Grana 43: 231–237, 2004

Taylor & Francis
Taylor & Francis Group

Spore wall ultrastructure in *Anogramma* species (Pteridaceae) from Argentina

JUAN P. RAMOS GIACOSA•, MARTA A. MORBELLI and GABRIELA E. GIUDICE

Ramos Giacosa, J. P., Morbelli, M. A. & Giudice, G. E. 2004. Spore wall ultrastructure in *Anogramma* species (Pteridaceae) from Argentina. – Grana 43: 231–237. ISSN 0017-3134.

The sporoderm ultrastructure of *Anogramma* Link species, which grow in Argentina, was studied using scanning and transmission electron microscopy. The species *A. chaerophylla* (Desv.) Link and *A. lorentzii* (Hieron.) Diels were studied with TEM for the first time. The spores of both species have a sculptured, apparently two-layered exospore. The perispore is uniformly thickened on the whole surface: in *A. chaerophylla* it is three-layered, while, in *A. lorentzii* it is single-layered with a complex structure. Spherules are present on the perispore surface or incorporated into the structure of *A. chaerophylla* while, globules exist on and within the perispore in *A. lorentzii*. The sporoderm ultrastructure in these two species was compared with other cingulate genera within the Pteridaceae. The characteristics found in this work, with respect to spore wall structure and general morphology, suggest that these characters may differentiate species within genus.

Juan P. Ramos Giacosa & Gabriela E. Giudice, Cátedra de Morfología Vegetal; Marta A. Morbelli, Cátedra de Palinología; Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, B1900FWA, La Plata, Argentina*
**Corresp. Author*
E-mail: jpramosgiacosa@hotmail.com.

(*Manuscript received 10 November 2003; accepted 2 August 2004*)

The genus *Anogramma* Link is represented by six species that are distributed through tropical and subtropical America, meridional Europe and New Zealand (de la Sota 1977). According to Ponce (1996), two species grow in Argentina: a tropical American species *A. chaerophylla* (Desv.) Link and *A. lorentzii* (Hieron.) Diels, which also grows in Brazil and Uruguay (Tryon 1962).

The vegetative and reproductive features of *Anogramma* were described by Tryon & Tryon (1982). The palynological aspects of this genus are described in a few Palynological Floras e.g. published by Large & Braggins (1991) and Lugardon (1963). A detailed synthesis of the spore morphology by SEM can be found in Tryon & Lugardon (1991).

In Argentina, Michelena (1989) studied the spores of *A. chaerophylla* with SEM for the Palynological Flora of the Buenos Aires Province. Subsequently, an analysis of both species was made by Ramos Giacosa et al. (2001) using light and scanning electron microscopy.

Tryon & Lugardon (1991) described the sporoderm ultrastructure of *Anogramma leptophylla* (L.) Link as composed of a two-layered exospore, the outer one forming the sculpture and a two-layered perispore. This species grows in tropical America, Africa, southern Europe and eastern New Zealand (Tryon 1962). However, the ultrastructure of the sporoderm of *A. chaerophylla* and *A. lorentzii* has not been studied yet.

The aim of this study is to analyze the sporoderm of *Anogramma* species that grow in Argentina with transmission and scanning electron microscopy, in order to contribute to the general knowledge of these species and to compare the ultrastructure of both species to find out if the characteristics of the ultrastructure are stable or vary at certain taxonomic level.

MATERIAL AND METHODS

The study was based on herbarium material from the following Argentinean institutions: Instituto Miguel Lillo, Tucuman (LIL), División Plantas Vasculares, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (LP) and Instituto de Botanica Darwinion, San Isidro (SI).

For SEM analysis, the material was treated with hot 3% sodium carbonate for two minutes, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates and finally coated with gold.

