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Spore wall ultrastructure in Aspleniaceae (Pteridophyta) from North–West Argentina

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Abstract

The spore wall ultrastructure of five species of *Asplenium* L. growing in North–West Argentina was studied with scanning and transmission electron microscopy. The spores are monolete, the major equatorial diameter is 30–55 μm. The exospore is 0.5–2.5 μm thick, smooth, apparently double-layered. The perispore is 0.3–3 μm thick, ornamented, perforate and folded (winged and ridged). The perispore in section is double-layered: the layer P1 constitutes the major part of this wall, is strongly contrasted, with a fibrous structure and camerate. Two different structure organisation of P1 were recognised: single-stratified with chambers restricted to the inner part of the folds and, triple-stratified with a middle stratum composed of a large continuous space of variable height. Within this space there are radial rods that have continuity with both the inner and outer strata. The perispore layer P2 is less contrasted than the layer P1 and has variable thickness. It covers the inner and outer surfaces of layer P1. Two different structures of P2 were recognised: multilamellate and granular discontinuous. Some perispore characteristics found in the selected species suggest affinities with characteristics also found in the spores produced by some other genera within the Aspleniaceae as well as in some other within the Dryopteridaceae and Oleandreaceae.

Keywords: Aspleniaceae; spores; ultrastructure; Argentina

1. Introduction

In a previous paper, Giudice et al. (2002) analysed with LM and SEM the spores of *Asplenium* that grow in North–West Argentina. According to these observations five different types of sporoderm organisation and structure were recognised out of seventeen species of this genus in the area of study. Those were selected according to perispore characteristics referred to its surface (ornamentation and fold features) and size and frequency of perforations as well as characteristics of this wall in section such as the nature and location of spaces and features of its middle stratum (thickness, structure).

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On these bases the species selected for the present ultrastructural study are: *Asplenium achalense* Hieron, *Asplenium auritum* Sw., *Asplenium praemorsum* Sw., *Asplenium pumilum* Sw. and *Asplenium serra* Langsd.

Previous studies about morphology and ultrastructure of *Asplenium* spores with SEM were those of Braggins and Large (1990) on material from New Zealand, Tryon (1990), Tryon and Lugardon (1991), Johns (2000) and Ganem et al. (2001).

With respect to species that grow in the area of study, the spores of *Asplenium praemorsum*, *Asplenium auritum*, *Asplenium monanthes* and *Asplenium serra* were described based on material from Colombia and Perú and illustrated with LM and SEM by Prada et al. (1989), those of *A. monanthes*, *A. resiliens*, *A. serra* and *A. auritum* were illustrated with SEM by Tryon and Tryon (1982) and Tryon and Lugardon (1991) and, those of *A. laetum* and *A. pumilum* by Tryon and Lugardon (1991).

Michelena (1993) studied with SEM the spores of *Asplenium* species that grow in Buenos Aires Province, Argentina, with SEM.

Pettitt (1966) studied with TEM the spore wall ultrastucture of *Asplenium adiantum-nigrum* L. and defined hollow central cavities "beneath the projections". Lugardon (1971, 1972, 1974) and Tryon and Lugardon (1991) studied the sporoderm in section with TEM in several species of *Asplenium*.

Morbelli et al. (2001) defined and illustrated the sporoderm ultrastructure of *A. achalense*, *A. serra*, *A. pumilum* and *A. praemorsum* with LM, SEM and TEM.

Our aim was to analyse with SEM and TEM the spore wall ultrastructure in the selected species in order to understand their sporoderm complexity and organisation. Special interest was focussed on the perispore organisation. Then the sporoderm structure in *Asplenium* will be compared with that previously reported in other genera of Aspleniaceae as well as in other families of Filicophyta.

