A–C.** *C. delgadii* spores: **A.** Proximal view; the surface is verrucate and it is covered with a network of rodlets; **B.** Equatorial view of a spore with perispore; **C.** Detail of a spore surface devoid of perispore; the exospore is verrucate and has perforations located in the base of the verrucae (arrow). **D–E.** *C. myriotricha* spores:

## Results

### General morphology

The spores of the *Cyathea* species studied here are trilete, triangular in polar view, with generally concave sides and rounded corners (Figures 1A, F, 2A, G, 3A, D). They are plane-hemispheric to convex-hemispheric in equatorial view (Figures 2B, I, 3B). Laesurae are straight and generally never reach the equator (Figures 1B, 2A, J, 3H, I). Spores are 29.0–55.6 µm in equatorial diameter and 21.6–48.1 µm in polar diameter. The exospore is 1.2–3.7 µm thick, psilate, perforate or verrucate, and the perispore is 0.3–0.6 µm thick (Table I).

### Perispore

The perispore is thin, 0.3–0.6 µm thick and appears dark brown when seen in LM. The spore surface bears a tri-dimensional network of rodlets with either free or fused distal edges, forming small spinules of variable sizes (Figures 1D, E, 2F, 3C). The surface of the rodlets is smooth or irregular (Figure 1I, J). Spores of *Cyathea hirsuta* and *C. leucofolis* (Figure 3E–H) were devoid of perispore or only had a fine deposition of perispore.

TEM studies of the perispore (P) show two clearly discernible layers, the inner layer (P1) and the outer layer (P2). The inner layer is composed of two strata, an inner and an outer stratum (iP1 and oP1) (Figure 4). The inner stratum is 20–90 nm thick with an undulate margin and is made up of slender, randomly fused threads, 10 nm in diameter (Figure 5C, D). In SEM, the surface of the inner stratum is granular (Figure 4D). The outer stratum is 50–500 nm thick and consists of small rods, *c.* 100 nm in diameter, circular in section, with a central channel, 10–20 nm in diameter (Figures 4C, F, 5C, D, 6). Basal rods are oriented tangentially to the spore surface, the more distal ones are perpendicular to the surface (Figure 5C, D) and may be fused at their tips to form spinules (Figure 5C). The substructure of the rods forming the oP1 layer is complex. Each rod is an independent unit with subunits helichoidally arranged around the central channel. These subunits consist of small threads, 10–20 nm in diameter (Figure 6). Finally,

the outer perispore layer (P2) is thin and has a high electrodensity. It is attached to the outer P1 layer (Figure 5C, D).

### Exospore

The exospore is 1.2–3.4 µm thick and appears yellowish when seen under LM. In *Cyathea delgadii*, the thickness of the exospore is variable due to the presence of verrucae. It is 2.9–3.7 µm thick in the verrucae, and 1.6–2.4 µm thick in between them. The exospore surface is variable. In *C. corcovadensis*, *C. hirsuta*, *C. leucofolis* and *C. villosa*, the surface is characterised by perforations of variable size (Figures 1G, H, 3C, E, G, H). Fractured surfaces and ultrathin sections show that the perforations are fovea, about 100 nm in depth (Figure 4A–C, F). In *C. atrovirens*, the entire exospore surface is psilate (Figures 1B, 5A, C, D), while the surface is proximally psilate and distally perforated in *C. phalerata* (Figure 2J, K). In *C. delgadii* and *C. myriotricha*, the exospore is verrucate (Figure 2A–F), which fuse and form complex structures, similar to short muri (Figure 2C, D). Verrucae have a parallel orientation at the laesurae, but are differently oriented at the surface of the laesurae (Figure 2A, E). The surface of the verrucae is smooth with perforations at their bases (Figure 2C). A common feature of all the studied species is the presence of perforations in the laesurae margins (Figures 1C, H, 2J, 3G, H).

The exospore consists of two layers (Figures 4C, E, F, 5A): the inner layer (iE) is thin with distinctly high electrodensity at the laesura base (Figure 4F) and the outer layer (oE) is lighter to electrons and thicker, the oE/iE relationship being 4:1. The ultrastructure of both exospore layers is massive, homogeneous and quite difficult to discern. Radial channels and cavities with a contrasted content are present all along the exospore, but they are more evident in the inner part of the outer exospore (Figure 5A, B) and in the middle and inner sides of the commissurae of each laesura (Figure 4F).

## Discussion and conclusions

The spores of *Cyathea* studied here are characterised by a three-dimensional network of rodlets with free distal edges, several of which are fused forming

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**D.** Distal view; the surface is verrucate and rodlets are visible between verrucae; **E.** Proximal view; superficial rods are visible. **F.** *C. delgadii*; detail of the distal surface covered with perispore; large rods are loosely intermixed between verrucae; at the verrucae surface are the rodlets intermixed in a compact way. **G–K.** *C. phalerata* spores: **G–I.** Spore surface covered by perispore rodlets, spore in proximal view (**G**), distal view (**H**) and equatorial view (**I**); **J–K.** Details of the surface in a spore devoid of perispore; **J.** Detail of the proximal surface of a spore with a smooth exospore; the perforations are restricted to the laesura margins (*arrow*); **K.** Detail of the exospore at the distal surface, completely covered with perforations variable in size; note the density of small perforations in the equatorial area (*arrow*). Scale bars – 10 µm (A, B, D, E, G, H, I), 2 µm (F), 5 µm (C, J, K).

Table I. Morphological data of *Cyathea* spores from southern South America (dimensions in  $\mu\text{m}$ ).