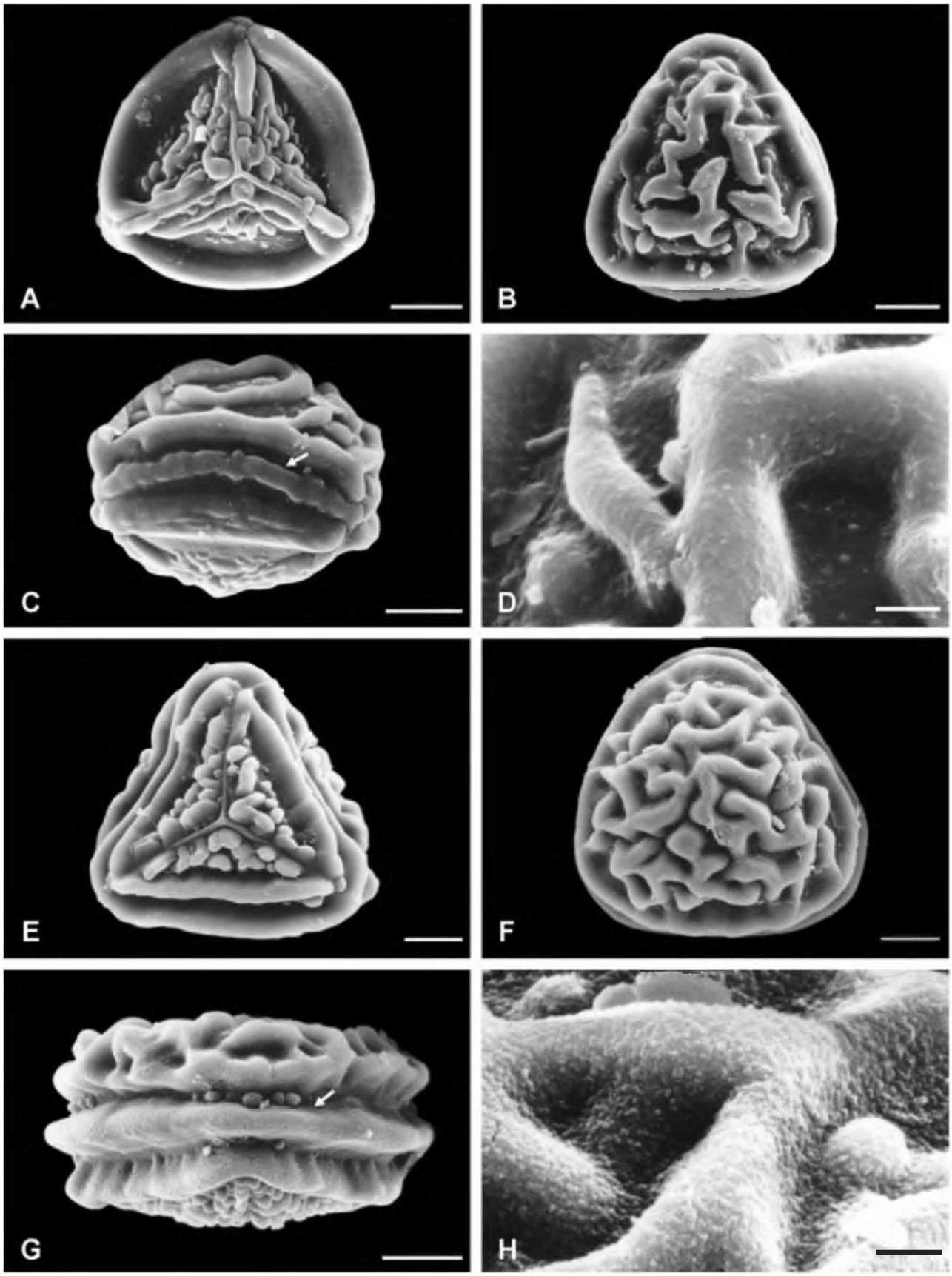
The observations were made with a JEOL JSMT-100 scanning electron microscope at the Museo de Ciencias Naturales de La Plata.

