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Spore morphology and wall ultrastructure of *Blechnum* L. species from North West Argentina

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ABSTRACT

Spores of Blechnaceae species that grow in northwest Argentina were studied. These are: *Blechnum australe* subsp. *auriculatum, B. austrobrasilianum, B. brasiliense, B. cordatum, B. laevigatum, B. mochaenum* var. *squamipes, B. occidentale, B penna-marina, B. sprucei* and *B. tabular*e. The study was performed with light microscope (LM), transmission electron microscope (TEM) and scanning electron microscope (SEM). The spores are monolete of 31.5–104 µm in equatorial diameter and 21–75 µm in polar diameter. The exospore is 0.6–3.5 µm thick, plane or scabrate and in section it is two-layered. According to the plane of sectioning, and especially in immature material, interwoven channels are evident. The exospore structure is blechnoid and similar in all taxa analysed. The perispore is 0.4–3.0 µm thick and plane, rugulated or folded. Its surface has verrucae, rugulae and perforations. In section it is two-layered. The outer layer is single stratified and the inner layer is three-stratified. Scattered spherules are also present on the surface and globules are detected in the perispore. In the analysed taxa the perispore has the greatest variability at specific and infraspecific levels and five structural types are observed. Differences were found among the studied taxa in the characteristics of the medium stratum. Additionally, in *B. cordatum* the structural elements are also superficially observed on and between the folds. The differentiated structural types would not find any correspondence with the ornamentation patterns of the species here analyzed. The diversification found in spore characteristics observed in this study, could be valuable for systematic and phylogenetic studies.

1. Introduction

The spores of *Blechnum* L. species were analysed as part of the study of Pteridophyta spores of northwest Argentina. This region includes the provinces of Salta, Jujuy, Catamarca and Tucumán, northwest La Rioja province, and the southwest of Santiago del Estero province (de la Sota, 1976). All of these provinces are included in the natural area known as "Yungas" (cloud forest).

Northwest Argentina is characterized by a high biodiversity of families, genera and species and is one of the main centres of concentration of Pteridophytes in Argentina (Zuloaga et al., 1999; Ramos Giacosa et al., 2004). The genus *Blechnum* (Blechnaceae, Filicophyta) has approximately 150–200 species, is cosmopolitan and is very well represented in the South Hemisphere (de la Sota, 1977; Tryon and Tryon, 1982; Kramer et al., 1990).

The Blechnaceae were originated in the Paleocene (Moran and Smith, 2001). Some impressions of *Blechnum* were found in the Eocene of Argentina by Frenguelli (1941) and Baldoni et al. (1985). However, fossil spores of *Blechnum* are difficult to distinguish from those of *Polypodium* (Couper, 1953).

Among the modern *Blechnum*, twelve taxa were reported as growing in northwest Argentina (Ponce, 1996; Ramos Giacosa et al., 2006; Durán, 1997). These are: *Blechnum australe* Linnaeus subsp. *auriculatum* (Cavanilles) de la Sota, *B. austrobrasilianum* de la Sota, *B. brasiliense* Desvaux, *B. cordatum* (Desvaux) Hieronymus, *B. laevigatum* Cavanilles, *B. mochaenum* G. Kunkel var. *squamipes* (Hieronymus) de la Sota, *B. occidentale* Linnaeus, *B. penna-marina* (Poiret) Kuhn, *B. sprucei* Christensen, *B. tabular*e (Thunberg) Kuhn, *B. australe* subsp. *auriculatum*×*B. occidentale* and *B. x pampasicum*.

Palynological studies of *Blechnum* based on particular areas are those of Large and Braggins (1991) for New Zealand and Nayar and Devi (1964) for India.

Lugardon (1972, 1974), analyzed the sporoderm structure of several species of *Blechnum* from different regions with SEM and TEM and described the ultrastructure of different types of exospores. Lugardon (1965, 1966) studied the wall structure and particularly the formation of the exospore of *Blechnum spicant* (Linnaeus) Smith. Later, Lugardon (1981) analyzed globules of Filicophyta including those of *Blechnum spicant*.

The *Blechnum* species growing in America were studied in several palynological studies with LM by Tschudy and Tschudy (1965) for Venezuela and Heusser (1971) for Chile, and with SEM by Kurita and Nishida (1985) for Bolivia and Southern Chile. The structure of the

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Table 1
Spore morphological data for *Blechnum* species from North West Argentina.

Taxa	Shape in equatorial view	Shape in polar view	Polar diameter	Equatorial diameter		Laesurae	Exospore	Perispore		
				Major	Minor	Length	Thickness	Thickness	Pattern	Surface
B. australe subsp. *auriculatum*	Concave–convex	Elliptic	20.7–29.7	37.8–50.4	22.5–32.4	24.3–39.6	3	0.4	Folded or plane	Verrucae, Perforate
B. austrobrasilianum	Plane–convex	Elliptic to oblong	23.8–31.5	38.7–49.5	21.6–32.4	27.4–35.5	3	0.5	Folded	Rugulae
B. brasiliense	Plane–biconvex	Elliptic	24.3–31.2	36.9–46.5	26.1–35.4	23.1–35.1	1	0.7	Rugulated	Rugulae
B. cordatum	Plane–biconvex	Elliptic	54.5–64.4	78.3–83.6	54.2–70.3	43.2–64.5	2	9	Folded	Threads
B. laevigatum	Concave–convex	Elliptic	21.6–31.5	31.5–48.6	21.6–30.6	19.8–37.8	2	0.4	Plane	Smooth
B. mochaenum var. *squamipes*	Plane–biconvex	Elliptic	21.6–33.3	35.1–43.2	25.2–34.2	22.5–38.7	1	2	Rugulated	Verrucae
B. occidentale	Concave–convex	Oblong	22.5–32.4	36–50.4	19.8–36.4	19.8–34.2	3	1	Plane	Perforate
B. penna-marina	Plane–concave–convex	Elliptic to subcircular	25.2–30.3	35.4–42.8	24.9–33.3	23.8–32.4	1	0.8	Folded	Rugulae
B. sprucei	Plane–convex	Elliptic	28.8–35.1	28.8–35.1	20.7–27	22.5–27	2.5	1.4	Folded	Rugulae
B. tabulare	Plane–convex	Elliptic	63.9–74.7	85.5–104.4	66.7–81	41.4–86.4	5	0.8	Folded	Rugulae

Dimensions in μm. Exospore and perispore thickness in mean values.

spores of some species that grow in America was analyzed with TEM by Tryon and Lugardon (1991).

Morbelli (1975, 1976, 1977, 1980) studied with LM the spores of *Blechnum* taxa growing in Argentina. Morbelli (1974) studied the characteristics of the spores produced by the hybrids that grow in Argentina: *Blechnum australe* Linnaeus subsp. *auriculatum* (Cavanilles) de la Sota × *B. laevigatum* Cavanilles and *B. australe* Linnaeus subsp. *auriculatum* (Cavanilles) de la Sota × *B. occidentale* Linnaeus, and described normal, abnormal and non-developed spores as well as spores with variations in shape, size, grouping and ornamentation. Morbelli also determined the percentage of viable spores produced by each studied specimen. Morbelli (1977) quoted the presence of orbicules on some ferns spores. The spores of *B. serrulatum* were illustrated and several tests were applied. Later, Morbelli (1976), as part of the study of the spores of *Blechnum* subgenus *Blechnum* concluded that four structural types of perispores were present.

Nevertheless, the spores of most species growing in northwest Argentina were not analysed with SEM and TEM. Thus the sporoderm ultrastructure still needs an accurate study in order to define its complex and diverse structure. The aim of this contribution is to analyse the morphologic characteristics and wall ultrastructure with LM, SEM and TEM of the spores of *Blechnum* taxa that grow in northwest Argentina.

2. Material and methods

Spores were obtained from herbarium specimens from the following Argentinean Institutions: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (BA), Instituto Miguel Lillo, Tucumán (LIL), 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 LM, SEM and TEM. For LM the spores were treated with hot 3% sodium carbonate for 2 min 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 TEM dry material from herbarium specimens was hydrated following the technique proposed by Rowley and Nilsson (1972) that consists 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 analysed with LM. Ultra-thin sections were stained with 1% uranyl acetate for 15 min followed by lead citrate for 3 min.