Species	Equatorial diameter	Polar diameter	Perispore thickness	Exospore thickness	Exospore surface
<i>Cyathea atrovirens</i>	42.3–(48.1)–55.6	41.5–(44.0)–48.1	0.6	2.0–2.8	Psilate
<i>Cyathea corcovadensis</i>	42.3–(51.5)–54.8	34.9–(42.3)–45.7	0.6	2.5–3.4	Perforate
<i>Cyathea delgadii</i>	39.0–(43.2)–44.8	32.0–(35.7)–39.0	0.3	1.6–3.7	Verrucate
<i>Cyathea hirsuta</i>	29.0–(32.4)–34.9	21.6–(24.9)–28.2	No	1.2–1.9	Perforate
<i>Cyathea leucofolis</i>	39.8–(41.5)–44.0	29.0–(31.5)–34.9	No	1.2–1.9	Perforate
<i>Cyathea myriotricha</i>	37.5–(38.8)–40.0	?	?	?	Verrucate
<i>Cyathea phalerata</i>	45.7–(51.5)–54.8	38.2–(41.5)–46.5	0.3	1.6–2.3	Distal: perforate Proximal: psilate

spines. This is in line with observation for *Cyathea* spores from other areas (Barth, 1975; Tryon & Lugardon, 1991; Lorscheitter et al., 1999), and the features may be used to support the *Cyathea* clade as indicated by earlier cladistic studies (Conant et al., 1996; Korall et al., 2007) and the systematic treatments of Barrington (1978), Gastony (1979), Tryon and Tryon (1982), and Holtum and Edwards (1983).

Along with information from acetolysis technique with NaOH by Gastony (1974), our TEM and SEM observations clarified that the ornamentation elements are part of the perispore formed from rodlets that are either smooth or with an irregular surface. Both smooth and irregular rodlets may occur on a single spore and may be related to different stages in spore maturation.

In spores of *Cyathea leucofolis* and *C. hirsuta*, the perispore is absent or partially deposited. Gastony (1979) reported that these two species in addition lack a perispore or have a not fully developed perispore. However, it would be interesting to study the ontogeny of the spores since there could be failures in perispore deposition.

The homogeneity found in the perispore sculpture and structure of the *Cyathea* species studied is in agreement with observations made by Gastony (1979), who suggested that spore features might reflect phylogenic relationships between *Trichipteris*, *Cyathea* and the *Sphaeropteris aterrima* group. The features of the perispore, in addition to the presence of marginate scales without an apical seta at the petiole base, together with results of recent phylogenetic analyses (Conant et al., 1996; Korall et al., 2007), would reinforce the concept for inclusion in the genus *Cyathea sensu* Lellinger (1987).

Three patterns were identified for the exospore surface: (a) laevigate, present in *Cyathea atrovirens*; (b) pitted in either one or both distal and proximal surfaces, present in *C. axilaris*, *C. corcovadensis*, *C. hirsuta*, *C. villosa* and only on the distal surface, in *C. phalerata*; and (c) verrucate, present in *C. delgadii* and *C. myriotricha*.

Recent studies in Selaginellaceae (Korall & Taylor, 2006), Dryopteridaceae (Moran et al.,

2007) and Cyatheaceae (Moran et al., 2008) showed that palynological characteristics are valuable in phylogenetic and systematic analysis of ferns and Lycophytes. Conant et al. (1996) correlated the different types of spores in Cyatheaceae with molecular phylogenetic trees and recognised three basic types of spores among the *Cyathea* clades: verrucate in the *Cyathea divergens* group, plain in the *Cyathea armata* and *Cyathea gibbosa* groups, and triporate in the *Cnemidaria* group. We compared the analysis made by Conant et al. (1996) with the spore morphology data provided by Gastony (1979), and identified the *Cyathea armata* group by the presence of exospore perforations, and the *Cyathea gibbosa* group by the absence of perforations. Therefore, we deduced that the absence of perforations would be a derived character state.

The exospore surface of the studied species has this variability. It can be inferred that the perforated surface of *Cyathea corcovadensis*, *C. hirsuta*, *C. leucolepis* and *C. villosa* spores is a plesiomorphic character, and therefore, these species would be placed in basal clades within the genus *Cyathea*. However, *C. atrovirens* and *C. phalerata* could belong to the most derived groups because they have spores with psilate exospores. Conant et al. (1996) stated that verrucate spores are a derived character because they were only present in the derived *C. divergens* group. However, Korall et al. (2007) recognised the presence of verrucate spores outside the *C. divergens* group and suggested that it might represent a plesiomorphic condition within *Cyathea*. Further phylogenetic studies on spore morphology can clarify the relevance of this character state.

TEM observations of the exospore revealed that inside the outer exospore (oE) is a zone with cavities and ramified channels. This zone was named a 'fissured stratum' by Lugardon (1971, 1974), typical for 'blechnoid' exospores. In the case of *Cyathea delgadii*, the outer exospore has a superficial stratum that constitutes the verrucae and is characterised by a high electron density. Nevertheless, ultrastructural differences were not noticed regarding the rest of the layer.