2. Materials and methods

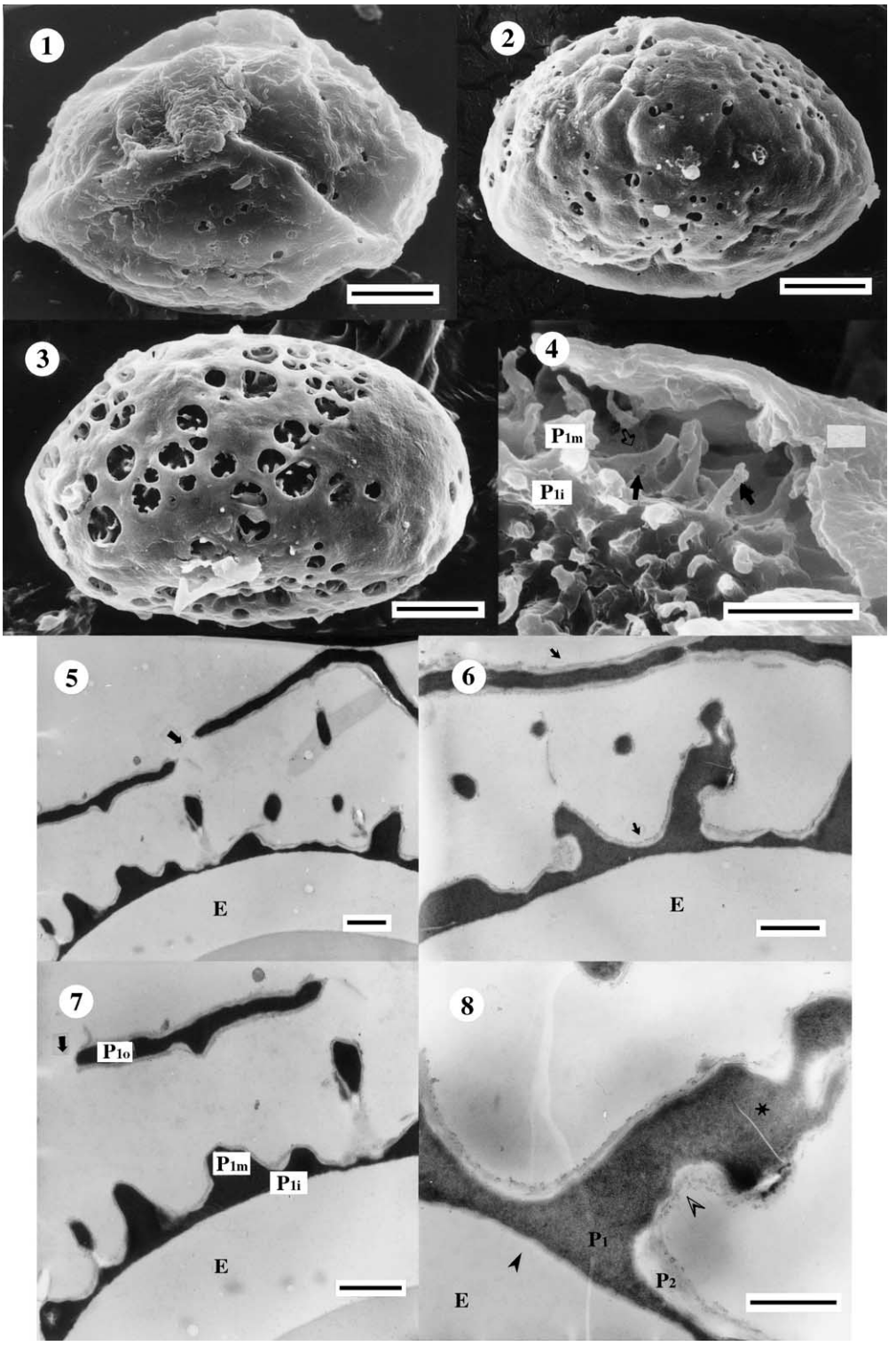
The study involved the analysis of herbarium material from the following institutions: Museo de Ciencias Naturales de La Plata (LP) and Museo de Ciencias Naturales de Salta (MCNS).

For observations with SEM the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates and coated with gold. The observations were made with JEOL JSMT-100 scanning electron microscope at the Museo de Ciencias Naturales de La Plata, Universidad Nacional de La Plata.

Plate I. Spores of *Asplenium achalense*

1–4.	SEM micrographs.
1–3.	Spores in equatorial view with variations in perforation size and frequency on the perispore surface.
4.	A fracture through the perispore that exposes the outer stratum P1o. the rods (arrows) of the middle stratum P1m are fractured. The rod bases connect with the inner stratum P1i.
5–8.	TEM micrographs.
5, 6 and 7.	Sections throughout the perispore and exospore that shows an exospore (E) with smooth surface and an homogeneous structure below a darkly contrasted perispore. The perispore layer P1 is very strongly contrasted and triple-stratified: the outer stratum P1o is folded and discontinuous (perforations marked with arrows in 5 and 7), the middle stratum P1m has structural elements with broad bases of different size. The bases of these elements have continuity with the inner stratum P1i.
8.	Magnification of a spot of (6) that shows the exospore (E) and part of the perispore. The layer P1 is darkly contrasted, with a fibrous structure. A high element (asterisk) of the stratum P1m has a broad base which shows a continuity with P1i. The element is branched outwards. A scarcely evident lamina (dark arrowhead) is present in the inner part of the perispore. Layer P2 is less contrasted than layer P1, it covers the middle and outer surfaces of layer P1 (arrows in (6), light arrowhead in (8)). The layer P2 has two levels. An inner multilamellate level and an outer discontinuous level composed of rodlets which are round in section.

Bars: 1–4: 10 μm, 5–8: 1 μm.


1
2
3
4
P1m
P1i
5
E
6
E
7
P1o
P1m
P1i
E
8
P1
P2
E

For studies with TEM dry material from herbarium specimens was hydrated following the technique proposed by Rowley and Nilsson (1972) that consist of the use of 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. Sections 3 μm thick 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 observations were made with a Zeiss T-109 transmission electron microscope at the Instituto de Biología Celular, Facultad de Medicina, Universidad Nacional de Buenos Aires.

2.1. Specimens investigated

The palynological samples are filed at the Laboratorio de Palinología, Facultad de Ciencias Naturales y Museo de La Plata, Universidad Nacional de la Plata.

Asplenium achalense: ARGENTINA. Salta, *La Caldera*, Palací 1036 (MCNS).

Asplenium auritum: ARGENTINA. Salta, *Orán*, MCNS 91 (MCNS).

Asplenium praemorsum: ARGENTINA, Salta, *Anta*, P.N. El Rey, Brown 437 (LP).

Asplenium pumilum: ARGENTINA, Tucumán, *Capital*, Venturi 817 (LP).

Asplenium serra: ARGENTINA, Salta, *Orán*, Palací 111 (LP).

3. Results

In general the spores produce by the selected species are monolete, 31–54 μm in equatorial diameter and 30–58 μm in polar diameter, the exospore is 0.5–2 μm thick, smooth and the perispore is 0.3–3 μm thick, ornamented, perforate and pleated.