The observations were performed with Olympus BH2 and BHB LMs, a JEOL JSMT-100 SEM and a Zeiss T-109 TEM.

The letters MP, in the list of specimens investigated indicate the reference number of each palynological sample filed in the Laboratorio de Palinología, Facultad de Ciencias Naturales y Museo de La Plata.

Since only one fertile specimen of *B. brasiliense* from northwest Argentina was available, a specimen from the northeast (Misiones Province) was included.

2.1. Material studied

Blechnum australe subsp. *auriculatum*: ARGENTINA: SALTA, Tartagal, Meyer 916 (BA), MP 3973.

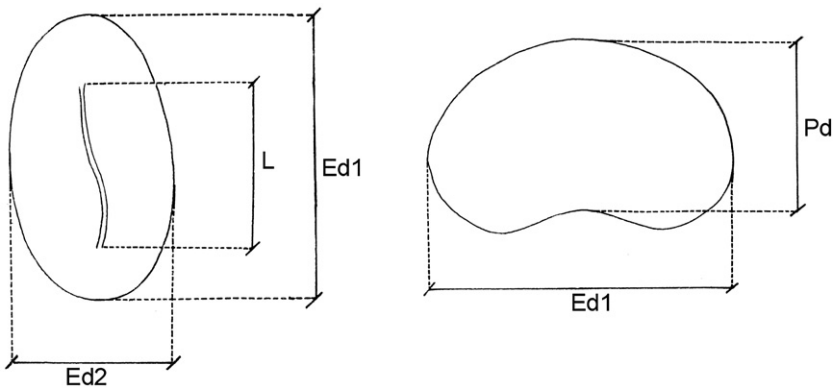

Fig. 1. Diagram of spore diameters. Ed1 = major equatorial diameter; Ed2 = minor equatorial diameter; L = laesurae length; Pd = polar diameter.

Plate I. Spores of *Blechnum sprucei* and *B. tabulare* with SEM. (see on page 188)

1–3. *B. sprucei*.
1. Proximal view of an elliptic spore. Scale bar = 10 μm.
2. Equatorial view of a plane–convex spore. The surface is composed of few folds and rugulae. Scale bar = 10 μm.
3. Detail of the perispore surface with rugulae and a fold (arrow). Scale bar = 2 μm.
4–6. *B. tabulare*.
4. Proximal view of an elliptic spore. Scale bar = 10 μm.
5. Equatorial view of a plane – convex spore with folded surface. The folds are fused forming an incomplete reticule. Scale bar = 10 μm.
6. This fracture exposes the three strata of the perispore: the inner (arrow) is thin and adhered to the exospore (E), the middle (Pm) has rodlets and the outer stratum (Po) with undulate margin. Scale bar = 2 μm.

Plate II. Spores of *Blechnum austrobrasilianum* and *B. laevigatum* with SEM. (see on page 189)

1–3. *B. austrobrasilianum*.
1. Proximal view of an elliptic to oblong spore. Scale bar = 10 μm.
2. Equatorial view of an elliptic to slightly oblong spore. There are small folds. Scale bar = 10 μm.
3. Spore surface with folds of different size and height, single or fused. Scale bar = 2.5 μm.
4–6. *B. laevigatum*.
4. Proximal view of an elliptic spore. Scale bar = 10 μm.
5. Equatorial view of a concave–convex spore. Scale bar = 10 μm.
6. Spore surface. The perispore is smooth and devoid of folds. Few small elements of variable shape are attached to the surface (arrowheads). Scale bar = 10 μm.

Plate III. Spores of *Blechnum mochaenum* var. *squamipes* and *B. penna-marina* with SEM. (see on page 190)

1–3. *B. mochaenum* var. *squamipes*.
1. Proximal view of an elliptic spore. Scale bar = 10 μm.
2. Equatorial view of a plane to slightly biconvex spore. The perispore is rugulate with short scattered rugulae. Scale bar = 10 μm.
3. Section through the perispore. This wall is composed of three-strata: the inner stratum (arrow) is adhered to the exospore (E), the middle stratum is composed of fused threads (Pm) and the outer stratum (Po) has an undulate margin. Scale bar = 2.5 μm.
4–6. *B. penna-marina*.
4. Distal view of an elliptic to subcircular spore. Scale bar = 10 μm.
5. Equatorial view of a plane–convex to concave–convex spore. Scale bar = 10 μm.
6. Detail of the spore surface. It is composed of small folds. Scale bar = 2.5 μm.

Plate IV. Spores of *Blechnum brasiliense* and *B. cordatum* with SEM. (see on page 191)

1–4. *B. brasiliense*.
1. Proximal view of an elliptic spore. Scale bar = 10 μm.
2. Equatorial view of a plane to biconvex spore. Scale bar = 10 μm.
3. Spore surface. The perispore is rugulated with scattered short folds. Scale bar = 2 μm.
4. Section through the perispore. The perispore (P) is composed of three strata of different thickness. The exospore (E) below the perispore (P) is evident. Scale bar = 1 μm.
5–8. *B. cordatum*.
5. Equatorial view of a plane to slightly biconvex spore. Scale bar = 10 μm.
6. Distal view. Folds of different size fuse forming an incomplete reticule. Scale bar = 10 μm.
7. Detail of the spore surface. It consists of numerous threads single or fused. Scale bar = 2 μm.
8. This fracture exposes the three-stratified nature of the perispore. A high number of threads can be seen in the middle perispore stratum (Pm). Threads with a similar structure are seen also on the surface of the outer perispore layer (star). Scale bar = 1 μm.

Plate V. Spores of *Blechnum australe* subsp. *auriculatum* and *B. occidentale* with SEM. (see on page 192)

1–4. *B. australe* subsp. *auriculatum*.
1. Proximal view of an elliptic spore. Scale bar = 10 μm.
2. Distal view. Scale bar = 10 μm.
3. Equatorial view of a concave–convex spore. Scale bar = 10 μm.
4. Detail of the surface with verrucae, rugulae, spheroids and perforations of variable sizes and distribution. Scale bar = 2 μm.
5–6. *B. occidentale*.
5. Distal view of an oblong spore. Scattered rugulae are seen on the surface. Scale bar = 10 μm.
6. Detail of the surface with scattered perforations (arrows) and spheroids single or grouped forming glomerules (arrowhead). Scale bar = 2.5 μm.

Plate VI. TEM images of the spores of *Blechnum australe* subsp. *auriculatum* and *B. brasiliense*. (see on page 193)

1–3. *B. australe* subsp. *auriculatum* (perispore of type 1).
1. Section through the sporoderm. The exospore (E) has a radial channel (arrow) with contrasted content. The perispore is two-layered. The inner layer P1 consists of three strata: the inner stratum (P1i, star) is adhered to the exospore, the middle stratum (P1m) is cavate pillared and the outer stratum (P1o) continuous and adhered to P2. The P2 layer is highly contrasted, homogeneous, continuous and with irregular surface. Cavities are also observed in the deepest area of the outer layer (arrow).Scale bar = 0.5 μm.
2. The inner stratum of layer P1 (star) is thick and the camerae of the middle stratum (P1m) are small. Spherules can be observed on the spore surface (arrows). Scale bar = 0.5 μm.
3. Spherules with the same structure and contrast as layer P2 (arrow) are attached to the perispore surface through structural threads (double arrow). Below layer P1. Scale bar = 0.5 μm.
4–6. *B. brasiliense* (perispore of type 2).
4, 6. Section through the sporoderm. The exospore (E) has numerous radial channels (white arrows) with contrasted content. The perispore is two-layered. The layer P1 consists of three strata: the inner stratum (P1i, star) adhered to the exospore, the middle stratum (P1m) is composed of rodlets and the outer stratum (P1o) has undulated margin. The perispore layer P2 (black arrow) which is less contrasted than P1, covers all the free surfaces of layer P1. Scale bar = 1 μm.
6. Section through the sporoderm at the aperture that consists of a strongly protruding fold. The inner exospore (Ei) is more contrasted than the outer exospore (Eo). Scale bar = 1 μm.

