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Morphology and ultrastructure of the spores of the Grammitidaceae from Argentina

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Abstract

Spores of Grammitidaceae that grow in Argentina were studied, including *Grammitis magellanica*, *G. patagonica*, *G. poeppigiana*, *Lellingeria tungurahue*, and *Melpomene peruviana*. The study was performed on herbarium material with a light microscope and scanning and transmission electron microscopes. The spores are trilete with circular outline in polar view, the equatorial diameter is 30–83 μm and the polar diameter is 28–64 μm. The exospore is 0.3–1.44 μm thick, tuberculate–papillate, verrucate and in some cases gemmulate. It is composed of two layers and includes radial channels and very small cavities with dark contents. The cavities are in the exospore outer layer, mostly arranged along the contact surface with the inner layer. They are tangentially aligned with respect to the inner exospore surface. The perispore is c.a. 270 nm thick. It is composed of one to several strata and has irregular, spaced concentrations of dense materials. Globules of different sizes, free or fused together and covered with the perispore were observed. The sporoderm organization and structure are similar in the three genera studied. Nevertheless, differences in spore size, general morphology and the wall thickness were found.

Keywords: spores; morphology; ultrastructure; Grammitidaceae; Argentina

1. Introduction

Grammitidaceae comprises approximately 750 species with a Tropical and Austral distribution (Parris, 1990, 1998). According to de la Sota et al. (2000), five species belonging to three genera are present in Argentina. They are: *Grammitis magellanica* Desvaux, *Grammitis patagonica* (C. Christensen) Parris, *Grammitis poeppigiana* (Mettenius) Pichi-Sermolli, *Lellingeria tungurahue* (Rosenstock) A. R. Smith and R. C. Moran and *Melpomene peruviana* (Desvaux) A. R. Smith and R. C. Moran.

The three genera are distributed as follows: *Grammitis* grows in the Andean Patagonic Woods, *Lellingeria* is restricted to Salta province, North-western Argentina, while *Melpomene* has a wider distribution and grows in Central and North-western Argentina.

Contributions on Grammitidaceae spore morphology were made by Large and Braggins (1991) and Nayar and Devi (1965), who analysed the material from New Zealand and India, respectively.