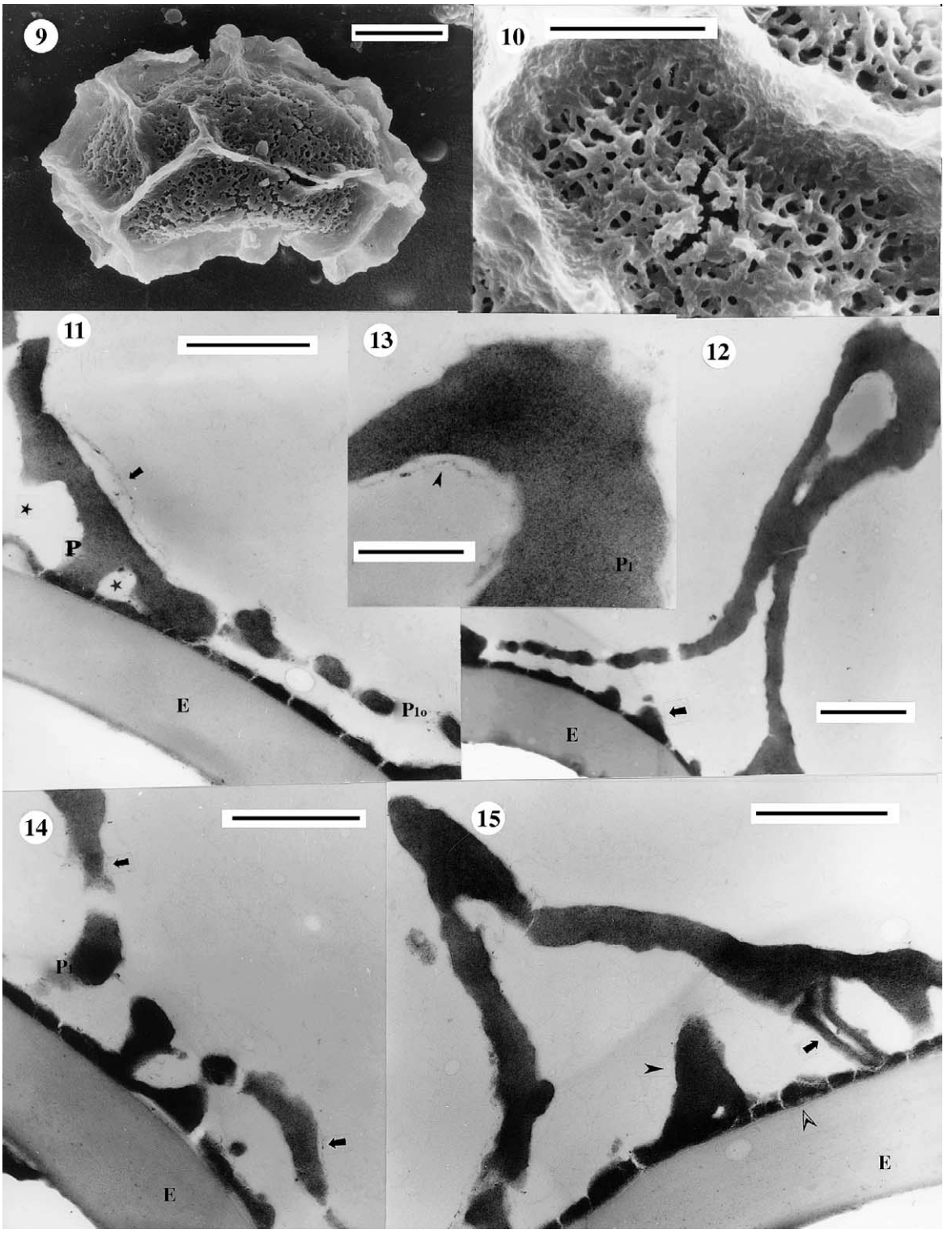
In every species, the exospore is less dense to the electrons than the perispore, apparently double-layered in section with homogeneous structure.

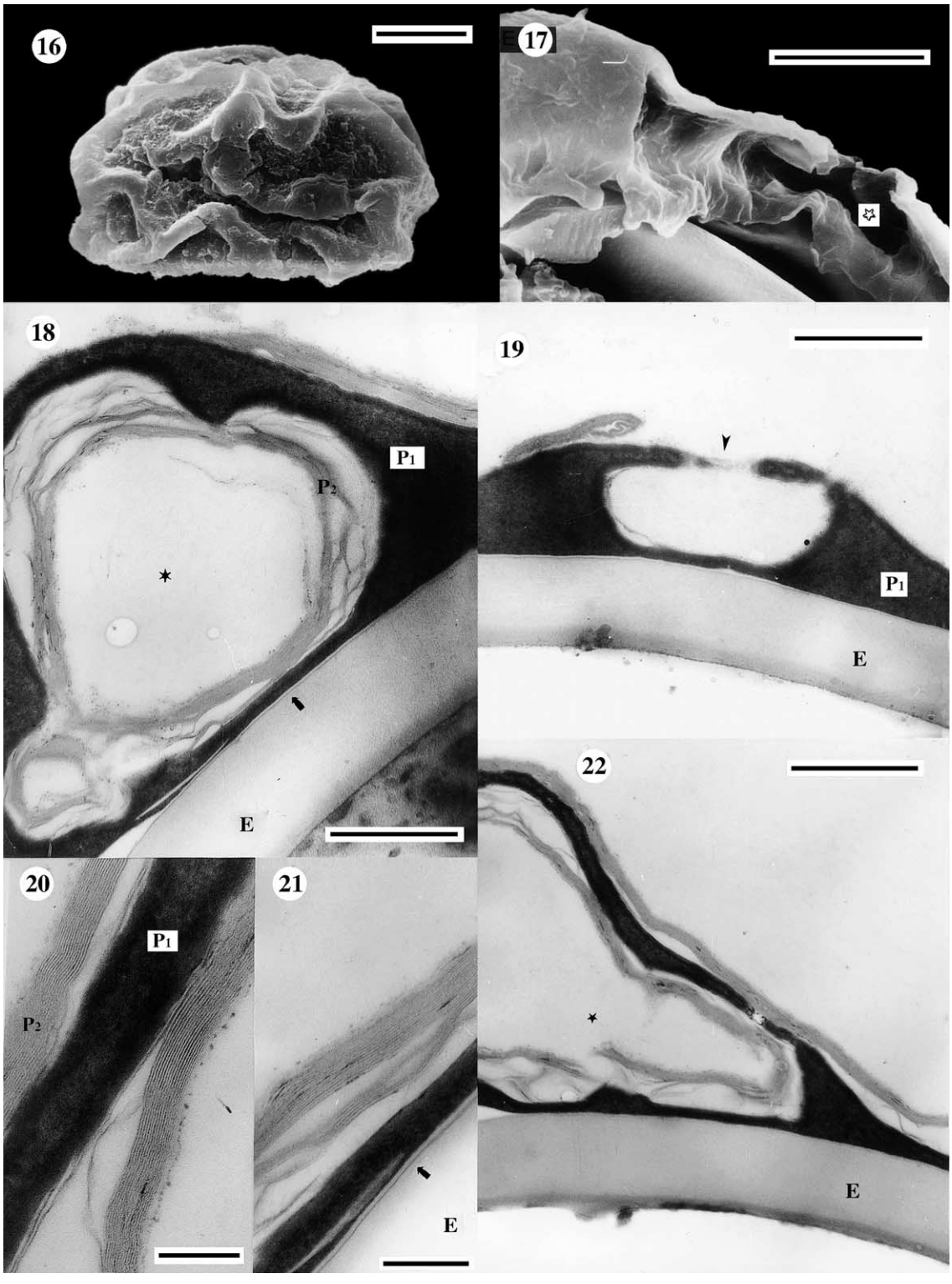
There are extensive contrasted laminae lying on the exospore at the inner part of the perispore in all the spores analysed (Plate I,8; Plate II,15; Plate III,18,19,21,22; Plate IV,24–26; Plate V, 30,32,33).