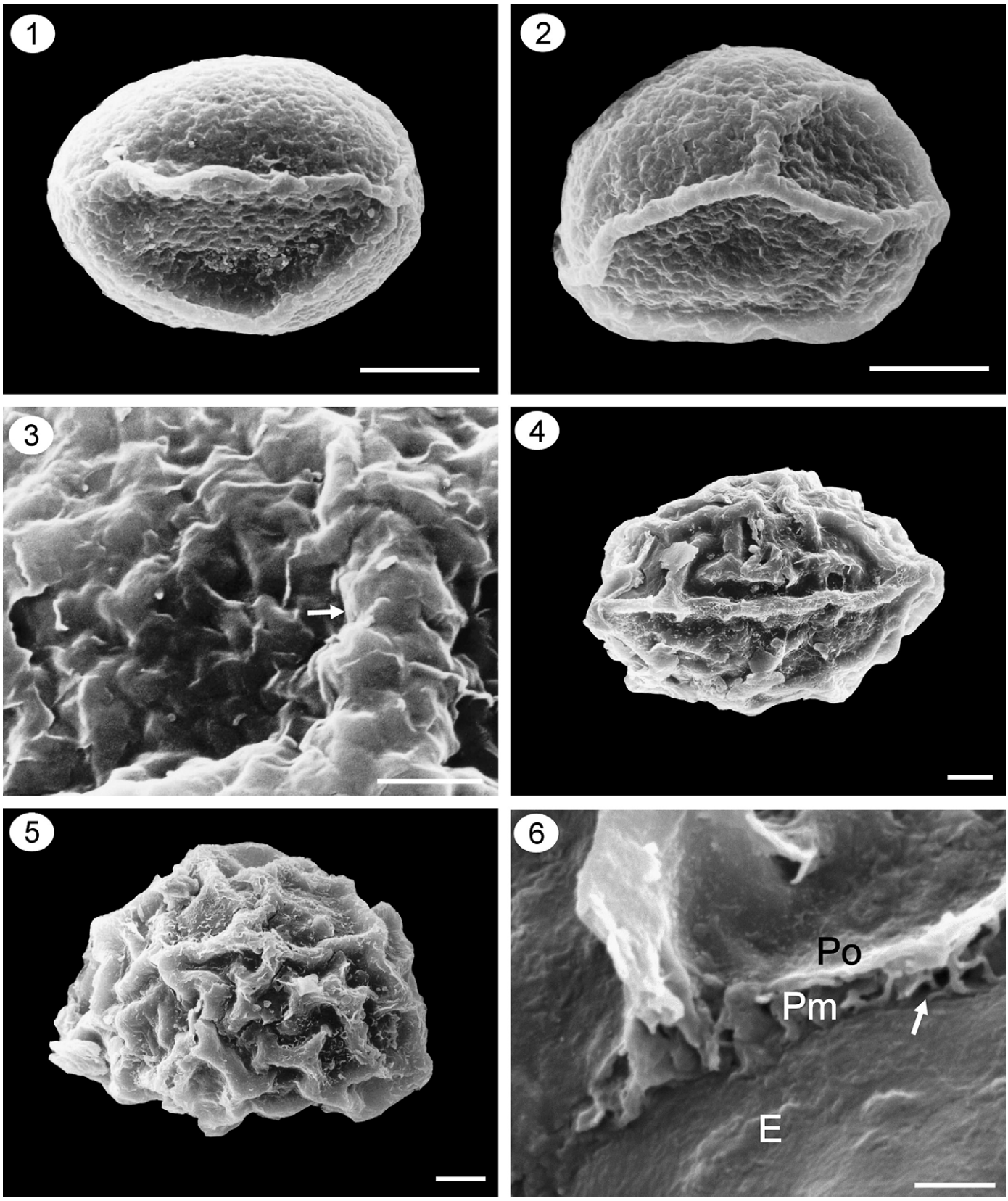

Plate I (*see caption on page 187*).

Blechnum austrobrasilianum: ARGENTINA: SALTA, Sta. Victoria, Pque. Nac. El Rey, Brown 816 (4) (LP), MP 3944; Idem, Los Toldos, de la Sota 7115 (LP), MP 3945; Jujuy, Dpto. Capital, Cabrera et al. 30641 (SI), MP 3971.

Blechnum brasiliense: ARGENTINA: TUCUMÁN, Capital, cultivado en LIL, Vervvorst 3303 (LIL), MP 3996; MISIONES, San Ignacio, Schwarz 5952 (LIL), MP 4098.

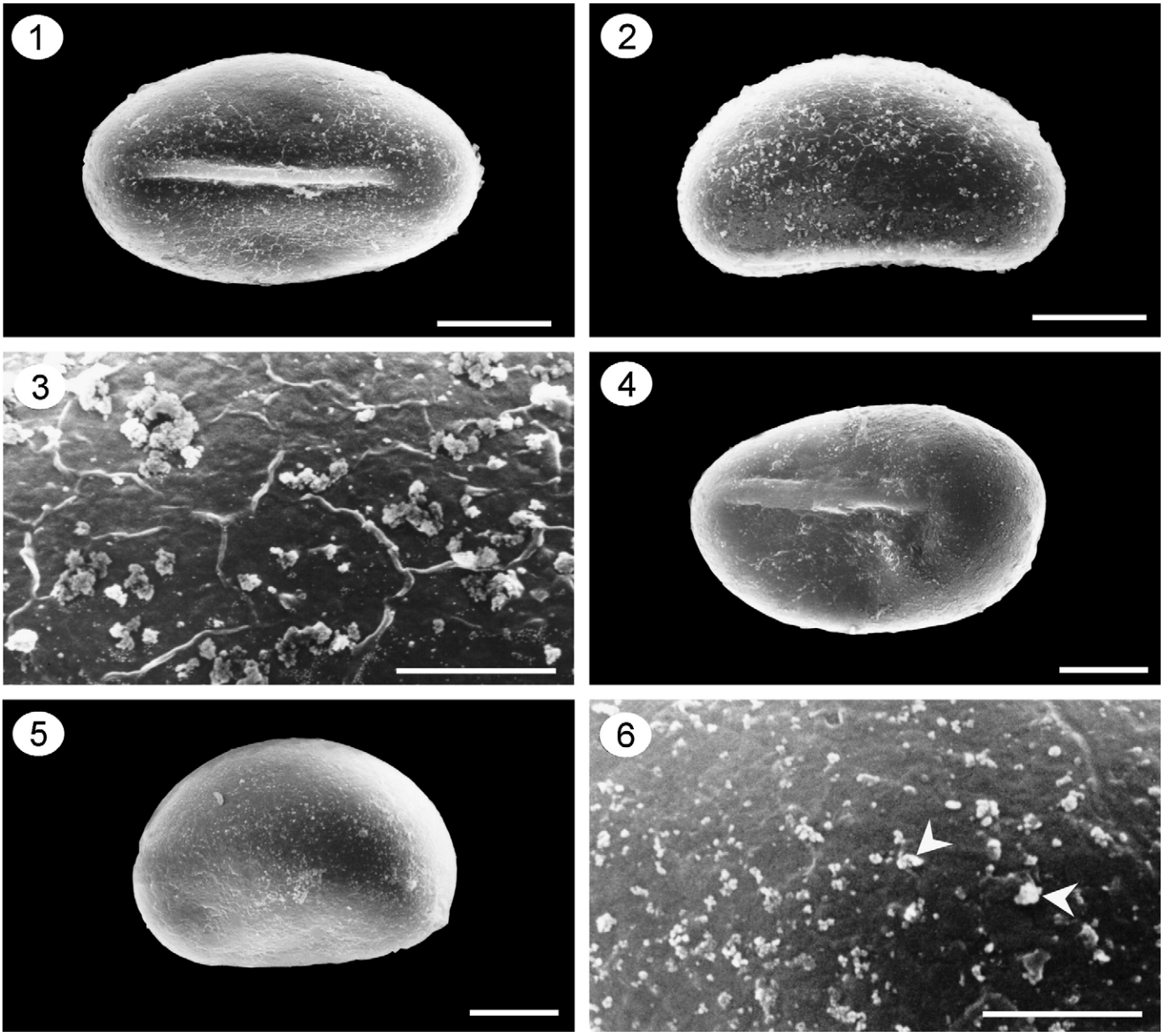

Plate II (*see caption on page 187*).