For TEM analysis, the spores removed from herbarium specimens were treated according to Rowley & Nilsson (1972): re-hydration with 0.1 M phosphate buffer and with 1% Alcian Blue for two hours followed by fixation with 1% glutaraldehyde + 1% Alcian Blue in phosphate buffer for 12 hours; washing for 15 min in phosphate buffer and post-fixation with 1% Osmium tetroxide in water plus 1% Alcian Blue for two hours. The spores were dehydrated in an acetone series (30 – 100%) and then embedded in Spurr's soft mixture. Ultrathin sections were stained with 1% uranyl acetate for 15 min followed by lead citrate for three min.

The observations were made with a Zeiss T-109 TEM at the Instituto de Biología Celular, Universidad de Buenos Aires.

• CONICET scholar

 ISSN 0017-3134
DOI: 10.1080/00173130410000767


A
B
C
D
E
F
G
H

RESULTS

Anogramma chaerophylla (Figs 1 A–D, 2 A–D)

The spores are trilete, triangular shape in polar view with straight to convex sides and round corners. An equatorial ridge is present (Fig. 1 A–C). There are two coarse, quite well defined ridges flanking each side of the equatorial ridge. The equatorial diameter is 35–52 μm, and polar axis is 28–50 μm.

When observed in SEM the sculpture appears verrucate with a few ridges. Number and size of these sculptural elements varies at the two poles with larger elements occurring distally (Figs 1 A–C).

The exospore is variable in thickness. As observed in TEM it is two-layered: a very thin layer (<100 nm) lining the inner surface of exospore and the apertural slit; an outer layer composing the major part of the wall forming the ridges, verrucae, and including in its innermost area a system of very minute cavities connected to the outer surface by channels. These channels are evident in the outer, in the inner and medium part, according to the plane of sectioning in each case (Fig. 2 A).

The perispore studied with SEM appears slightly sculptured with few small verrucae (Fig. 1 D).

TEM studies show that the perispore is 270–410 nm thick and composed of three layers that differ in thickness, contrast and structure. The inner (Pi), adjacent to the exospore is 80–120 nm thick; it strongly contrasts with the exospore especially in its outer surface and exhibits dark spots. The middle layer (Pm) is 130–190 nm thick, with less contrast than the other layers (Pi, Po), and shows striations which coincide with the irregularities of the inner layer (Pi). The outer layer (Po) is 60–100 nm thick with three internal laminations, the middle lamination has less contrast than the other two strata (Fig. 2 A–D).

Spherules (*sensu* Tryon & Lugardon 1981, p. 9) are seen both on the perispore surface and/or embodied in its structure at the level of the outer layer (Po; Fig. 2 A–D). They are 200–400 nm in diameter, mostly circular and have a concentric arrangement of the different perisporal materials. The spherules are single or grouped, being in the last case coated with a thin layer of the perispore lamella (Fig. 2 C).

Anogramma lorentzii (Figs 1E–H, 3 A–F)

The spores are trilete, with triangular shape in polar view with straight sides and round corners. An equatorial ridge is present (Fig. 1 E–G). There are two coarse, quite well defined ridges flanking each side of the equatorial ridge. Equatorial diameter is 60–76 μm, and polar axis is 47–67 μm.

The exospore varies in thickness. In SEM the sculpture shows verrucae and ridges. Distally the exospore has fused ridges, giving partially reticulated appearance with a few verrucae (Fig. 1 E–H).

As seen with TEM it seems single-layered with a homogeneous structure. Radial channels, with a darkly contrasted inner structure, are evident in the medium and outer parts, according to the plane of sectioning in each case (Fig. 3 A, C).

When observed with SEM, the perispore surface has small verrucae and granules (Fig. 1 H).

As seen with TEM, the perispore is 500–600 nm thick and has more contrast than the exospore. Dark spots and condensed material are evident in particular areas. The perispore is apparently single-layered with a complex compact structure. This wall is laxer towards the outer surface, which is highly irregular. The structure could be interpreted as composed of threads with a helicoidal substructure. These threads are visible in both longitudinal and transverse sections. The transverse section is circular (Fig. 3 A–F).