Plate II. Spores of *Asplenium auritum* (see page 134).

9–10. SEM micrographs.
9. Equatorial view of a spore with a winged, fenestrate perispore.
10. Detail of the spore surface showing a fenestrated area between folds.
11–15. TEM micrographs.
11. A section through the sporoderm. It exposes the perispore structure between and below the folds. The outer stratum P1o has large perforations between folds. There is a chamber (stars) within the middle stratum P1m. The discontinuities of P1i were considered as artefacts produced in the course of section making. The perispore layer P2 is less contrasted than P1 and scarcely evident at the surface of stratum P1 (arrow).
12. A section through the sporoderm. The exospore surface is smooth and its structure is homogeneous. The perispore layer P1 has an outer stratum P1o perforated and folded. A high, winged fold is evident. Spiny elements are present below the fold (arrow).
13. Magnification of a spot of (12), that shows the fibrous structure of layer P1 and the thin layer P2 (arrowhead). The layer P2 seems to be composed of a series of osmiophilic granules that alternate with other less contrasted ones.
14. Section of the sporoderm in an area between folds. The perispore layer P1 has an outer stratum discontinuous (perforate); the middle stratum P1m has structural elements of variable shape showing a continuity with the inner stratum P1i that is thin. The layer P2 is scarcely differentiated (arrows). The discontinuities of P1i were considered as artefacts produced in the course of section making.
15. Section through the sporoderm at the place where a fold raises. The fold is conic in section. A cone-shaped element is present at its base (arrowhead). The structural elements of the middle stratum P1m (arrow) have continuity with both inner and outer strata. The discontinuities of P1i were considered as artefacts produced in the course of section making. An extensive lamina scarcely evident lies on the exospore (white arrowhead).

Bars: 9: 10 μm, 10: 5 μm, 11, 13, 14, 15: 1 μm, 12: 0.5 μm.


9
10
11
P
E
P1o
13
P1
12
E
14
P1
E
15
E


16
17
18
P1
P2
E
19
P1
E
22
E
20
P1
P2
21
E

3.1. *Asplenium achalense* (Plate I,1–8)

The spores of this species are bi-convex in equatorial view. The surface is pleated with few large folds and has perforations of varied size, distribution and frequency. All the variations mentioned could be distinguished in different spores produced by the same specimen (Plate I,1–4).

In this species the exospore is 1–2 μm thick. It shows an especially low contrast.

The perispore is 0.4–0.7 μm thick between the folds and double-layered. The main layer (P1) is darkly contrasted and triple-stratified. Its structure consists of contrasted fibres within a less contrasted matrix (Plate I,8). The outer stratum (P1o) is 360 nm thick, perforate and folded. The middle stratum (P1m) is 3 μm height where the folds arise, camerate, with thick threads (high elements with a wide base and curved sides, branched at several levels including their bases which are often connected with contiguous elements), 0.5–2.3 μm height × 0.6–1.4 μm wide. According to the plane of sectioning some threads are seen as branched outwards (Plate I,6,8). The inner stratum (P1i) is 150–200 nm thick and lies on the exospore. The elements of the stratum P1m show a continuity with both the strata P1i and P1o.

The second layer (P2) is less contrasted than the layer P1, it is ca. 100 nm thick, composed of two structural levels: an inner level multilamellate, and the other level (ca. 20 nm thick) composed of rodlets that appear round in section (Plate I,6,8). The layer P2 covers both the outer surface of layer P1 and all the surfaces of the stratum P1m, (Plate I,5–8).

The exospore/perispore ratio is 1:1–1:1.5, in areas between folds and 1:2–1:3 in areas where the folds arise.