Blechnum cordatum: ARGENTINA: SALTA, Santa Victoria, *Palací 514* (LP), MP 4088; Idem, (MCNS) MP 4089.

Blechnum laevigatum: ARGENTINA: SALTA, Sta. Victoria, Los Toldos, Martínez, Ganem y de la Sota 659 (LP), MP 3969.

Blechnum mochaenum var. *squamipes*: ARGENTINA: TUCUMÁN, Chicligasta, Estancia Las Pavas, Venturi 300 (LIL, SI), MP 3972; idem, Venturi 3001, (SI), MP 3972; SALTA, Sta. Victoria, Los Toldos, Finca "El Nogalar", Cassá 255 (LP), MP 3946.

Blechnum occidentale: ARGENTINA: JUJUY, Ledesma, Dinelli s/n° (LIL 41714), MP 3876; TUCUMÁN, Horco Molle, L.M.R. Nalls s/n° (LIL 444143), MP 516.

Blechnum penna-marina: ARGENTINA: JUJUY, Capital, Laguna Yala, Schulz 6609 (LP), MP 3947; Capital, El Cucho, Cerro Labrado, de la Sota 4366 (LP), MP 3948.

Blechnum sprucei: ARGENTINA: JUJUY, Ledesma, Abra de las Cañas, 1700 m.sm., de la Sota 4451 (LP), MP 487 ; Capital, El Cucho, Cerro Labrado, de la Sota 4366 (LP), MP 3948; TUCUMÁN, Chichigasta, Estancia Las Pavas, Venturi 2994 (SI), MP 3968.

Blechnum tabulare: ARGENTINA: SALTA, Dpto. Santa Victoria, San José, Martínez, Ganem & de la Sota 603 (LP), MP 3949; JUJUY, Ledesma, camino a Valle Grande, Legname y Cuezzo 8219 c (LP), MP 3950; Valle Grande, Legname y Cuezzo 8609 (LP), MP 3951.

3. Results

3.1. Morphology

The palynological characteristics of the analysed taxa are summarized in Table 1. The spore shape in equatorial view is concave–convex, plane–convex or plane–biconvex and elliptic. They are oblong or subcircular in polar view. The major equatorial diameter (Fig. 1) is 31–104 µm, the minor equatorial diameter is 20–80 µm, and the polar diameter is 21–75 µm. The laesura in all the taxa analysed is tenuimarginate and 20–86 µm long. The exospore can be observed at LM as light brown or dark-brown. Its surface is smooth to slightly scabrate and single-layered in section.

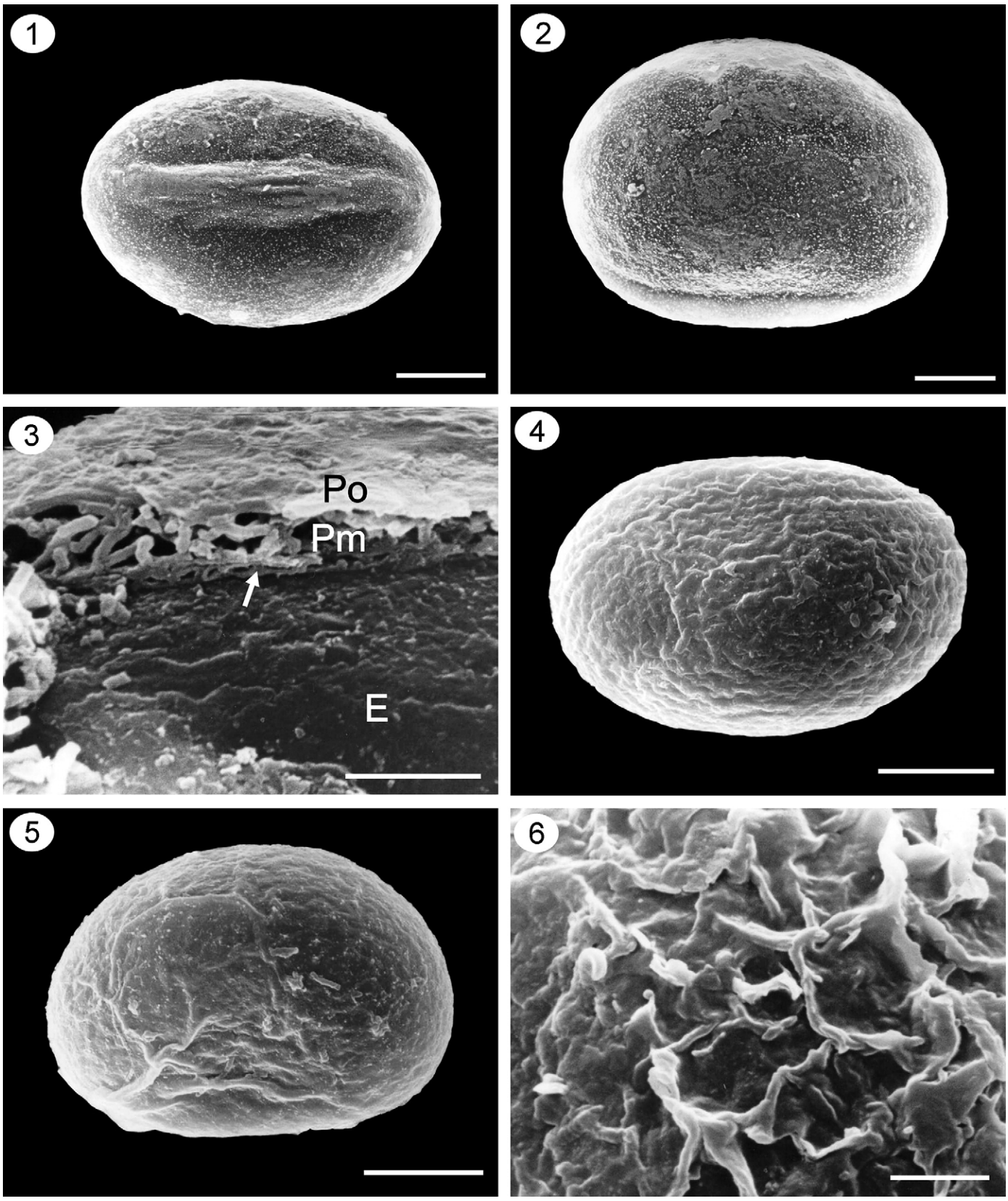

Plate III (*see caption on page 187*).

The perispore in *Blechnum cordatum* may reach a thickness of 12 µm in the folds. It is plane, rugulated or folded. If it has folds, these may be robust like in *B. tabulare* (Plate I, 4, 5), small like in *B. austrobrasilianum* and *B. penna-marina* (Plate II, 2, 3; Plate III, 4–6) or crested like in *B. cordatum* (Plate IV, 5–7). In addition, these folds may fuse and form an incomplete reticulum (Plate IV, 5–7; Plate I, 4, 5).