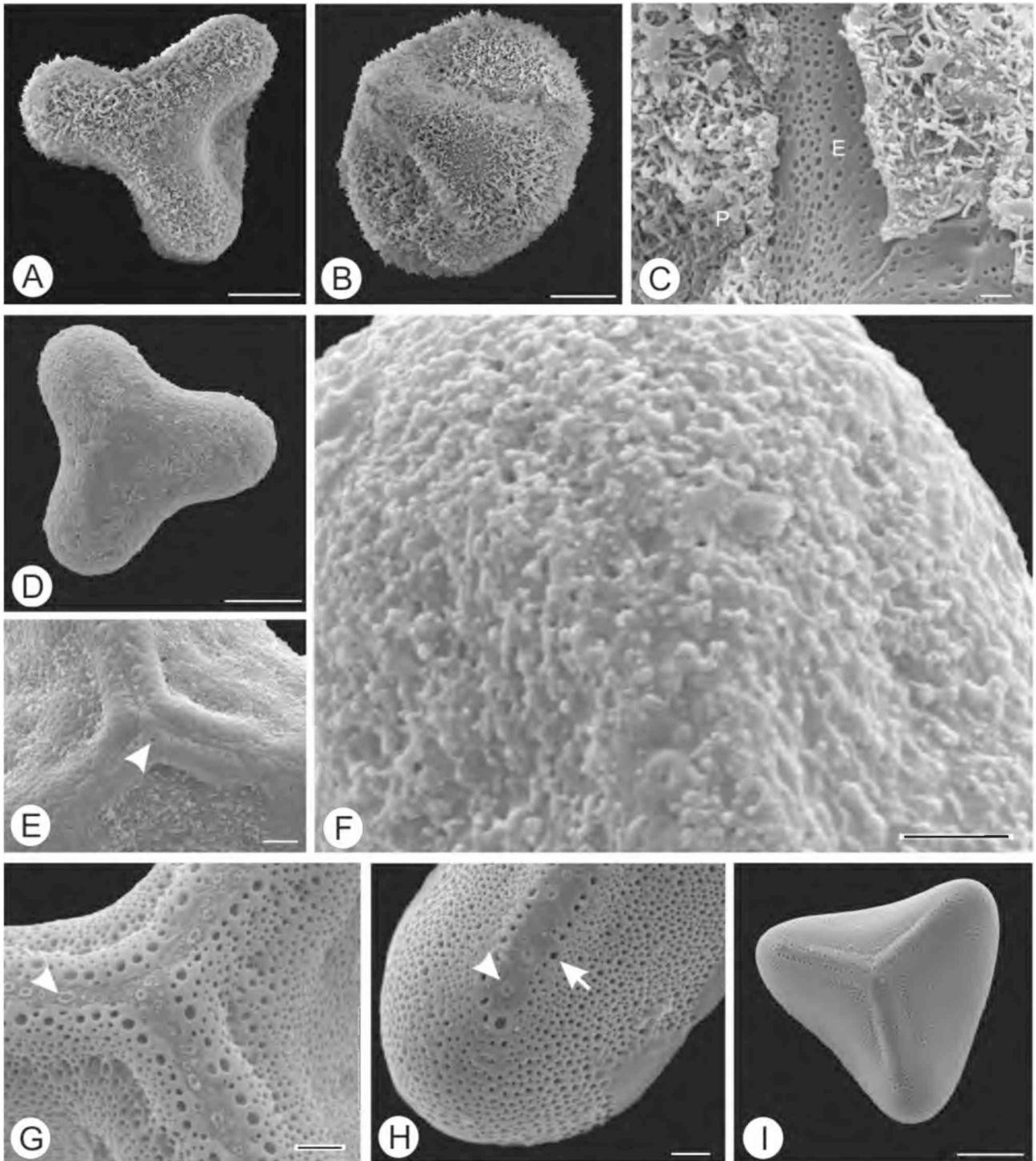
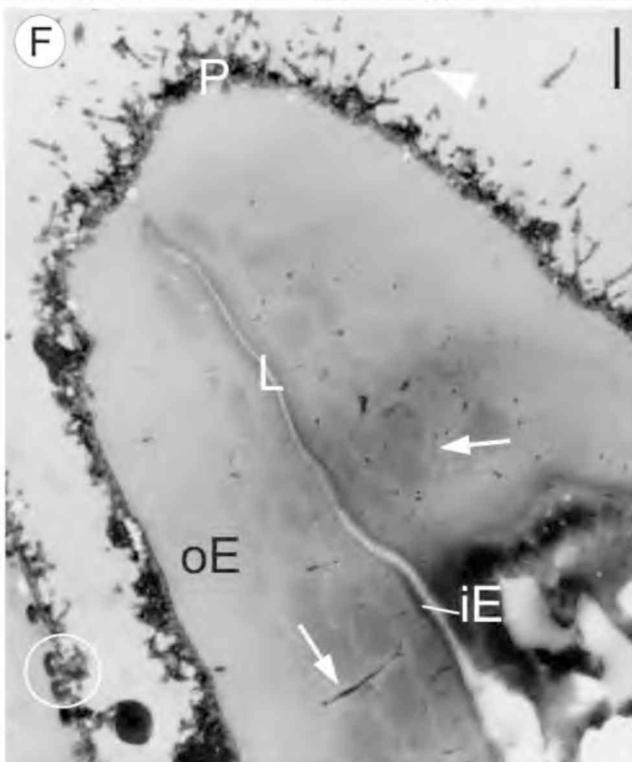
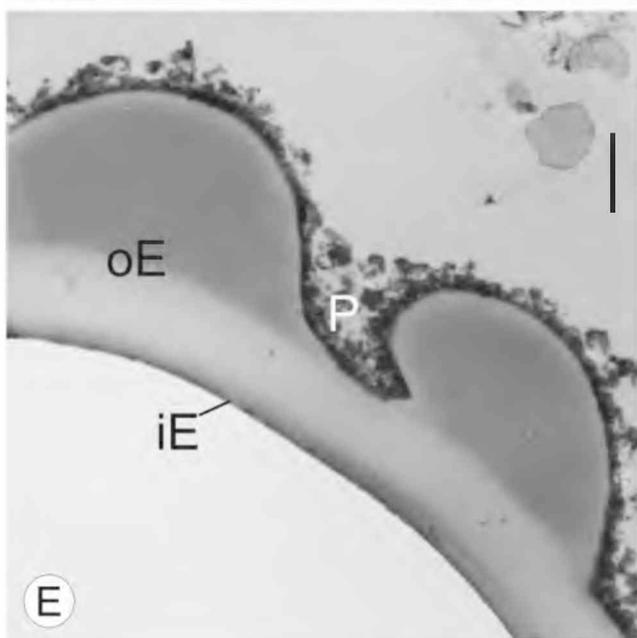
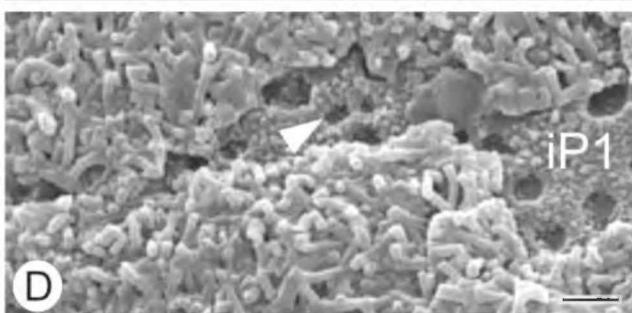
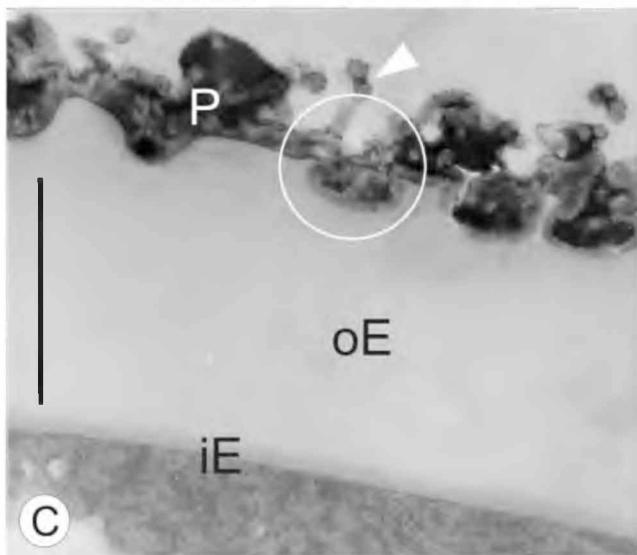
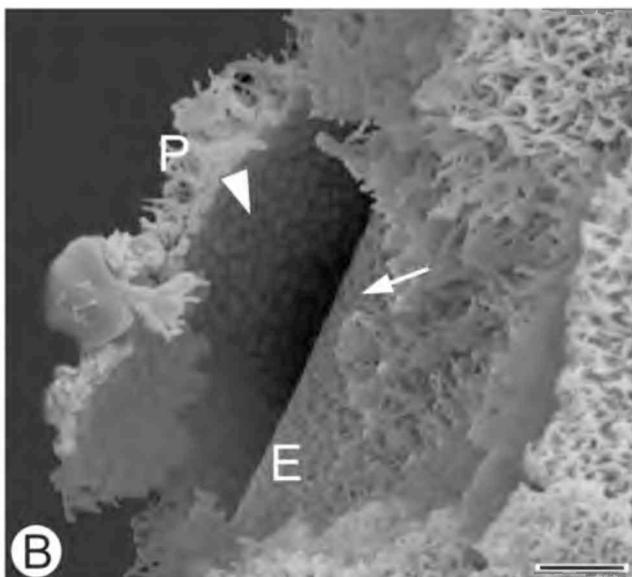
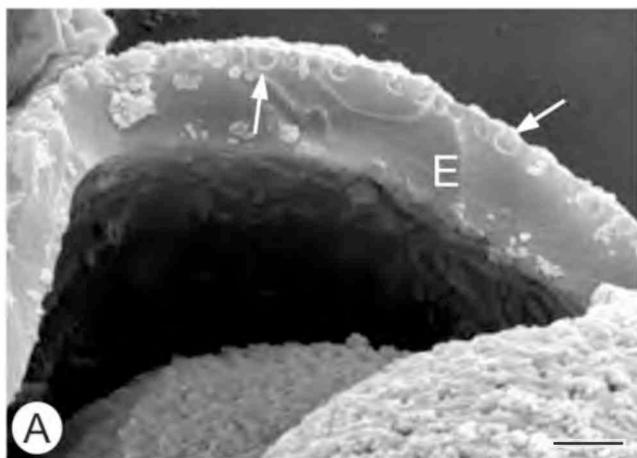