There are globules (*sensu* Lugardon 1981, Tryon & Lugardon 1991, p. 8) of 1.9 to 2.1 μm in diameter. They have a central mass with less contrast made up of similar material to the exospore, wrapped in an envelope with the same features (structure, contrast and surface) as those of the perispore (Fig. 3 A, B). The globules are on the perispore surface (Fig. 3 A, B) or included into the inner part of it (Fig. 3 D, E). They are fused or slightly attached to the wall by thin threads (Fig. 3 A, B).

DISCUSSION AND CONCLUSIONS

The spores of *Anogramma* species that grow in Argentina are very similar to those of the related genus *Pityrogramma* Link in sculpture and in the presence of an equatorial ridge (Ramos Giacosa et al. 2001) and exospore ultrastructure.

Anogramma when analyzed with TEM has a two-layered exospore and a three- or single-layered perispore. On the other hand, these spores differ from those of *Pityrogramma*, which have a two-layered perispore (Tryon & Lugardon 1991).

The species *A. leptophylla*, which does not grow in Argentina, studied by Tryon & Lugardon (1991, p. 133, fig. 35.6, 35.7) has a two-layered perispore. These results contrast with the characteristics found in the species studied in the present work.

Anogramma species that grow in Argentina have an exospore with similar structure. In the exospore of both species there are channels associated with the laesurae and others radially oriented with a contrasted helicoidal content. These channels possibly facilitate transport across the exospore, during development by this wall to the perispore. Channels with a similar structure and orientation have been found in pollen grain exines by Rowley et al. (1987), as well as in the exospore of *Selaginella kraussiana* (Lycophyta) by

Fig. 1. SEM of *Anogramma chaerophylla* and *A. lorentzii* spores. A–D. *A. chaerophylla*. (A) Proximal view of a triangular-shaped spore with convex sides; (B) Distal view of a verrucate spore with a few ridges; (C) Equatorial view of a spore with an equatorial ridge (*arrow*); (D) Surface in detail that shows the exospore ridges covered by the perispore with small verrucae. E–H. *A. lorentzii*. (E) Proximal view of a triangular-shaped spore with straight sides; (F) The ornamentation of the distal face is ridged, partially reticulate; (G) Equatorial view of a spore with a continuous ridge all along this zone (*arrow*); (H) Surface in detail that shows the exospore ridges covered by the perispore with small verrucae and granules (Globules). Scale bars – 10 μm (in *A–C, E–G*); 2 μm (in *D, H*).