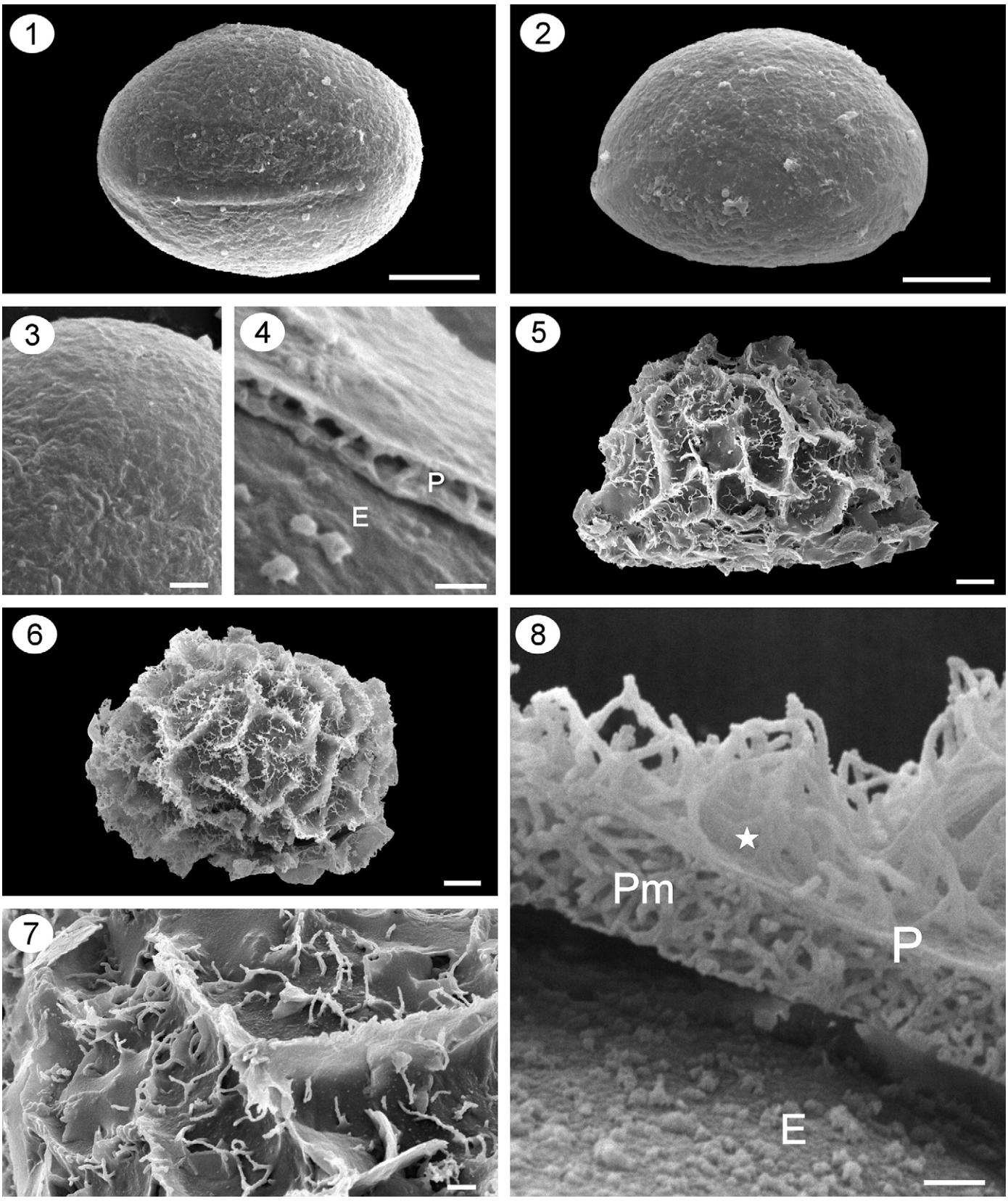

Plate IV (*see caption on page 187*).

Some perforations can be observed on the perispore like in *B. occidentale* (Plate V, 6).

In *Blechnum australe* subsp. *auriculatum* the perispore is plane or folded. When folds are present they are variable in shape, size and number. The surface has verrucae, rugulae and perforations. Spheroids were occasionally present on the perispore surface in *B. australe* subsp. *auriculatum* (Plate V, 1–4) and *B. occidentale* (Plate V, 6). A few small elements of variable shape

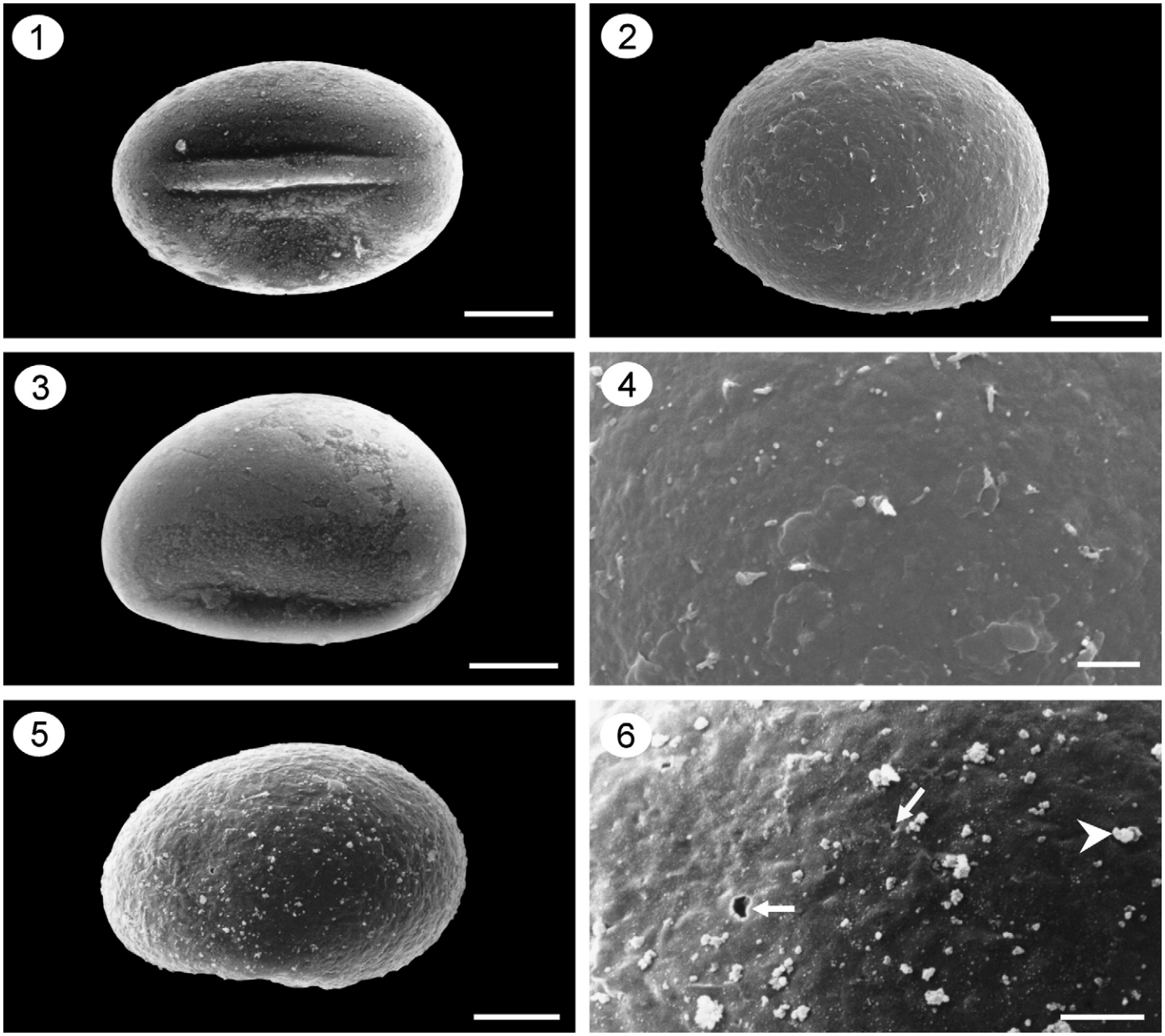

Plate V (*see caption on page 187*).

are scattered and attached to the surface in *B. laevigatum* (Plate II, 6).

3.2. Ultrastructure

For studies with TEM the following species were selected: *Blechnum australe* subsp. *auriculatum*, *B. brasiliense*, *B. cordatum*, *B. mochaenum* var. *squamipes* and *B. tabulare*. The selection was based on the variability in the structural characteristics observed with LM and SEM such as ornamentation, stratification, number and characteristics of the layers, thickness and complexity of the middle stratum.