Figure 3. Spores of *Cyathea villosa*, *C. hirsuta* and *C. leucofolis* by means of SEM. **A–C.** *C. villosa* spores: **A, B.** The rodlets of the perispore cover the whole surface, proximal view (**A**), equatorial view (**B**); **C.** Fracture in the proximal zone that exposes the exspore (**E**), with perforations; small rods are seen on the perispore surface (**P**). **D–G.** *C. hirsuta* spores: **D.** Distal view; a granular deposit is visible on the surface; **E.** Detail of the proximal surface in the place where the laesurae join; perforations with a thick margin are present on the laesurae surfaces (*arrowheads*); **F.** Detail of the proximal surface; a granular deposit covers the spore surface; **G.** Detail of the proximal surface of a spore devoid of perispore, in the place where the laesurae join; note the perforation with a thick margin (*arrowhead*). **H, I.** *C. leucofolis* spores: **H.** Detail of the proximal surface of a spore with a perforate exspore; the perforations have larger diameters on the laesurae margins (*arrow*); the perforations on the commissure (*arrowheads*) have a thick margin; **I.** Proximal view of a spore devoid of perispore. Scale bars – 10  $\mu\text{m}$  (**A, B, D, I**), 2  $\mu\text{m}$  (**E–H**), 1  $\mu\text{m}$  (**C**).