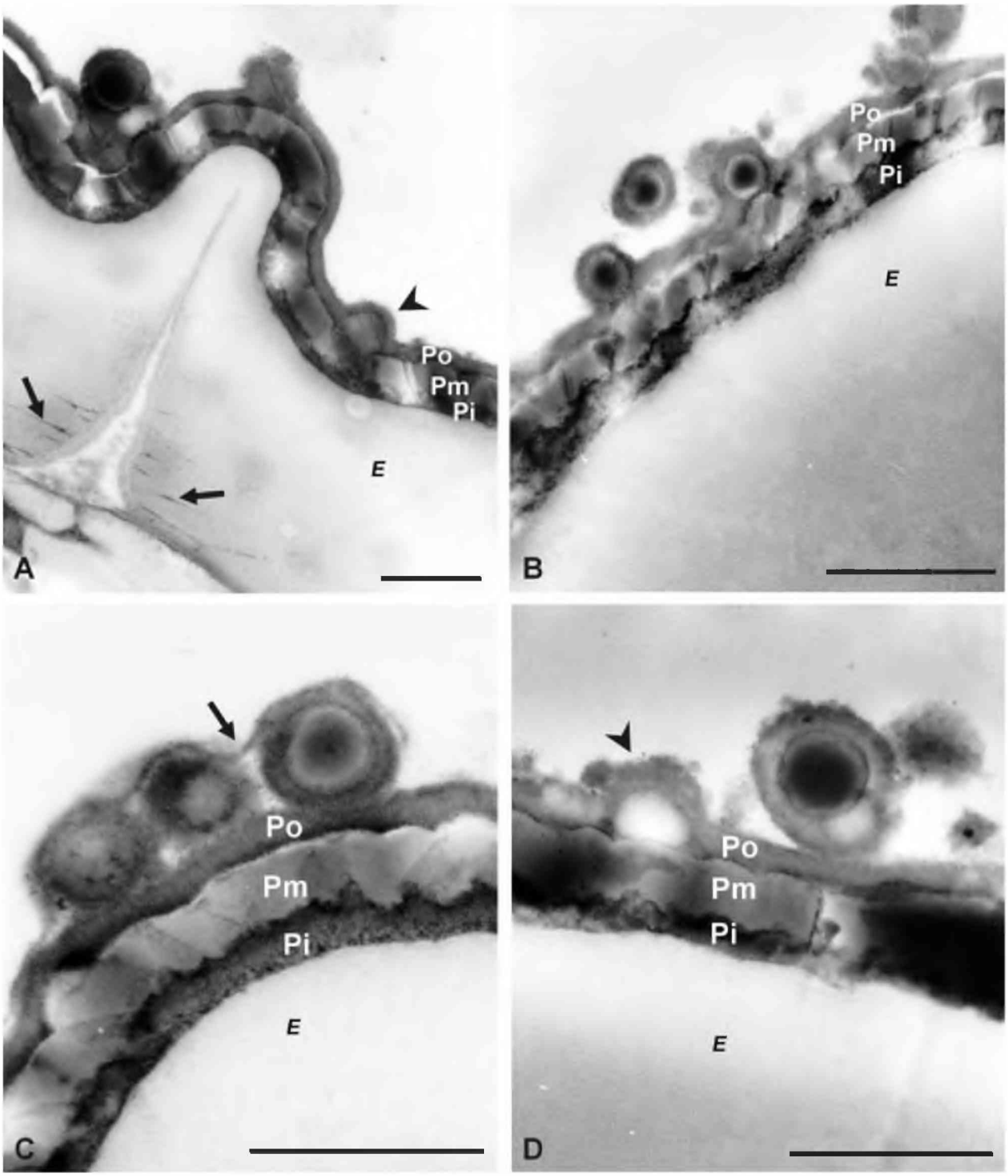


Fig. 2. TEM of the spore wall structure in *Anogramma chaerophylla*. (A) General view of a section at the level of the laesurae. The exospore (*E*) is two-layered. There are channels with a contrasted content associated with the laesurae in the middle and inner part of the exospore (*arrows*). The perispore, more contrasted than the exospore, is three-layered (*Pi, Pm, Po*). Spherules with a similar structure to the perispore are seen on the surface or included into the outer layer (*arrowhead*); (B, C, D) Details of the perispore above the exospore. There are three layers (*Pi, Pm* and *Po*). *Pi* and *Po* are more contrasted than *Pm*. *Pi* has dark spots within a less contrasted matrix. Its surface is irregular and those irregularities match with the striations of the layer *Pm*. *Pi* and *Po* apparently have three levels of different density. Spherules with the same structure of the sporoderm are free, attached or embodied within the layer *Po* (D; *arrowhead*). Some of them are grouped and coated with a thin perispore layer as seen in C (*arrow*). Scale bars – 0.5 μm.