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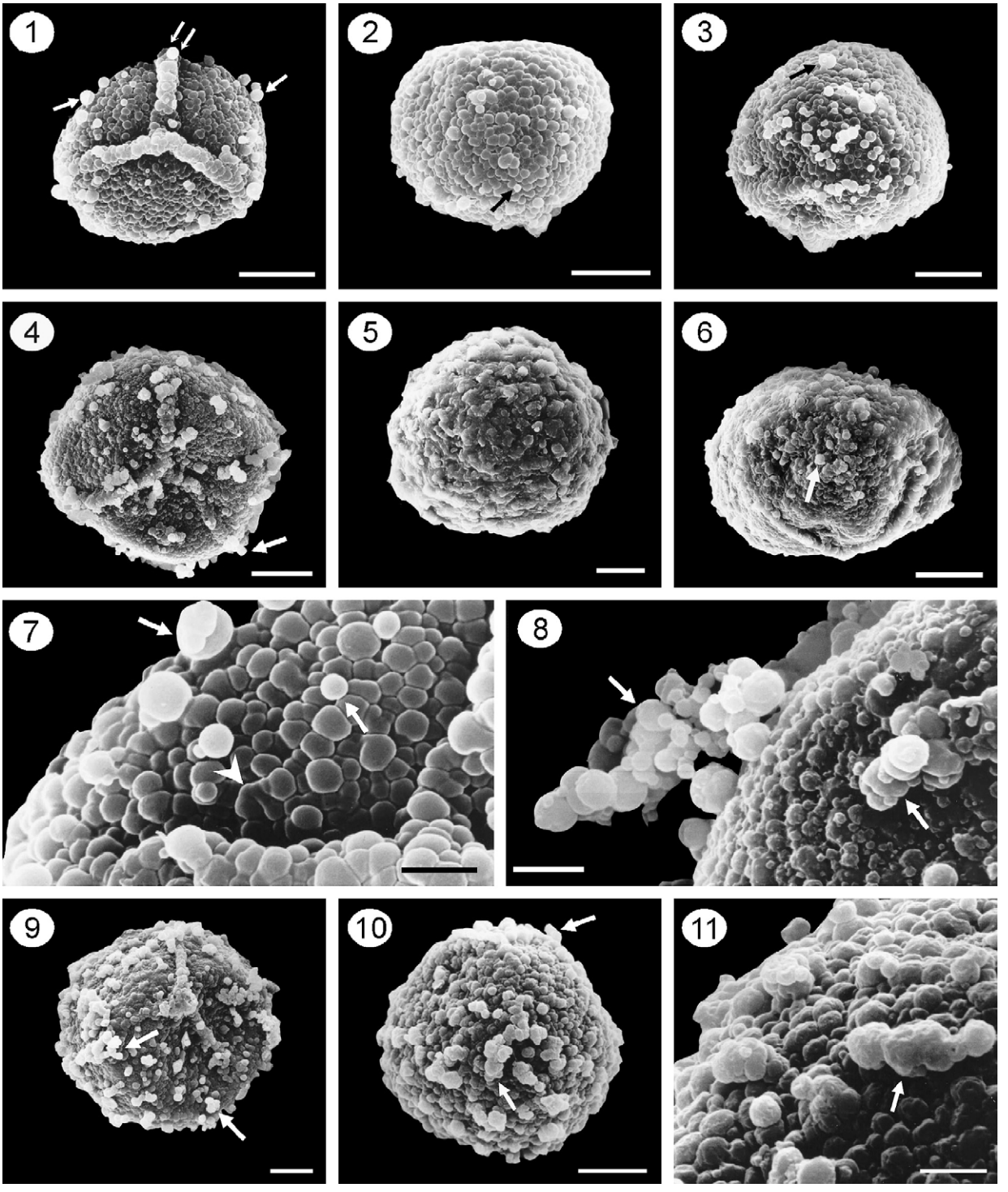
With respect to studies on American taxa, general descriptions are found in floristic studies such as those of Tryon and Tryon (1982) and Rodríguez Ríos (1974, 1995). The latter author, who analysed specimens from Chile, first described the spore ornamentation of *Grammitis magellanica* and *G. poeppigiana* as verrucate, and that of *G. patagonica* as papillate. Later (Rodríguez Ríos, 1995) he described the spore ornamentation in the three species of *Grammitis* as papillate.



Another study concerning this family is that of Wagner (1985) who analysed mainly material of *Grammitis* from Costa Rica and defined the spore ornamentation as tuberculate.

Tryon and Lugardon (1991) carried out studies with scanning electron microscopy (SEM) of *Grammitis* and *Melpomene* (this last one under the name *Grammitis*) species and defined the spore ornamentation as papillate or tuberculate. They also studied some species of *Grammitis* and *Lellingeria* (this last one under the name *Grammitis*) that grow in America or in the Old World by using scanning and transmission electron microscopy.

Regarding Argentina, only general descriptions in de la Sota (1966, 1977) and in the Patagonia Flora (de la Sota et al., 1998) were found.

Morbelli (1980) studied with light microscopy (LM) the species of *Grammitis* that grow in the Andean Patagonic Woods of Argentina and Chile. The author described the spores as having varied processes such as verrucae, gemae, clavae, granules and papillae.

No studies have been conducted on *Melpomene peruviana* and *Lellingeria tungurahue* with transmission electron microscopy (TEM). *Lellingeria tungurahue* has been quoted recently for the region by de la Sota et al. (2000) and has not been studied yet with scanning or transmission electron microscopy.

The aim of this study is to analyse the spores by using LM, SEM and TEM of the Grammitidaceae taxa that grow in Argentina as a contribution to the knowledge about the general morphology and the wall ultrastructure of the spores and assess if those characteristics could be useful for systematic proposes.

2. Materials and methods

The study was based on herbarium material from the following Argentinean institutions: Laboratorio de Botánica del Ministerio de Agricultura de la Nación, Buenos Aires (BAB), Instituto Miguel Lillo, Tucumán (LIL), División Plantas Vasculares, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata (LP), and Instituto de Botánica Darwinion, San Isidro (SI).