The type of inner perispore structure (including sub-unit structure) is similar to cylindrical units described by Wittborn et al. (1998) for *Fagus sylvatica* and *Lycopodium clavatum*. According to these authors, the sub-structural units would also be formed of sub-units that are helichoidally arranged. This hypothesis is reinforced by the size of the structural units and sub-units, their spatial arrangement and a typical striation. The use of more powerful microscopes would provide more accurate information about the fine structure of these elements. Our results support the hypothesis by Rowley (1981, 1988, 1990, 1995) and Rowley and Dahl (1982) that the wall sub-structures of spore/pollen are built by helichoidal sub-structural units. Thus, this contribution differs from the interpretations by Southworth (1986) and Tryon and Lugardon (1991), who considered that the basic sub-structural units of the perispore are granules.

Finally, our results suggest that there is homogeneity within the perispore of the spores in the genus *Cyathea*. In addition, this ultrastructural analysis provides original data that could be of help for evolutionary estimations and functionality of the sporoderm.

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## Specimens investigated

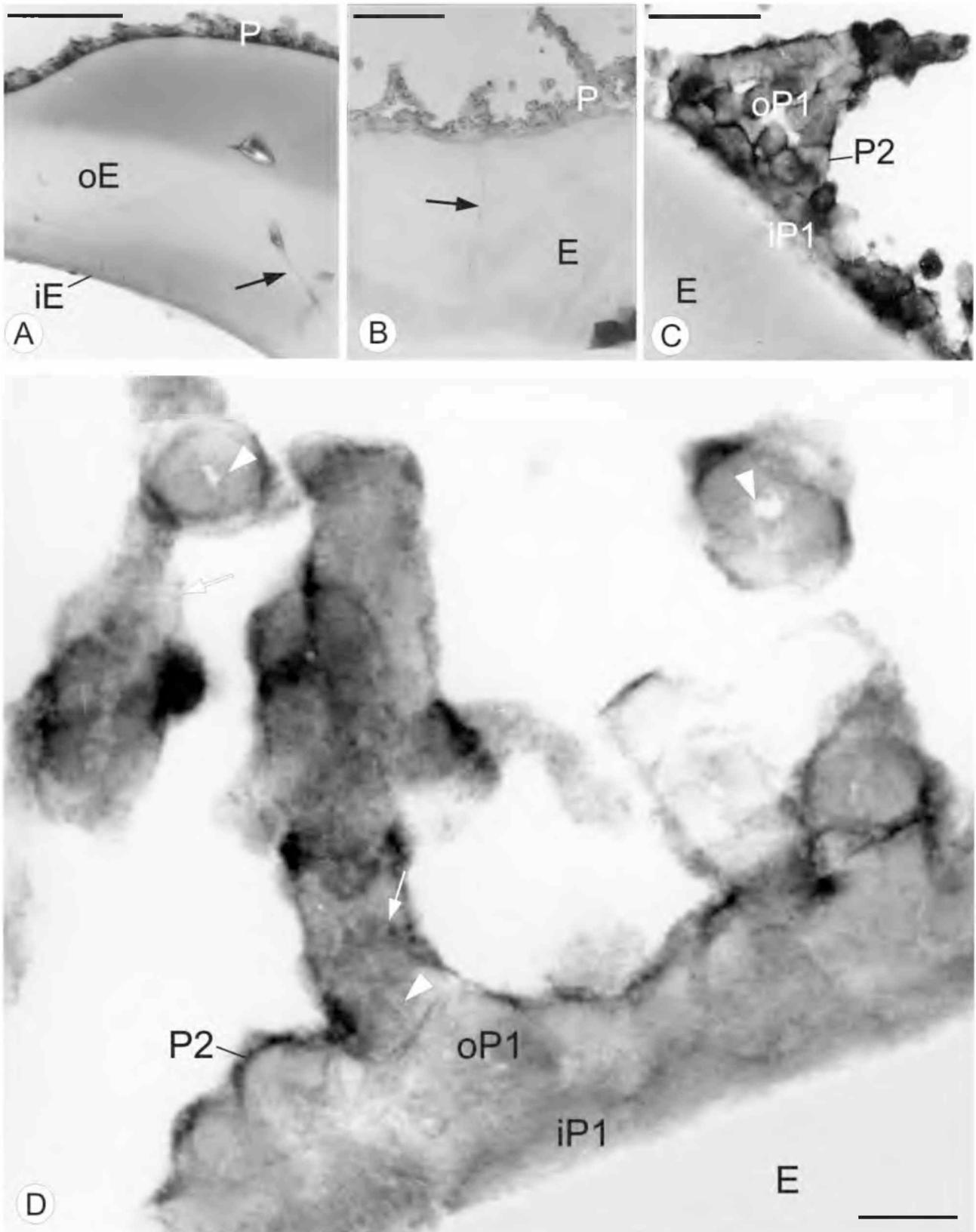
- Cyathea atrovirens* (Langsd. & Fisch.) Domin. Argentina: Corrientes, Ituzaingó, Tressens et al. 372 (LP, CTES), MP 4084; idem, Misiones, Gral. Manuel Belgrano, Partridge s/n (BA 70619 a-b-c), MP 4099; idem, Iguazú, *Rodriguez 430* (SI, BA), MP 4078; idem, Guaraní, 28/4/1997, Morrone et al. 2181 (SI), MP 4080; idem, San Pedro, P. P. Piñalito, Márquez & Carrión 181 (LP); idem, San Ignacio, P. P. Teyu Cuare, Márquez et al. 230 (CTES, LP); idem, San Antonio, Capurro 935 (BA). Brazil: Rio Grande do Sul, Rio Pardo, Jürgens s/n (Rosenstock 257) (SI). Paraguay: San Pedro, Col. Guayaibí, Krapovickas et al. 14282 (SI).
- C. corcovadensis* (Raddi) Domin. Brazil: Parana, Pereira 8224 (LP), MP 4105; idem, Curitiba, Krapovickas et al. 23143 (LP), MP 4104; idem, Guaratuba, Dusén 13729 (SI), MP 4102; Santa Caterina, Lages, Spanagel s/n (Rosenstock 240) (LP), MP 4103.
- C. delgadii* Sternb. Argentina: Corrientes, Ituzaingó, Meyer 6278 (US 2361678). Brazil: Santa Caterina, Sao Jose, Fernandes 1132 (SPF); Parana, Paranagua, Fernandes et al. 1117 (SPF), MP 4127; idem, Piraquara, Fernandes 1115 (SPF).
- C. hirsuta* C. Presl. Brazil: Parana, Paranagua, Fernandes 1123 (SPF); Santa Caterina, Rio do Sul, Fernandes 1150 (SPF); idem, Ilha Santa Catarina, Sehnem 786 (PACA); idem, Morro da lagoa, Sehnem 8398 (PACA).
- C. leucofolis* Domin. Brazil: Parana, Morretes, Hatschbach 30390 (PACA); idem, Guaratuba, Krieger 11129 (PACA).