3.2. *Asplenium auritum* (Plate II,9–15)

The spores of this species are concave–convex in equatorial view. The surface is folded. The folds are short, winged, with an irregular margin. Some of them are fused forming luminae of varied size and form. The surface between folds is fenestrate. Within those areas there are varied elements that constitute the ornamentation (echinulae, baculae, capitate processes, etc.) (Plate II,9,10).

In this species the exospore is 0.5–1 μm thick (Plate II,11,12,14,15).

Plate III. Spores of *Asplenium praemorsum.*

16–17. SEM micrographs.
16. Equatorial view of a spore with a folded and perforate perispore.
17. A fracture through the sporoderm that shows a chamber of the perispore (star) restricted to the inner part of a projection.
18–22. TEM micrographs.
18. Section through the sporoderm showing an smooth homogeneous exospore less contrasted than the perispore. It is a chamber (asterisk) within a perispore fold. The perispore layer P1 is darkly contrasted and the layer P2 covers the surfaces of layer P1. An extensive lamina (arrow) is evident in the inner part of the perispore laying on the exospore. The chamber is restricted to the inner part of a projection.
19. The micrograph corresponds to the same place recorded in (17). A section through the sporoderm that shows a chamber restricted to a fold. A perforation is present in the outer part of P1 (arrowhead).
20. Detail of the perispore layers on a side of the chamber in (18). The layer P1 has a fibrous structure. The perispore layer P2 has a multilamellar level covered with a succession of osmiophilic granules. The layer P2 covers the inner and outer surfaces of layer P1.
21. Detail of a section at the exospore/perispore boundary. An extensive contrasted lamina (arrow) lies on the exospore (E). A multilamellate layer P2 is partially separated from the layer P1.
22. A section through the sporoderm. The exospore (E) structure is homogeneous. The perispore was sectioned just in a fold and shows a chamber (asterisk) in its inner part. P1 is darkly contrasted and perforated. A multilamellate layer P2 covers the inner and outer surfaces of P1.

Bars: 16: 10 μm, 17: 5 μm, 18, 19, 21, 22: 1 μm, 20: 0.25 μm.

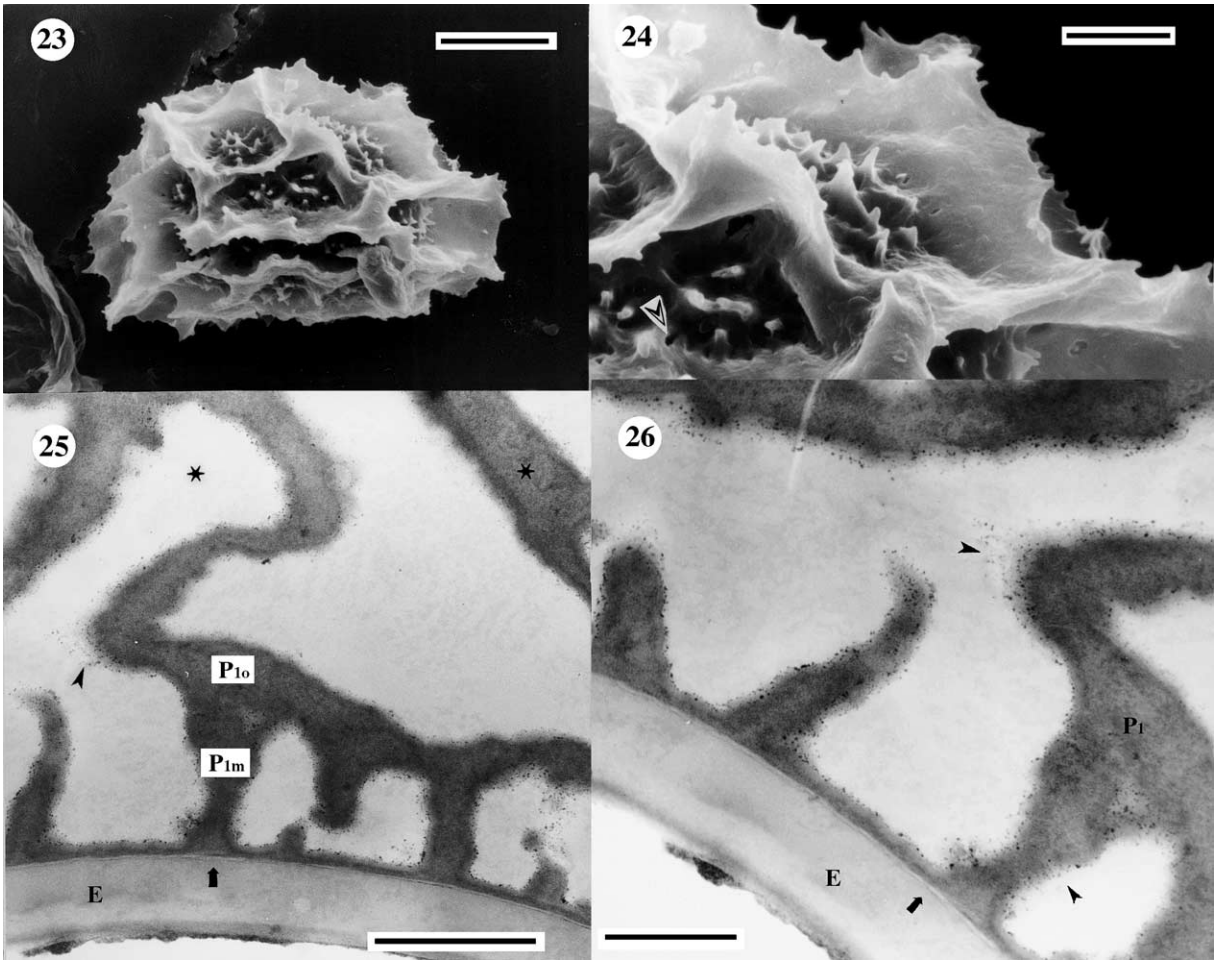


Plate IV. Spores of *Asplenium pumilum.*

23–24. SEM micrographs.