The exospore (E) is 0.6–3.5 μm thick. In all the taxa analyzed it is two-layered: the inner layer (Ei) is 100–160 nm thick and the outer layer (Eo) is 650–840 nm thick with less contrast than the inner one. The apertural area consists of a strongly protruding fold. In the base of the apertural area the exospore thickens and has a progressive reduction towards the final part of the fold (Plate VI, 6; Plate VII, 4). Radial channels with a content of high contrast are observed in both layers of the exospore and are especially abundant near the aperture (Plate VI, 4–6). Cavities are also observed in the deepest area of the outer layer (Plate VI, 1).

In all the species, the exospore is smooth except in *Blechnum australe* subsp. *auriculatum* where it is scabrate (Plate VI, 1–3).

Spherules with similar structure and contrast to the perispore are observed on the surface (Plate VI, 2, 3; Plate VIII, 8).

Spheroids with the same structure and contrast as the exospore and perispore can be observed immersed in the perispore. Several short portions of membranes (scales) are seen on their surface (Plate VII, 4, 5).

Besides the exospore, a perispore can be observed. This wall is 0.4–3.0 μm thick and two-layered. Five types of structural perispores were observed and are described below:

Type 1, represented by *Blechnum australe* subsp. *auriculatum* (Plate VI, 1–3):

The perispore is two-layered: P1 and P2. The inner layer (P1) consists of three strata: the inner stratum (P1i) is 60–80 nm thick and

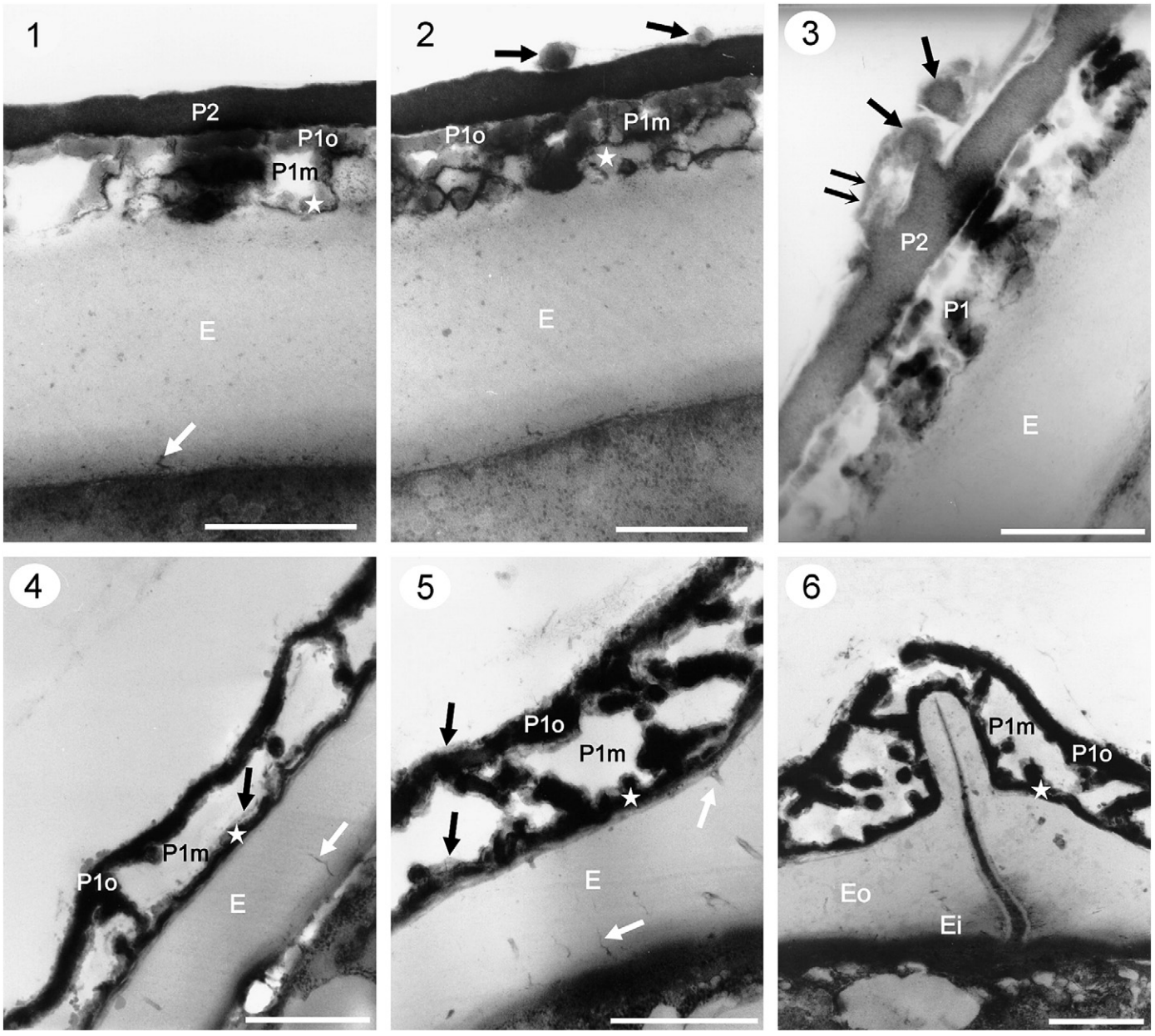

Plate VI (*see caption on page 187*).

adhered to the exospore; the middle stratum (P1m) is 230–250 nm thick, cavate pillared, with rods arranged in several directions in a compact way; the outer stratum (P1o) is 60–90 nm thick. The outer layer (P2) is 130–200 nm thick, homogeneous, continuous, with irregular surface and more contrast than the inner one.

Type 2, represented by *Blechnum brasiliense* (Plate VI, 4–6):

The perispore is two-layered: P1 and P2. The inner layer (P1) is three-stratified: the inner stratum (P1i) is 80–120 nm thick, continuous and adhered to the exospore, the middle stratum (P1m) is 470–600 nm thick, cavate, pillared and composed of radial threads; the outer stratum (P1o) is 110–280 nm thick and has discontinuities. The outer layer (P2) is 40–70 nm thick, less contrasted than P1 and is just coating all around the different elements forming P1.

Type 3, represented by *Blechnum cordatum* (Plate VIII, 1–5):

The perispore is two-layered: P1 and P2. The inner layer (P1) is three-stratified: the inner stratum (P1i) is 90–170 nm thick and adhered to the exospore; the middle stratum (P1m) is 160–200 nm thick, constituted of interwoven, ramified and fused threads, and in the bases of folds the threads are laxly distributed forming large cameras (Plate VIII, 3); the outer stratum (P1o) is 100–200 µm thick with threads similar to those of the middle stratum on the surface of this stratum (these structural components are simple or ramified and fused to other similar components of the same stratum) (Plate IV, 8; Plate VIII, 2, 4, 5). The outer layer (P2), is 40–90 nm thick, less contrasted than P1 and covers the inner and outer surfaces of P1 (Plate VIII, 5).

Type 4, represented by *Blechnum mochaenum* var. *squamipes* (Plate VIII, 6–8):

The perispore is two-layered: P1 and P2. The inner layer (P1) is three-stratified: the inner stratum (P1i) is 90 to 200 nm thick and adhered to the exospore; the middle stratum (P1m) is 900–1200 nm thick, with abundant threads ramified and fused in different directions (Plate VIII, 6–8); the outer stratum (P1o) is 150–200 nm thick, including perforations on the surface that traverse this stratum (Plate VIII, 8). The outer layer (P2) is 50–70 nm thick, less contrasted than P1, and is essentially a coating all

around the different elements forming P1 including the inner surface of the perforations (Plate VIII, 8).

Type 5, represented by *Blechnum tabulare* (Plate VII 1–5):

The perispore is two-layered: P1 and P2. The inner layer P1 is three-stratified: the inner stratum (P1i) is 30–70 nm thick, with some scales evident just on the exospore surface (Plate VII, 3); the middle stratum (P1m) is 1000–5000 nm thick, and is composed of threads; the outer stratum (P1o) is 120–170 nm thick (Plate VII, 1–5). The outer layer (P2) is 50–60 nm thick, less contrasted than P1 and is essentially a coating all around the different elements forming P1 including the inner surface (Plate VII, 1–3). In places where the folds rise the middle stratum (P1m) shows a lax structure bearing cameras (Plate VII, 1).