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Figure 4. Spore wall structure (SEM) and ultra-structure (TEM) in species of *Cyathea*. **A, B.** *C. villosa* (SEM): fracture of the wall with pointed faveoli (**A**) at the outer margin (*arrows*) of the exospore (E), and a perispore (**B**) detached from the exospore (E); the protuberances (*arrowheads*) visible in the inner surface of the perispore (P) exactly match the perforation (*arrows*) bases of the exospore surface. **C, D.** *C. corcovadensis* (SEM and TEM): **C.** TEM image of the inner exospore (iE), the outer exospore (oE) and the perispore (P); note the fovea (*circle*) in the exospore margin; the perispore was deposited within the foveoli; a rodlet of the perispore (*arrowhead*); **D.** Fracture of the perispore (EM), where the inner stratum (iP1) of the inner layer of the perispore covers the perforate surface (*arrowhead*) of the exospore and the outer stratum is formed by rodlets. **E.** *C. delgadii* (TEM): wall section with large verrucae that are characteristic of the exospore in this species; the latter consists of two layers: the inner layer (iE) and the outer layer (oE); the perispore (P) covers the exospore surface completely. **F.** *C. corcovadensis* (TEM): section of the sporoderm through the laesura; the inner exospore (iE), the outer exospore (oE) and the perispore (P) with rodlets (*arrowhead*) are visible; in the exospore, cavities and straight and fused channels (*arrows*) are present close to the laesura (L). Note that the exospore is continuous on the commissure. Note the foveoli of the exospore (*circle*) in another spore, at the bottom left corner of the figure. Scale bars – 2 µm (B), 1 µm (A; D–F), 500 nm (C).

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Figure 5. Spore wall ultra-structure in *Cyathea* species by means of TEM. **A.** *C. delgadii*, the inner exospore (iE) and the outer exospore (oE) with a ramified channel (*arrow*) are visible; rodlets constitute the perispore (P) on the surface. **B–D.** Wall sections of *C. atrovirens*: **B.** The exospore (E) and the perispore (P) are visible; a radial channel (*arrow*) is evident within the exospore; in the perispore, the inner stratum is of uniform thickness and the outer stratum is composed of rods; **C.** A spine formed by fusion of several rodlets of the perispore outer stratum (oP1); in the limit between perispore and exospore (E) the less electron-dense inner stratum (iP1) is visible; the outer perispore (P2) is evident as electron-dense layer with a thin cover on the rods; **D.** Each rod has inner channels in longitudinal and transversal sections (*arrowhead*) with threads coiled around the channels (*arrows*); (iP1) inner stratum, (oP1) outer stratum, (P2) outer perispore, (E) exospore. Scale bars – 1 µm (A, B), 500 nm (C), 100 nm (D).