For LM analysis, the material was acetolyzed (cf. Erdtman, 1960) after treatment with hot 3% sodium carbonate for 2 min and was analysed with Olympus BH2 and CH2 microscopes.

For SEM study, the material was treated with hot 3% sodium carbonate for 2 min, 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 as proposed by Rowley and Nilsson (1972) as follows: rehydrated with 0.1 M phosphate buffer and with 1% Alcian Blue for two hours; then fixed with 1% glutaraldehyde + 1%

Plate I. Spores of *Grammitis* with SEM.

1–3, 7 *Grammitis magellanica*.

1. Proximal view of a globose spore with a circular outline. Spheroids, single or grouped, are evident on the surface (arrows). The laesurae are covered by verrucae and spheroids (double arrow). Scale bar: 10 μm.
2. Distal view of a spore with a verrucate–papillate–tuberculate ornamentation. There are also spheroids on the surface (arrow). Scale bar: 10 μm.
3. Equatorial view of a spore. Numerous spheroids are seen on the surface (arrow). Scale bar: 10 μm.
7. Detail of the surface of spore in fig. 1. The ornamentation is verrucate–papillate–tuberculate. The tubercles are irregular in form and size, and some of them are fused (arrowhead). Spheroids of variable size are seen adhered to the surface (arrows). Scale bar: 2.5 μm.