4. Discussion and conclusions

Based on the data given here greater dimensions are found in the spores of *Blechnum cordatum* and *B. tabulare* (greater than 78 µm in equatorial diameter) than in the rest of the analyzed taxa which show small to medium sizes (31–50 µm). These sizes coincide with the data given by Morbelli (1974, 1976, 1980) and Kurita and Nishida (1985). Differences in size are usually related to differences in ploydia. However, such differences have not been reported in the studied taxa. It may be pointed out that these taxa in particular are the only ones which show an arborescent or subarborescent habit among those which grow in the studied area.

Based on the analyses made with LM and SEM, the spores of the *Blechnum* species studied have smooth, rugulate or folded perispore. However, in some species they show variations such as folded–rugulated or smooth with some isolated folds.

The short rugulae observed in spores of several species (e.g., *Blechnum australe* subsp. *auriculatum* and *B. occidentale*) in some cases could be due to dehydration, considering that they represent dry herbarium specimens. Thus, working with dry specimens could lead to erroneous interpretations regarding ornamentation in these species. The folds observed with TEM are elevations of the perispore involving all its layers and strata. These observations confirm the presence of folds in *B. cordatum, B. penna-marina, B. spruce* and. *B. tabulare*.

Spheroids present on the perispore surface of *Blechnum australe* subsp. *auriculatum* have the same structure and contrast as one or more layers of the perispore. This is the reason why they are considered "spherules" according to the concept and terminology proposed by Tryon and Lugardon (1991, p. 9).

Other spheroids observed with TEM included in the perispore of *Blechnum tabulare* have the same structure as the exospore and the perispore. Due to their characteristics, location and the definition proposed by Lugardon (1981), we consider them as "captive globules". These globules were frequently found on the spore surface of Filicophyta and homologized with Ubisch Bodies of the Spermatophytes by Morbelli (1977) and Lugardon (1981).

The few small elements attached to the surface observed in *Blechnum laevigatum* and *B. occidentale* could be due to tapetal remnants that remain adhered to the perispore surface.

Based on the studies carried out here with TEM, the characteristcs of the exospore correspond to the "blechnoid" type defined by Lugardon (1972). According to this author, it would be formed by two layers: the outer layer with greater thickness and with a fractured stratum in contact with the thin inner layer.

In all cases, the perispore consists of two layers (layer P1 and layer P2) with three different strata are found in layer P1. Even if similarity as regards stratification was observed among the studied taxa, differences were found among the studied taxa in the characteristics of the medium stratum of P1, as regards the number of threads, degree of fusion and their ramifications.

According to their ultrastructural features and according to those defining the five types identified and listed above, the 10 species of *Blechnum* which have been studied in the present work can be divided as follow: Type 1: *B. australe* subsp. *auriculatum*, *B. austrobrasilianum*, *B. laevigatum*; Type 2: *B. brasiliense*, *B. occidentale*; Type 3: *B. cordatum*; Type 4: *B. mochaenum* var. *squamipes*, *B. penna-marina*, *B. sprucei*; Type 5: *B. tabulare*.

Blechnum cordatum is the only case in which threads are observed on the P1 layer; they are similar in structure to those of the middle stratum. These threads constitute the structural elements that are superficially observed on and between the folds.

The differentiated structural types do not find any correspondence with the ornamentation patterns (plane, rugulated or folded) of the species here analyzed.

We observed in this study with TEM that the perispore of *Blechnum australe* subsp. *auriculatum* is composed of two layers (P1 and P2). The inner layer P1 consists of three strata. Nevertheless, Morbelli (1976) with LM recognized one perispore layer in the same species.

The perispore stratification observed with TEM in *Blechnum brasiliense* is coincident with the perispore structure described by Morbelli (1976) based on observations with LM. Elsewhere, the ultrastructure observed in this work in *B. cordatum* is similar to that

Plate VII. TEM images of the spores of *Blechnum tabulare* (perispore type 5). (see on page 195)

1, 2. Section through the sporoderm. The exospore is two-layered. The inner layer (Ei) is slightly darker and thinner than the outer layer (Eo). The perispore is two-layered. The layer P1 consists of three strata: the inner stratum (P1i, white arrows) is adhered to the exospore, the middle stratum (P1m) is cavate (c) and has rodlets and the outer stratum (star) is continuous and homogeneous. The layer P2 (black arrows) less contrasted than P1, it covers all the free surfaces of layer P1. Scale bars = 1 µm.

3. Short portions of membranes (= scales) are evident on the inner part of the inner perispore stratum (P1i). Scale bar = 0.5 µm.

4. Section through the sporoderm at the aperture that consists of a strongly protuding fold. Two layers (Ei) and (Eo) are distinguishable in the exospore. In the perispore (P), within the P1m stratum a globule (double arrow) is distinguishable. Its centre has a similar contrast than the exospore. Scale bar = 1 µm.

5. Detail of the globule in 4. Note that its centre has the same structure and contrast as the exospore and that there are several scales (double arrows) distributed tangentially to it. Scale bar = 0.5 µm.

Plate VIII. TEM images of the spores of *Blechnum cordatum* and *B. mochaenum* var. *squamipes*. (see on page 196)

1–5. *B. cordatum* (perispore type 3).

1, 2. Section through the sporoderm. The perispore is two-layered. The layer P1 consists of three strata: the inner stratum (P1i, star) is adhered to the exospore (E), the middle stratum (P1m) is composed of a high number of interwoven threads and the outer stratum (P1o) with threads outside its surface (arrowhead). The layer P2 (arrow) is less contrasted than P1, it covers all the free surfaces of layer P1. Scale bars = 1 µm.

3. At the base of some folds the threads of the middle stratum (P1m) are laxly arranged delimiting a camera (c). Scale bar = 1 µm.

4, 5. Outside of the outer stratum (P1o), similar threads than the middle stratum (P1m) can be observed (arrowheads). The threads are circular in transverse section (double arrow). The layer P2 (arrows) is less contrasted than P1 and covers all the free surfaces of layer P1. Scale bars = 4: 1 µm, 5: 0.5 µm.

6–8. *B. mochaenum var squamipes* (perispore type 4).

Section through the sporoderm. The perispore, is two-layered and more contrasted than the exospore. The inner perispore layer P1 consists of three strata: the inner stratum (P1i, star) is adhered to the exospore (E), the medium stratum (P1m) has abundant rodlets frequently fused and the outer stratum (P1o) with few perforations (white arrow). The layer P2 (arrow) is less contrasted than layer P1 and covers its surface. Scale bars = 6: 1 µm, 7–8: 0.5 µm.