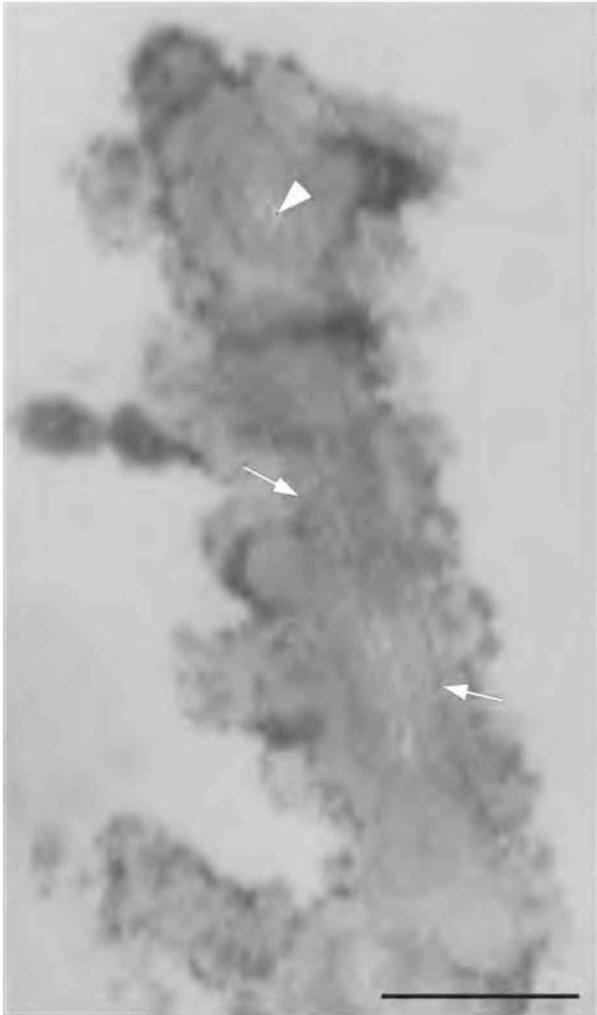


Figure 6. General structure of a perispore thread: a rod of the perispore (here of *Cyathea atrovirens*) with the sub-units (arrows) coiled around the inner channel (arrowhead). Scale bar – 100 nm.

*C. myriotricha* (Baker) R. C. Moran & J. Prado. Brazil: Parana, Jaguariaíva, próximo ao Rio Jaguariaíva Schwartzburd et al. 846 (UPCB).

*C. phalerata* Mart. Brazil: Parana, Paranaguá, Fernandes & Abreu 1118 (SPF), MP 4135; idem, Ponta Grossa, Krieger 10818 (SPF); Santa Caterina, Angelina, Fernandes 1133 (SPF); idem, Rio do Sul, Fernandes 1148 (SPF); idem, Ilhota, Fernandes 1140 (SPF), MP 1140.

*C. villosa* Willd. Brazil: Parana, Senges, Hatschbach 26771 (CTES), MP 4125; idem, Furnas, Reitz & Klein 17509 (US).

## References

Barth, O. M. (1975). Principais tipos de esporos de pteridofitos encontrados em sedimentos do Ar do Rio de Janeiro. *Leandra*, 6, 55–64.

Barrington, D. S. (1978). A revision of the genus *Trichipteris*. *Contributions from the Gray Herbarium of Harvard University*, 208, 3–93.

Conant, D. S., Raubeson, L. A., Attwood, D. K. & Stein, D. B. (1995). The relationships of Papuanian Cyatheaceae to New World tree ferns. *American Fern Journal*, 85, 328–340.

Conant, D. S., Stein, D. B., Valinski, A. E. C., Sudarsanam, P. & Ahearn, M. E. (1994). Phylogenetic implications of chloroplast DNA variation in the Cyatheaceae. I. *Systematic Botany*, 19, 60–72.

Conant, D. S., Raubeson, L. A., Attwood, D. K., Perera, S., Zimmer, E. A., Sweere, J. A. & Stein, D. B. (1996). Phylogenetic and evolutionary implications of combined analysis of DNA and morphology in the Cyatheaceae. *Proceedings of the Holtum Memorial Pteridophyte Symposium* (p. 231–247). Kew: Royal Botanic Gardens.

Christenhusz, M. J. M. (2009). New combinations and an overview of *Cyathea* subg. *Hymenophyllopsis* (Cyatheaceae). *Phytotaxa*, 1, 37–42.

Erdtman, G. & Sorsa, P. (1971). *Pollen and spore morphology/plant taxonomy. Pteridophyta (text and additional illustrations)*. Stockholm: Almqvist & Wiksell.

Esteves, L. M. & Felipe, G. M. (1985). Germination and morphology of spores of *Trichipteris corcovadensis*. *American Fern Journal*, 75, 92–102.

Gastony, G. J. (1974). Spore morphology in the Cyatheaceae, 1. The perine and the sporangial capacity: general considerations. *American Journal of Botany*, 61, 672–680.

Gastony, G. J. (1979). Spore morphology in the Cyatheaceae, 3. The genus *Trichipteris*. *American Journal of Botany*, 66, 1238–1260.

Gastony, G. J. & Tryon, R. (1976). Spore morphology in the Cyatheaceae, 2. The genera *Lophosoria*, *Metaxya*, *Sphaeropteris*, *Alsophila* and *Nephelea*. *American Journal of Botany*, 63, 738–758.

Harris, W. F. (1955). A manual of the spores of New Zealand Pteridophyta. *New Zealand Department of Scientific and Industrial Research Bulletin*, 116, 1–186.

Holtum, R. E. & Sen, U. (1961). Morphology and classification of the tree ferns. *Phytomorphology*, 11, 406–420.

Holtum, R. E. & Edwards, P. J. (1983). The tree-ferns of Mount Roraima and neighbouring areas of the Guayana Highlands with comments on the family Cyatheaceae. *Kew Bulletin*, 38, 155–188.

Korall, P. & Taylor, W. A. (2006). Megaspore morphology in the Selaginellaceae in a phylogenetic context: a study of the megaspore surface and wall structure using scanning electron microscopy. *Grana*, 45, 22–60.