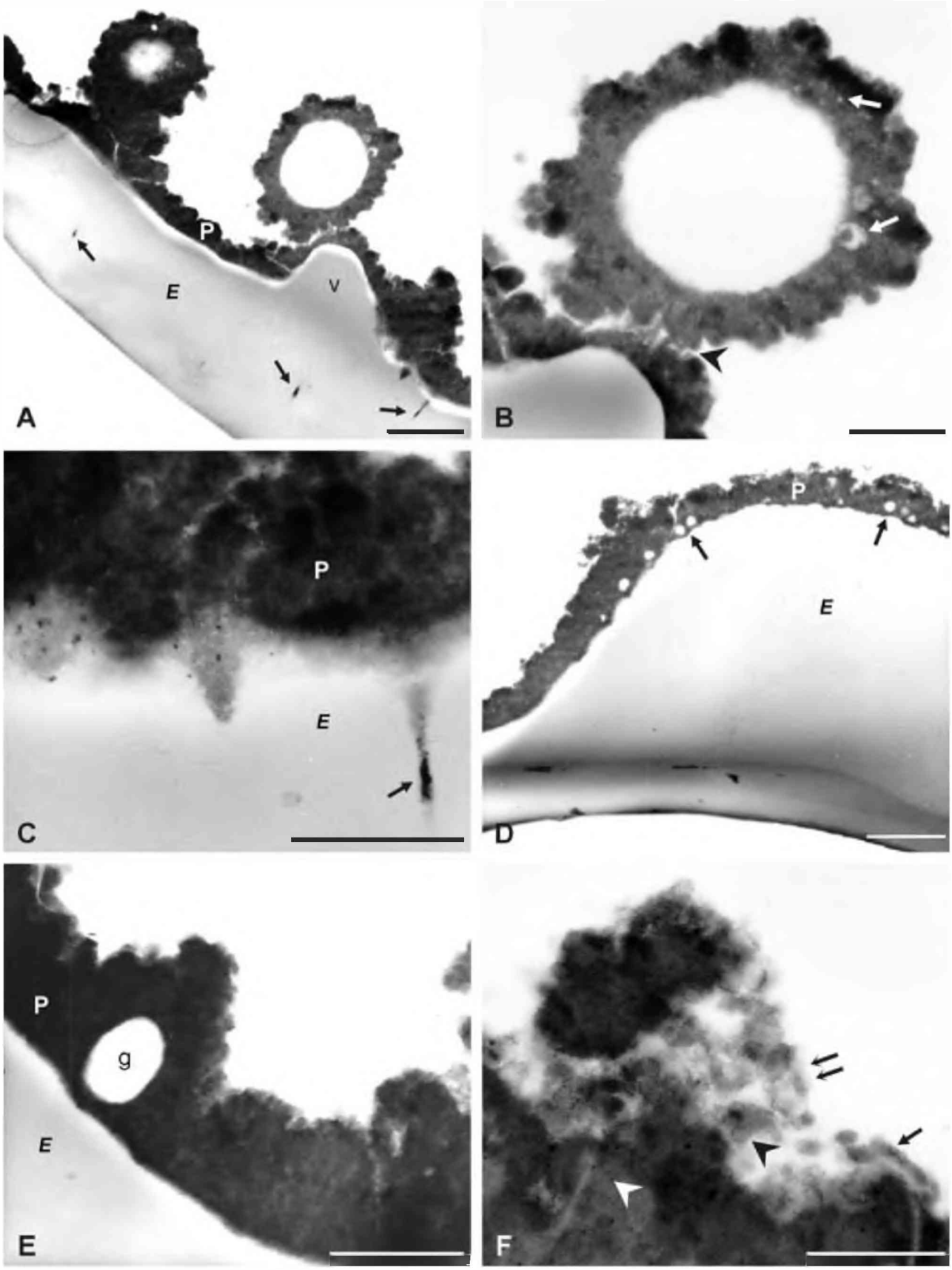

P
E
V
A
B
P
E
C
P
E
D
P
g
E
E
F

Fig. 3. TEM of wall structure in *Anogramma lorentzii*. (A) Section through the sporoderm. The exospore (*E*) is compact, single-layered with radial channels with dark content (*arrows*). A verrucae or ridges that form the sculpture in this species is sectioned (*v*). The perispore (*P*) has irregular thickness. Two globules are seen on the perispore surface. The globule on the left is attached by a solid stalk. The globule on the right is slightly attached to the perispore surface by threads; (B) Detail of the globule on the right in (A) of 2 µm of diameter. Its general structure is similar to that of the sporoderm. It has an inner less contrasted core of 1µm of diameter and a dark contrasted outer layer with similar structure to that of the perispore; round structures (*arrows*) of different sizes are present within this wall. This globule is attached to the perispore by means of structural threads (*arrowhead*); (C) Detail of the outer part of the exospore and perispore. Channels are seen within the exospore next to its surface. In one of the channels there is a contrasted inner content (*arrow*); (D) Section through a ridge. The exospore is compact, single-layered with a variable thickness depending on the different parts of the spore analyzed. The perispore is single-layered with irregular surface. Several globules are incorporated into the inner part of the perispore (*arrows*). They can be distinguished also in (E); (E) Detail of the perispore above the exospore; the spaces between the structural units are broader outwards. A globule (*g*) is evident in the inner part of this layer; (F) Detail of the perispore. The structural threads are visible in longitudinal (*arrow*) and transverse section (*arrowhead*). One helicoid can be seen within the outer lax part of this wall (*double arrows*). Dark spots are evident within the structure (*white arrowhead*). Scale bars – 1 µm (*in A, D*); 0.5 µm (*in B, C, E*); 0.2 µm (*in F*).

Rowley et al. (2002) and in the exospore of several genera within the Filicophyta by Tryon & Lugardon (1991).

Differences found in the exospore are related to the distal sculpture being ridged; partially reticulate in *A. lorentzii* and verrucate with a few ridges in *A. chaerophylla* (Ramos Giacosa et al. 2001).

The perispore is the wall that shows the greatest variety in the ultrastructural analysis. These variations are related to its sculpture and structure and in the last case especially referred to thickness and stratification.