23. A spore in equatorial view. It has a winged, echinulate and perforate perispore.

24. A detail of the spore in (23). The perispore has wings with margins echinulate. The surface between wings is echinulate and perforate (arrowhead).

25–26. TEM micrographs.

25. Section through the sporoderm. The exospore is less dense to the electrons than the perispore. It is single-layered in section and its structure is homogeneous. Its surface is smooth. The perispore layer P1 is triple-stratified. Wings (asterisks) of the outer stratum P1o are evident; the middle stratum P1m is camerate and has structural elements wich have continuity with the inner and outer P1 strata. In the inner part of the perispore, there is an extensive contrasted lamina that lies on the exospore surface (arrow). The perispore layer P2 covers the middle and outer surfaces of P1. It is composed of a succession of osmiophilic granules which alternate with other granules less contrasted (arrowhead).

26. A magnification of a spot in the section in (25) in the place where the fold arises. The layer P1 has a fibrous structure. The layer P2 covers the outer surface of P1 and the elements and chambers within stratum P1m (arrowheads). An extensive lamina lies in the inner part of the perispore on the exospore (arrow).

Bars: 23: 10 μm, 24: 2.5 μm, 25: 1 μm, 26: 0.5 μm.

The perispore is 0.4–0.8 μm thick, double-layered. The layer P1 is darkly contrasted, of variable thickness according to the area of the spore sectioned, with three strata in section and camerate. The structure of P1 has a network of dark short fibres in a massive lighter contrasted matrix (Plate II,13). The outer stratum (P1o) is 100–250 nm thick, it expands in certain areas forming folds, papillate to conic in section. This stratum is discontinuous in areas between folds (Plate II,11,14). The middle stratum (P1m) is variable in height, it ranges between 250 and 450 nm, it has rods (short straight elements, round in section, radially oriented), 160–400 nm high (Plate II,11,12,14,15). Spiny elements are present inside the chambers located within the folds (Plate II,12,15). The structural elements of P1m show continuity with both strata P1i and P1o. The inner stratum (P1i) is 80–150 nm thick (Plate II,11,14,15).

The perispore layer P2 is 6–10 nm thick, less contrasted than layer P1, it is composed of a succession of osmiophilic granules that alternate with other granules less contrasted. It covers the whole surface of layer P1 (Plate II,13).

The exospore/perispore ratio is 1:1–1:1.5 in areas between folds and 1:4–1:6 in areas where the folds arise.

3.3. *Asplenium praemorsum (Plate III,16–22)*

The spores of this species are plane–convex in equatorial view. The surface is pleated with ridged folds, which are fused forming irregular areas. The surface between folds has short rugulae and few perforations (Plate III,16,17).

The exospore of this species is 1–1.5 μm thick (Plate III,18,19,22).

The perispore is 0.6–1 μm thick, double-layered. The layer P1 is darkly contrasted, of variable thickness (100–200 nm) between folds according to the area of the spore sectioned, with an fibrous structure and single-stratified. It has chambers 2.5–3 μm high that are restricted to the inner part of the folds (Plate III,17–19 and 22).

The perispore layer P2 is 70–200 nm thick, composed of two structural levels: an inner level multilamellate, and the other level composed of rodlets that appear round in section. The layer P2 covers the inner and outer surfaces of layer P1 (Plate III,18,20–22).

The exospore/perispore ratio is 1.5:1 in areas between folds and 1:4 in areas where the folds arise.

3.4. *Asplenium pumilum (Plate IV,23–26)*

The spores of this species are plane–convex in equatorial view. The surface is pleated. The folds are winged with echinulate margins and fused forming a reticulate design. The luminae have echinulae and perforations (Plate IV,23,24).

In this species the exospore is 0.5–1.5 μm thick (Plate IV,25,26).

The perispore is 0.4–1 μm thick. In section it is composed of two layers. The layer P1 is darkly contrasted, of variable thickness according to the area of the spore sectioned with a fibrous structure. It has three strata in section. The outer stratum (P1o) is 150–300 nm thick, perforate and folded (Plate IV,24–26). The middle stratum (P1m) is 500–800 nm high between folds. It is camerate and has elements (rods) of variable shape with broad bases (Plate IV,24–26). The inner stratum (P1i) is 50–90 nm thick. The bases of the elements of stratum P1m show continuity with this stratum.

The perispore layer P2 is 20–80 nm thick and not consolidated. It is composed of a succession of osmiophilic granules which alternate with other granules less contrasted. It covers the whole surface and the elements present in the stratum P1m (Plate IV,24–26).