Korall, P., Pryer, K. M., Metzgar, J. S., Schneider, H. & Conant, D. S. (2006). Tree ferns: Monophyletic groups and their relationships as revealed by four protein-coding plastid loci. *Molecular Phylogenetics and Evolution*, 39, 830–845.

Korall, P., Conant, D. S., Metzgar, J. S., Schneider, H. & Pryer, K. M. (2007). A molecular phylogeny of scaly tree ferns (Cyatheaceae). *American Journal of Botany*, 94, 873–886.

Lellinger, D. B. (1987). The disposition of *Trichopteris* (Cyatheaceae). *American Fern Journal*, 77, 90–94.

Lorscheitter, M. L., Ashraf, A. R., Windisch, P. G. & Mosbrugger, V. (1999). Pteridophyte spores of the Rio Grande do Sul flora, Brazil. Part II. *Palaeontographica Abt. B*, 251, 71–235.

Lugardon, B. (1971). *Contribution à la connaissance de la morphogénèse et de la structure des parois sporales chez les Filicinaées isosporées*. Toulouse: Université Paul Sabatier, PhD Diss.

Lugardon, B. (1974). La structure fine de l'exospore et de la perispore des Filicinaées isosporées. *Pollen et Spores*, 16, 161–226.

Márquez, G. J. (2009). *Estudios morfológicos y ultraestructurales en esporas de Cyatheaceae del Cono Sur*. La Plata: Facultad de Ciencias Naturales y Museo, Universidad de La Plata, PhD Diss.

Márquez, G. J., Morbelli, M. A. & Giudice, G. E. (2007). Análisis comparativo de las esporas del género *Alsophila*

- (Cyatheaceae) del Cono Sur. *Boletín de la Sociedad Argentina de Botánica*, 42, 123.
- Márquez, G. J., Morbelli, M. A. & Giudice, G. E. (2008). Morfología y ultraestructura de las especies de *Cyathea* (Pteridophyta) del Cono Sur de América meridional. *Boletín de Resumos, XII Simpósio Brasileiro de Paleobotânica y Palinología, Florianópolis, Brasil*, 137.
- Márquez, G. J., Morbelli, M. A. & Giudice, G. E. (2009). Comparative analysis of spores of *Alsophila* (Cyatheaceae) species from southern South America. *Review of Palaeobotany and Palynology*, 156, 165–176.
- Moran, R. C., Garrison Hanks, J. & Rouhan, G. (2007). Spore morphology in relation to phylogeny in the fern genus *Elaphoglossum* (Dryopteridaceae). *International Journal of Plant Sciences*, 168, 905–929.
- Moran, R. C., Prado, J., Labiak, P. H., Garrison Hanks, J. & Schuettpelz, E. (2008). A “new” tree fern species from southeastern Brazil: *Cyathea myriotricha* (Cyatheaceae). *Brittonia*, 60, 362–370.
- Murillo, M. T. & Bless, M. J. M. (1974). Spores of recent Colombian Pteridophyta. I. Trilete spores. *Review of Palaeobotany and Palynology*, 18, 223–269.
- Nayar, B. K. & Devi, S. (1966). Spore morphology of the pteridoid ferns. *Grana Palynologica*, 6, 476–503.
- Rowley, J. R. (1981). Pollen wall characters with emphasis upon applicability. *Nordic Journal of Botany*, 1, 357–380.
- Rowley, J. R. (1988). Substructure within the endexine, an interpretation. *Journal of Palynology*, 24, 29–42.
- Rowley, J. R. (1990). The fundamental structure of the pollen exine. *Plant Systematics and Evolution, Supplement*, 5, 13–29.
- Rowley, J. R. (1995). Are the endexines of pteridophytes, gymnosperms and angiosperms structurally equivalent? *Review of Palaeobotany and Palynology*, 85, 13–34.
- Rowley, J. R. & Nilsson, S. (1972). Structural stabilisation for electron microscopy of pollen from herbarium specimens. *Grana*, 12, 23–30.
- Rowley, J. R. & Dahl, A. O. (1982). A similar substructure for tapetal surface and exine “tuft”-units. *Pollen et Spores*, 24, 5–8.
- Schuettpelz, E. & Pryer, K. M. (2007). Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon*, 56, 1037–1050.
- Simabukuro, E. A., Esteves, L. M. & Gil Felipe, M. (1998). Fern spore morphology and spore rain of a preserved Cerrado region in southeast Brazil (Reserva Biológica e Estação Experimental de Moji Guaçu, São Paulo). *American Fern Journal*, 88, 114–137.
- Southworth, D. (1986). Substructural organization of pollen exines. In S. Blackmore & I. K. Ferguson (Eds), *Pollen and spores. Form and function* (p. 61–69). London: Academic Press.
- Tryon, A. F. & Lugardon, B. (1991). *Spores of the Pteridophyta. Surface, wall structure and diversity based on electron microscope studies*. New York: Springer Verlag.
- Tryon, R. M. (1970). The classification of the Cyatheaceae. *Contributions from the Gray Herbarium of Harvard University*, 200, 3–53.
- Tryon, R. M. (1976). A revision of the genus *Cyathea*. *Contributions from the Gray Herbarium of Harvard University*, 206, 19–98.
- Tryon, R. M. & Tryon, A. F. (1982). *Ferns and allied plants with special reference to tropical America*. New York: Springer Verlag.
- Wittborn, J., Rao, K. V., El-Ghazaly, G. & Rowley, J. R. (1998). Nanoscale similarities in the substructure of the exines of *Fagus* pollen grains and *Lycopodium* spores. *Annals of Botany*, 82, 141–145.