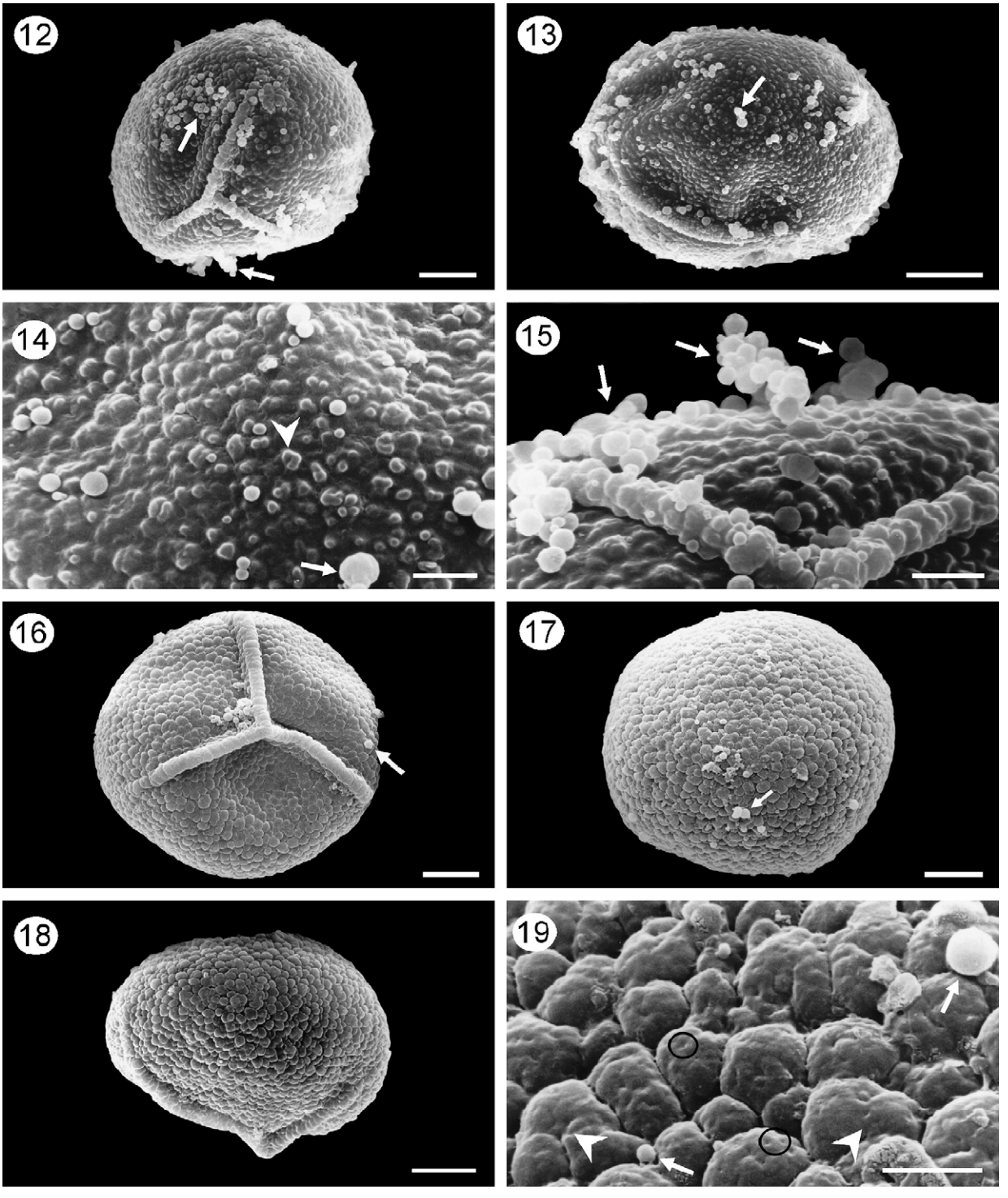
4–6, 8 *Grammitis patagonica.*

4. Proximal view of a globose spore with a circular outline. Many single or grouped spheroids are seen on the surface (arrow). The laesurae are covered by verrucae and spheroids. Scale bar: 10 μm.
5. Distal view of a spore with a verrucate–gemmulate ornamentation. Scale bar: 10 μm.
6. Equatorial view of a verrucate–gemmulate spore with spheroids on the surface (arrow). Scale bar: 10 μm.
8. Detail of the spore surface. The ornamentation is verrucate–gemmulate. The verrucae are frequently fused one another. Numerous spheroids fused together forming glomerules can be observed on the surface (arrows). Scale bar: 2.5 μm.

9–11 *Grammitis poeppigiana*.

9. Proximal view of a globose spore with a circular outline. Spheroids, single or grouped, are seen on the surface (arrows). The laesurae are covered by verrucae and spheroids. Scale bar: 10 μm.
10. Distal view of a spore with a verrucate ornamentation. Numerous spheroids are evident on the surface (arrows). Scale bar: 10 μm.
11. Detail of the spore surface. On the verrucae numerous spheroids are fused forming glomerules (arrow). Scale bar: 2.5 μm.

Alcian Blue in phosphate buffer for 12 h; then washed for 15 min in phosphate buffer and postfixed with 1% Osmium tetroxide in water plus 1% Alcian Blue for 2 h. The spores were dehydrated in an acetone series (30–100%) and then embedded in Spurr soft mixture. Ultra thin 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 TEM at the Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires.



Grammitis magellanica and *G. poeppigiana* were selected as representative for the study with TEM due to the high morphological homogeneity of the genus *Grammitis*.

The following features were analysed: shape, diameter, laesura, ornamentation and ultrastructure.

For spore descriptions, the terms proposed by Morbelli (1980), Tryon and Lugardon (1991), and Punt et al. (1994) were used.

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

2.1. Material studied

Grammitis magellanica: Argentina: Tierra del Fuego, Puerto Cooke, 30/XI/1967, M. A. Torres 1180, (LP), MP 462.

Grammitis patagonica: Argentina: Santa Cruz, Estancia Fitz Roy, Chorro de agua, 26/XII/1950, Sleumer 1316, (LIL), MP 753; Chubut, Dpto Chushamen, Lago Esperanza, Pobl. Cárdenas, 31/I/1996, Jonson 625, (SI), MP 3965.

Grammitis poeppigiana: Argentina: Neuquén, Parque Nacional Nahuel Huapi, naciente del Arroyo Estacada 1600 m, 24/II/1953, Boeckle and M. Correa 7188, (BAB), MP 478; Tierra del Fuego: Ushuaia, 29/II/1896, Alboff 860, (LP), MP 463; Río Olivia, 22/VI/1974, Pichi Sermolli and Bizarri 7547, (SI), MP 3967; Canal de Beagle, Isla Redonda en frente a Lapataia, 22/VI/1974, Pichi Sermolli and Bizarri 7528, (SI), MP 3966.