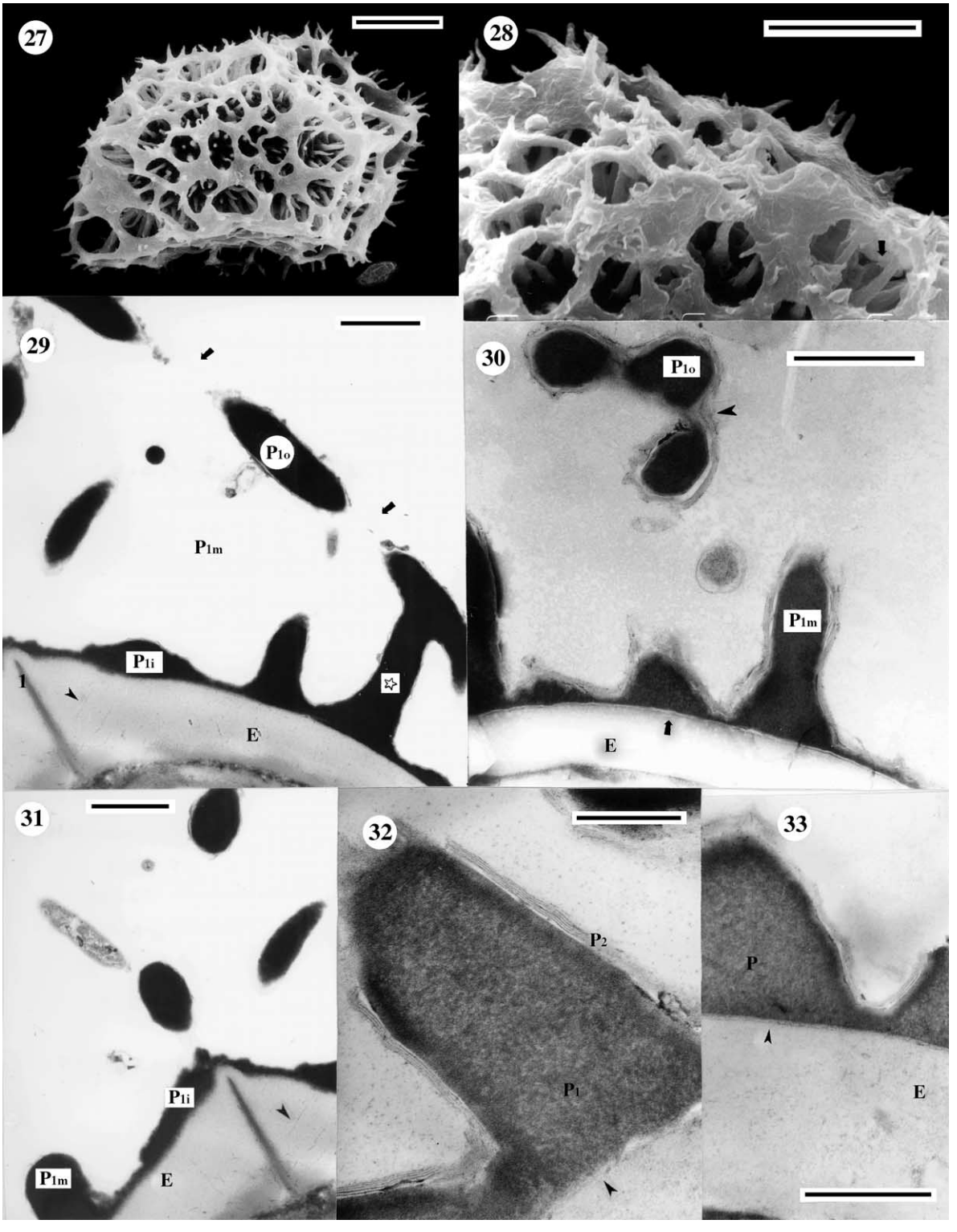
(see page 134) The exospore/perispore ratio is 1:1.5 in areas between folds and 1:5.5 in areas where the folds arise.

3.5. *Asplenium serra (Plate V,27–33)*

The spores of this species are plane–convex in equatorial view. The surface is perforated and pleated. The folds are few, winged with an echinulate margin. The perforations are large and numerous determining a reticulate design (Plate V,27,28).

In this species the exospore is 0.5–2 μm thick. Some channels are observed at different levels in the exospore (Plate V,29,30,31,33).

The perispore is 0.6–3 μm thick. In section it is composed of two layers. The layer P1 is darkly contrasted, of variable thickness according to the area


27
28
29
P1o
P1m
P1i
1
E
30
P1o
P1m
E
31
P1i
P1m
E
32
P2
P1
33
P
E

of the spore sectioned (Plate V, 29, 31). It has a structure made of dark fibres within a less contrasted matrix (Plate V,32,33) and with three strata in section. The outer stratum (P1o) is 300–550 nm high, discontinuous and folded (Plate V, 27–31). The middle stratum (P1m) is 0.3–2.6 μm high, camerate, with elements of variable shape and height together with pillars (high, strong, straight elements with a wide base and round in section) fused to both, inner and outer strata. Along with the large processes, shorter ones exist. They do not connect with the outer stratum (P1o) and are situated in the areas in which P1o is discontinuous (Plate V,29,30,31). The inner stratum (P1i) is 20–100 nm thick.

The perispore layer P2 is 10–70 nm thick, with a multilamellate structure. This layer covers the inner and outer surfaces of layer P1 (Plate V,30,32,33).

The exospore/perispore ratio is 1:1.3 in the areas between folds and 1:6 in the areas where the folds arise.

4. Discussion and conclusions

There are few references about the structure of the exospore in *Asplenium*. Pettitt (1966) described the exospore in *Asplenium adiantum-nigrum* L. as homogenous, granular or completely undifferentiated. Lugardon (1974) described the exospore in Aspleniaceae as "blechnoide" type. Nevertheless in the analysed species we observed (in magnifications of 50,000 to 140,000), short, slender, dark fibrils embedded within a less contrasted matrix. With respect to its apparent double- stratification mentioned it could be said that this is more evident in certain specimens.

The perispore ultrastructure in the species analysed here was compared with those in records given for other genera of Filicophyta in former studies. Thus Tryon (1990) considered the perispore structure in *Asplenium serra* as pillared and pointed out that this particular structure resembled the columellate characteristic typical of the Angiosperm pollen exine.

The ultrastructure of *Asplenium serra* was found similar to that of *Oleandra articulata* (Sw.) Pr. (Oleandraceae) according to the records given by Tryon and Lugardon (1991, p. 504, Plate 196: 10, 11).