In *A. chaerophylla*, the perispore surface has small verrucae. In section, it is three-layered. On the other hand, in *A. lorentzii*, the perispore surface has granules and verrucae, the wall is thicker than in *A. chaerophylla* and has a one-layered perispore with a very complex structure. It is apparently composed of interwoven threads with a helicoidal substructure.

Moreover, the perispore in both species has single, scattered or grouped spherules in *A. chaerophylla* and globules in *A. lorentzii* on the surface or included within it.

The spherules are small bodies with a concentric arrangement of the different perisporal materials as defined Tryon & Lugardon (1991, p. 9) in *Schizaea* J. E. Smith and *Salpichlaena* J. Smith. Similar bodies have been shown in *Schizaea pectinata* (L.) Sw. from South Africa by Parkinson (1994).

Lugardon (1981) defined globules with a similar structure as free or captive and mentioned that they could be found in many genera of Filicophyta.

The large size of globules in spores of *A. lorentzii* could be correlated with its polyploid condition reported by Gastony & Baroutsis (1975).

It was observed that when the perispore is single-layered such as in *A. lorentzii*, the globules are on its surface or included in the inner part of the perispore. However, in the three-layered perispore of *A. chaerophylla*, the spherules are on the surface and they are also present between the medium and outer layers.

Globules with characteristics similar to those described for *Anogramma lorentzii* were frequently found on the spore surface of Filicophyta and homologized with the Ubisch bodies of the Spermatophytes by Lugardon (1981).

For the above-mentioned reasons, the ultrastructural characters of the perispore, and those of the perispore and exospore sculpture could have significant differences, which should be considered, together with other palynological and vegetative characteristics of the sporophyte, in order to aid differentiations at species level as well as to establish possible relationships with other Pteridaceae.

ACKNOWLEDGEMENTS

The authors thank the technicians of the Servicio de Microscopía Electrónica of the Museo de Ciencias Naturales de La Plata and the Servicio de Microscopía Electrónica of the Instituto de Biología Celular, Facultad de Medicina of the Universidad de Buenos Aires. The first author also thanks Carlos Cioce for his useful comments of plates making. This study was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires (PIP 5044) and Universidad Nacional de La Plata for Project 363.