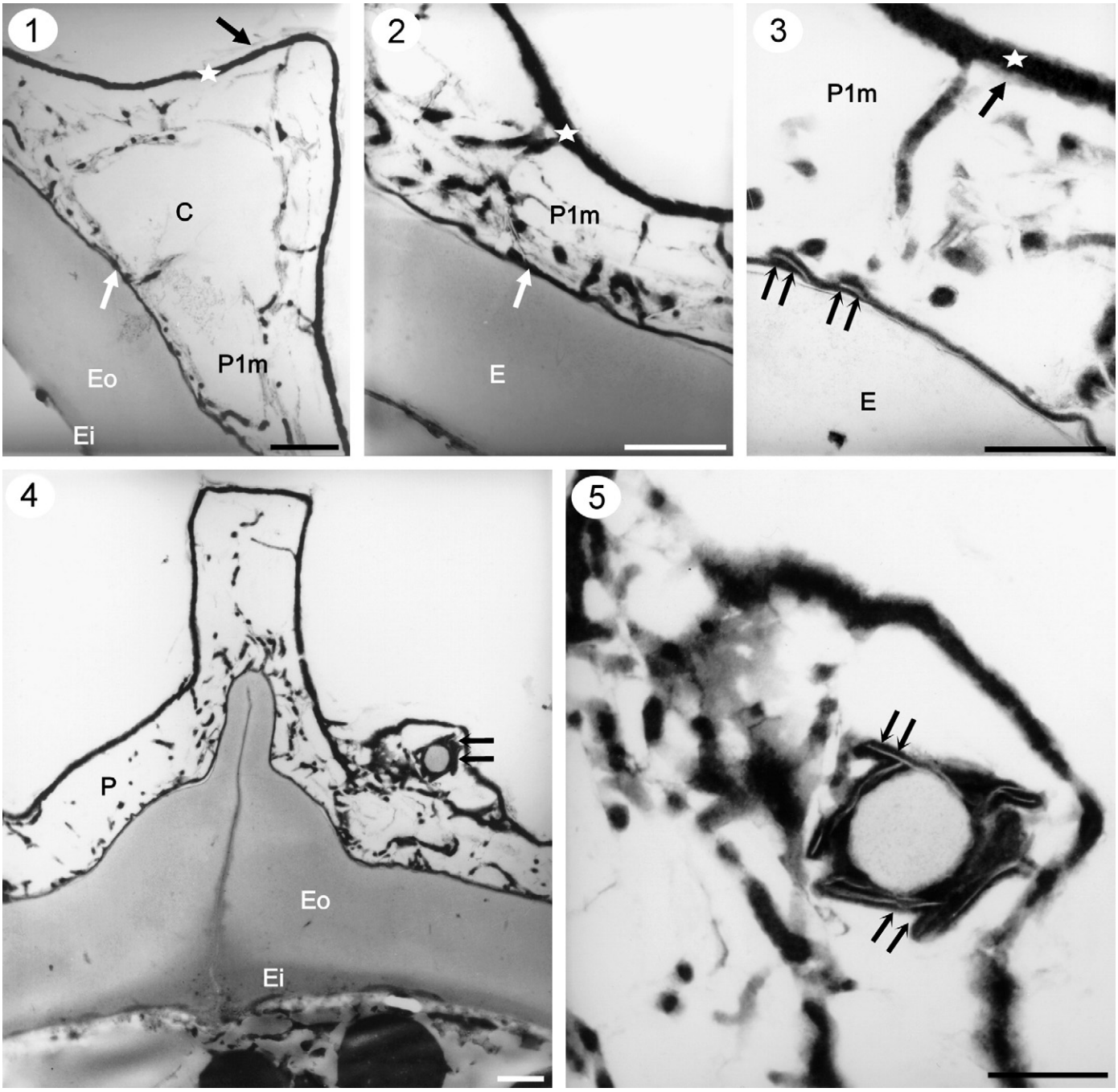

Plate VII (*see caption on page 194*).

observed in SEM fractures in the same species based on material from Bolivia by Kurita and Nishida (1985).

For other species of *Blechnum* that grow in other areas Tryon and Lugardon (1991) described the perispore ultrastructure as alveolate, lacunose and cavate pillared. According to this classification, two of the five species selected for the ultrastructural types recognized in Argentina (*B. australe* subsp. *auriculatum* and *B. brasiliense*) would correspond to the cavate pillared type defined by Tryon and Lugardon (1991).

The scales observed in the perispore inner stratum and surrounding the globules in *Blechnum tabulare* could be related to membrane units which are responsible for wall deposition. The presence of scales was extensively noted by Tryon and Lugardon (1991) in the basal part of the perispore and surrounding the globules. Authors such as Blackmore and Barnes (1987) in in their studies about origin, development and homologies of Embryophte spore walls and Uehara et al. (1991) in spores of Isoetaceae commented on sporopollenin deposition during the wall formation.

According to the results obtained in this contribution it can be said that the perispore is the wall that has the greatest variability at specific and infraspecific levels. Those variations are related to: characteristics of the structural elements of the P1 middle perispore stratum (density, fusion and ramification) and folds (presence/absence, and if present to its height, length and frequency of fusion). Thus, this observation is partially in agreement with the generalization proposed by Tryon and Tryon (1982) who suggested that the "type of surface and stratification of the perispore is usually characteristic of genera or infrageneric groups".

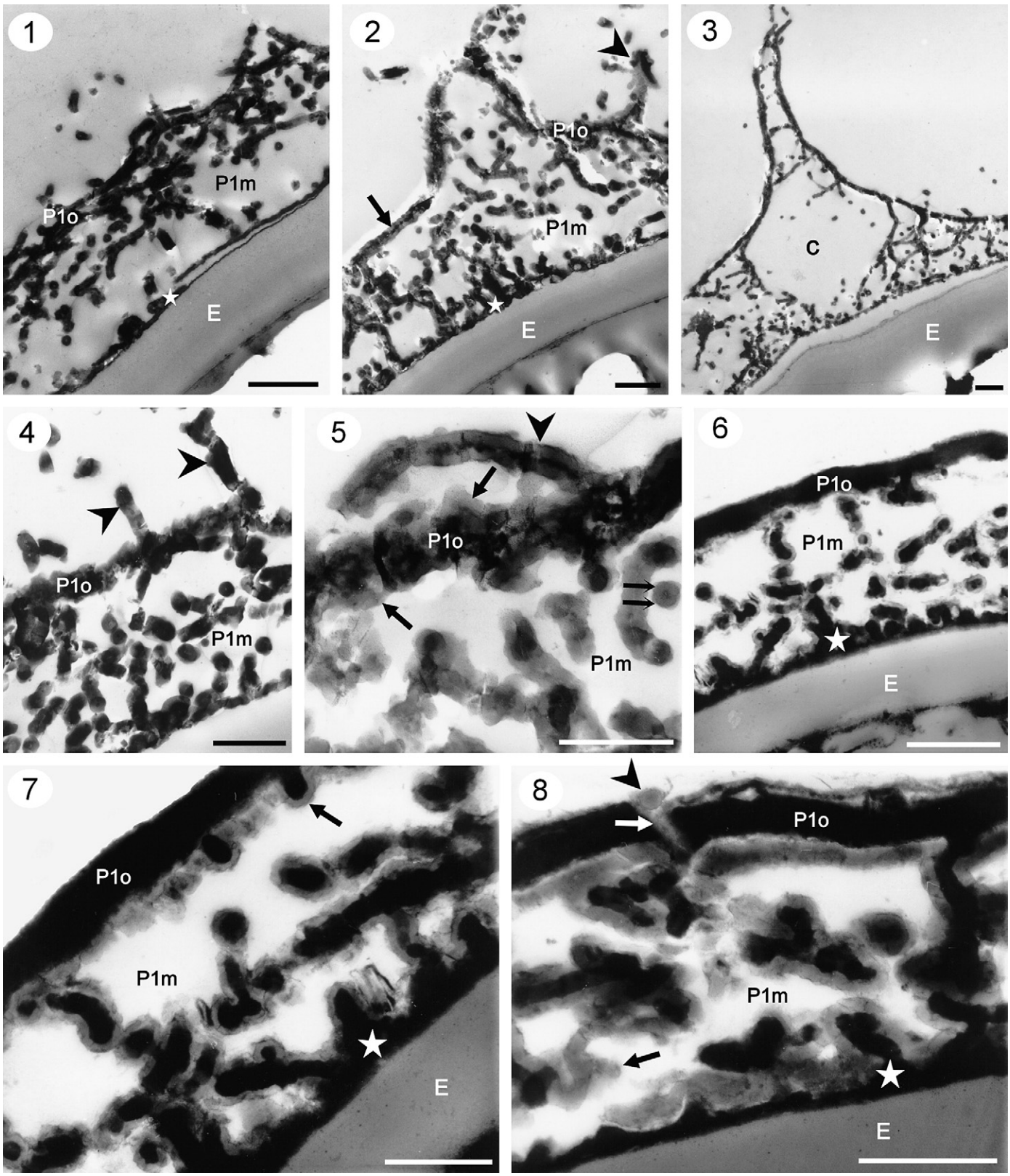

Plate VIII (*see caption on page 194*).

According to a molecular phylogenetic analysis by Cranfill (2001), the genus *Blechnum* is paraphyletic and should be divided into several small monophyletic genera. The great diversification found in spore characteristics in our study could be interpreted as a consequence of the paraphyletic nature of *Blechnum*. Thus, the results obtained in this study contribute to further palynological ecological, systematic and phylogenetic studies for a better knowledge of genus *Blechnum* and its interspecific relationships.

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