Lellingeria tungurahue: Argentina: Salta: Dpto Santa Victoria, camino Los Toldos a Lipeo, Vallecito, Quebrada Honda, 13-XII-1997, Cassá et al. 281, (LP), MP 3942.

Melpomene peruviana: Argentina: Tucumán: Dpto. Tafí, Tafí del Valle, II-1965, Eskuche 129, (LP), MP 3858; Jujuy: Dpto. Santa Bárbara, Sierra de Santa Bárbara, 13-XII-1962, de la Sota 2908, (LP), MP 3859; Salta: Dpto. Santa Victoria, Santa Victoria, 27-II-1966, de la Sota 4191, (LP), MP 3955.

3. Results

3.1. Morphology

3.1.1. Grammitis

The spores of the different species of *Grammitis* studied here share general characteristics that are summarized below.

They are trilete, with a circular outline in polar view. In equatorial view, the proximal face usually appears strongly convex and the distal face is hemispheric. The laesurae bear dense verrucae analogous to those covering the various spore regions (*G. magellanica* Plate I, 1, 7; *G. patagonica* Plate I, 4; *G. poeppigiana* Plate I, 9)

There are numerous spheroids, single or combined together forming glomerules, fused to the spore surface (Plate I).

3.1.2. Grammitis magellanica (Plate I, 1–3, 7)

The equatorial diameter is 31–41 μm and the polar diameter is 29–38 μm. The laesurae are 11–38 μm long and reach the equator.

Observed with SEM the exospore is verrucate–papillate–tuberculate, with tubercles in high density, which sometimes are fused to one another (Plate I, 1–3). The perispore is not evident under SEM.

Plate II. Spores of *Lellingeria tungurahue* and *Melpomene peruviana* with SEM.

12–15. *Lellingeria tungurahue*.

12. Proximal view of a globose spore with a circular outline. Spheroids, single or grouped, are seen on the surface (arrows). A series of verrucae are seen on the laesurae. Scale bar: 10 μm.
13. Equatorial view of a spore with a papillate–tuberculate ornamentation. Numerous spheroids are seen on the surface (arrow). Scale bar: 10 μm.
14. Detail of the spore surface. The papillae and tubercles are frequently fused one another (arrowhead). Some spheroids are seen on the spore surface (arrow). Scale bar: 2.5 μm.
15. Detail of the papillate–tuberculate proximal surface. The papillae and tubercles are low. Two laesurae with a similar ornamentation to the rest of the spore surface are evident. Numerous spheroids fused forming glomerulae are seen (arrows). Scale bar: 2.5 μm.

16–19. *Melpomene peruviana*.

16. Proximal view of a globose spore with a circular outline. Few spheroids are seen on the surface (arrow). Scale bar: 10 μm.
17. Distal view of a spore with a tuberculate ornamentation. Few spheroids are seen on the surface (arrow). Scale bar: 10 μm.
18. Equatorial view of a spore with tubercles in high density. Scale bar: 10 μm.
19. Detail of the spore surface. The ornamentation is tuberculate. The tubercles are irregular in size and form. They are frequently fused one another (arrowheads). Puncta on tubercle surface (circled areas) and few spheroids (arrows) are visible. Scale bar: 2.5 μm.

3.1.3. Grammitis patagonica (Plate I, 4–6, 8)

The equatorial diameter is 36–58 μm and the polar diameter is 31–47 μm. The laesurae are 10–28 μm long and have verrucae on their surfaces.

Observed with SEM, the exospore is verrucate–gemmulate. The verrucae are irregular and often fused one another. (Plate I, 4–6). The perispore is not evident under SEM.

Besides the usual trilete spores, monolete spores have been observed in both specimens of which spores have been analysed.