SPECIMENS INVESTIGATED

The letters MP in the following list and in the figure legends indicate the reference number of each palynological sample which is filed in the Cátedra de Palinología, Facultad de Ciencias Naturales y Museo de La Plata.

Anogramma chaerophylla (Desv.) Link. Buenos Aires, Ensenada, Isla Santiago, Abbiattii 1076 (LP), MP 2632; Chaco, Schulz 63 (SI), MP 3790; Entre Ríos, Uruguay, Arroyo Urquiza, Nicora 2965 (SI), MP 3802; Jujuy, Ledesma, Capital, Dinelli 5119 (LIL), MP 3797; Salta, Tartagal, San Pedro, Capurro 378 (LIL), MP 3789.

Anogramma lorentzii (Hieron.) Diels. Chaco, 1° de Mayo, Colonia Benítez, Schulz 7351 (LP), MP 3979; Jujuy, Ledesma, forest of Calilegua, H. Bartlett 20370 (SI), MP 3791.

REFERENCES

Gastony, G. J. & Baroutsis, J. G. 1975. Chromosome numbers in the genus *Anogramma*. – Am. Fern J. 65(3): 71–75.

Large, M. F. & Braggins, J. E. 1991. Spore atlas of New Zealand ferns & fern allies. – N. Zeal. J. Bot. Suppl. 29: 38.

Lugardon, B. 1963. Les spores des Ptéridacées de France. – Pollen Spores 5: 325–336.

Lugardon, B. 1981. Les globules des Filicinées, homologues des Corps dUbisch des Spermaphytes. – Pollen Spores 23: 93–124.

Michelena, I. G. 1989. Esporas de Adiantaceae (Pteridophyta) de la Provincia de Buenos Aires, Argentina. – Bol. Asoc. Latinoam. Paleobot. Palinol. 12: 25–31.

Parkinson, B. M. 1994. Morphological and ultrastructural variations in *Schizaea pectinata* (Schizaeaceae: Pteridophyta). – Bothalia 24: 203–210.

Ponce, M. M. 1996. Pteridophyta. – In: Catálogo de las Plantas Vasculares de la República Argentina I. (ed. F. Zuloaga, & O. Morrone), pp. 1–79. – Monogr. Syst. Bot. 60, Mo. Bot. Gard., St. Louis MO..

Ramos Giacosa, J. P., Morbelli, M. A. & Giudice, G. E. 2001. Estudio palinológico de las especies de *Anogramma* y *Trachypteris* (Pteridaceae, Pteridophyta) de Argentina. – Bol. Soc. Argent. Bot. 36: 283–288.

Rowley, J. R., El-Ghazaly, G. & Rowley, J. S. 1987. Microchannels in the pollen grain exine. – Palynology 11: 1–21.

Rowley, J. R., Morbelli, M. A. & El-Ghazaly, G. 2002. Microspore wall structure in *Selaginella kraussiana* (Lycophyta). – Taiwania 47: 115–128.

Rowley, J. R. & Nilsson, J. 1972. Structural stabilization for electron microscopy of pollen from herbarium specimens. – Grana 12: 23–30.

Sota de la, E. R. 1977. Pteridophyta. – In: Flora de la Provincia de Jujuy (ed. A. Cabrera). – Colecc. Ci. Inst. Nacl. Tecnol. Agropec. 13: 1–275.

Tryon, A. F. & Lugardon, B. 1991. Spores of the Pteridophyta. – Springer, New York.

Tryon, R. M. 1962. Taxonomic fern notes II. *Pityrogramma* (including *Trismeria*) and *Anogramma*. – Contrib. Gray Herb. Harvard Univ. 189: 52–79.

Tryon, R. M. & Tryon, A. F. 1982. Ferns and allied plants, with special reference to Tropical America. – Springer, New York.