The perispore structure of *Asplenium praemorsum* as described here was found as strikingly similar to that of *Lastreopsis* (Dryopteridaceae) according to the

Plate V. Spores of *Asplenium serra*.

27–28. SEM micrographs.
27. Equatorial view of a spore with a winged reticulate perispore.
28. Detail of the perispore surface in the same spore. It has large perforations and wings with margins that are echinulate. The structural elements of the middle stratum (arrow) can be seen within the lumina.
29–33. TEM micrographs.
29. Section of a spore at a side of a laesura (l). The exospore (E) is less dense to the electrons than the perispore, its structure is homogeneous and some channels are evident according to the plane of sectioning (arrowhead). The perispore layer P1 is darkly contrasted and is triple-stratified. The outer stratum P1o has large discontinuities (arrows). The middle stratum P1m has structural elements of variable shape, all of them have broad bases and variable height. A pillar, within P1m, is fused to both, inner and outer strata (white asterisk).
30. Section through the sporoderm. In the middle stratum P1m there are irregular structural elements like cones and capitate processes. The outer stratum P1o is discontinuous. The perispore layer P2 has multilamellate structure and can be seen all around of a section of P1o (arrowhead). In the inner part of the perispore there is an extensive lamina lying on the exospore surface (arrow).
31. A detail of the apertural area in section. In the exospore at the side of the laesura there are channels (arrowhead). Layer P1 is darkly contrasted. Part of the three strata of the perispore are evident. The inner stratum P1i has variable thickness and the structural elements of P1m are sectioned on different orientations.
32 and 33. Magnifications of elements of the middle stratum P1m that show their structure. They are composed of dark fibres within a less contrasted matrix. The multilamellate layer P2 covers the surface of the elements. An extensive contrasted lamina (arrowheads) at the inner part of the perispore is evident.
Bars: 27: 10 μm, 28, 29, 30, 33: 1 μm, 31: 0.25 μm, 32: 0.5 μm.

records shown by Tryon and Lugardon (1991, p. 466, Plate 179.15 and 16). It is here referred for the first time that both the outer surface and the chambers surface are covered by a multilamellate perispore layer.

Cavities similar to those described for layer P1 in *Asplenium praemorsum* and *A. pumillum* were also defined in *Asplenium adiantum-nigrum* L. by Pettitt (1966) and for *Asplenium trichomanes* L. by Lugardon (1971, 1974).

The presence of laminae in the inner part of the perispore as shown in this work was also recorded for spores of other Aspleniaceae such as *Ceterach officinarum* Willd. and *Asplenium marinum* L. by Tryon and Lugardon (1991, pp. 551 and 553) and *Asplenium trichomanes* L. and *Scolopendreum vulgare* Smith by Lugardon (1971, 1974).

With regard to the perispore layer P2 observed here in all the studied species, Lugardon (1971, 1974) described in some species of Aspleniaceae also a layer with similar characteristics. According to Lugardon (1974, p. 220) the materials forming P2 are frequent against the free surfaces of the fern spores, and do not belong to the perispore proper and very likely can be related to some of the materials deposited on the free surfaces of pollen grain exines.

We observed two different structures of layer P2 in the analysed species. A structure unconsolidated composed of a succession of osmiophilic granules that alternate with other less contrasted granules, which can be distinguished in *Asplenium auritum* and *Asplenium pumilum*. In *Asplenium serra*, *Asplenium achalense* and *Asplenium praemorsum* the structure of P2 is multilamellar and with an outer unconsolidated layer. In all the cases this layer P2 covers all the inner and outer surfaces of P1, even in the discontinuities (perforations) of the outer stratum (P1o). Probably these differences in the structure of P2 could represent different stages of spore maturation.

We observed that the perispore layer P1 in all the spores analysed has a fibrous structure. Nevertheless Lugardon (1971, 1974) referred to the same characteristics as granular. We consider this distinction to be interpretative rather than a difference in structure.

Tryon and Lugardon (1991) used the term cavate to describe the perispore structure in spores produced by several species within the genera *Asplenium* as well as in *Dryopteris* and *Thelypteris.* We considered appropriate to introduce in *Asplenium* also the term **camerate** to refer to the perispore structure in which radial processes (pillars and rodlets) are present between two strata of layer P1. We consider that the term **cavate** could be reserved to those cases in which the spaces (chambers) are devoid of processes and restricted to the inner part of the folds.

Two types of perispore structure organisation were distinguished in the analysed spores. In the **camerate** perispore, the layer P1 has three strata in section with a broad continuous space all around the spore in its middle stratum (P1m). Within this space in P1m are high threads, some of them are branched outwards as in *Asplenium achalense*, rods as in *Asplenium auritum* and *Asplenium pumilum* or pillars as in *Asplenium serra*. In *A. auritum*, *A. pumilum* and *A. serra* the space in P1m has variable height. It is low between folds and high in places were projections exist. Within this space there are rods or pillars that show continuity with both strata P1o and P1i. The spores of *A. serra* differ from those of the other species by its discontinuous outer stratum (P1o) with large perforations and the presence of pillars as well as irregular elements like cones, spines and clavate, capitate processes.

In the **cavate** perispore, the layer P1 is single-stratified. This layer has chambers restricted to the inner part of the elevations. It was only recognised in *Asplenium praemorsum*.

The characteristics shared by all the species are: (1) the general structure and stratification of the exospore; (2) the presence of laminae on the exospore at the inner part of the perispore; (3) the fibrous structure of perispore layer P1; (4) a perispore layer (P2), which covers all the surfaces of perispore layer P1; and (5) the existence of discontinuities (perforations) on the perispore surface.

Studies of spore development are needed in order to understand the sequence of events that determine the formation of the perispore layers and strata as well as to understand their possible functions.

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