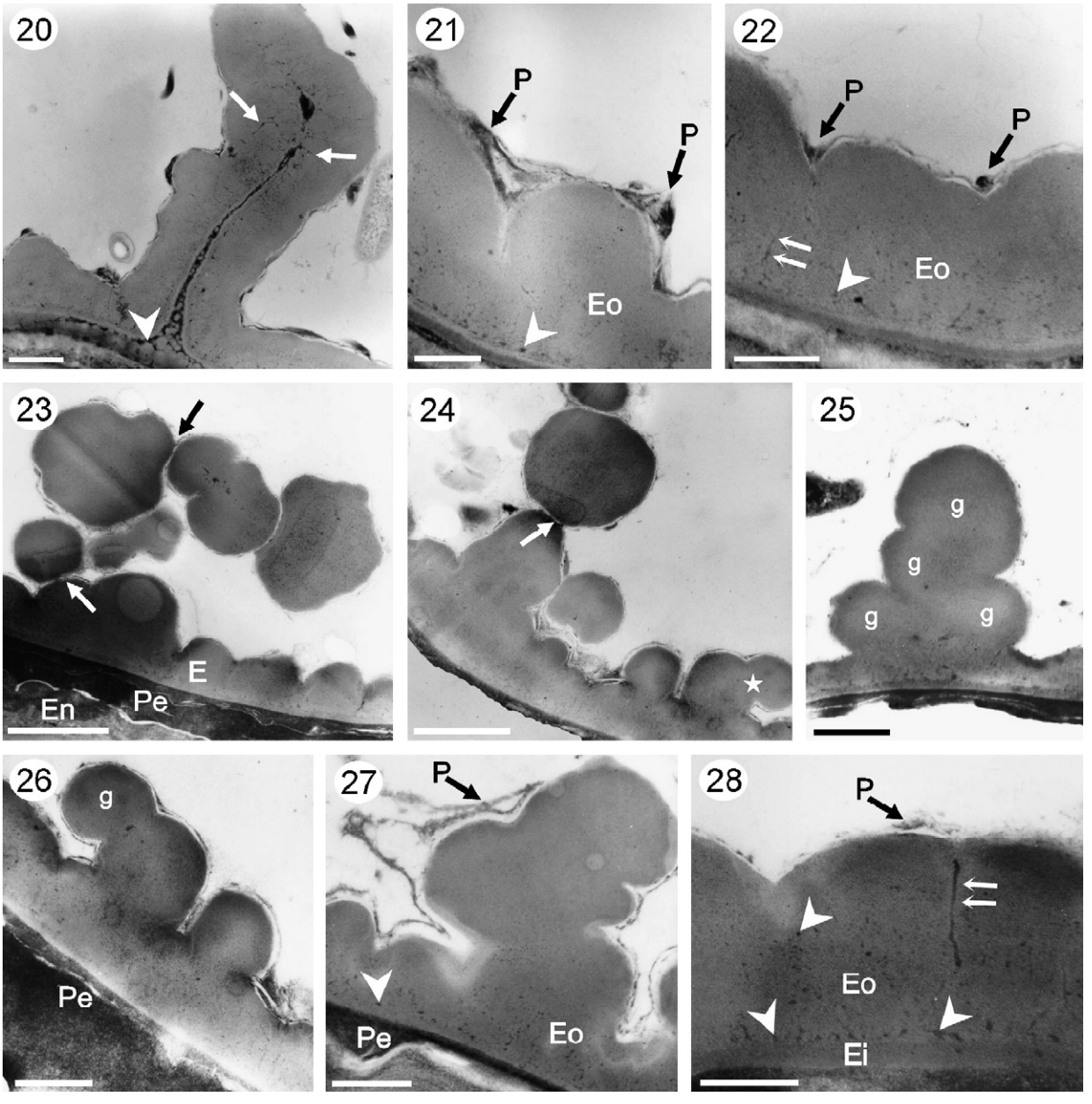
3.1.4. Grammitis poeppigiana (Plate I, 9–11)

The equatorial diameter is 37–58 μm and the polar diameter is 29–38 μm. The laesurae are 15–27 μm long and reach the equator.

Observed with SEM, the exospore is verrucose with some verrucae fused to one another (Plate I, 9–11). The perispore is not distinguishable under SEM.

3.1.5. Lellingeria tungurahue (Plate II, 12–15)

The spores are trilete, globose with a circular outline in polar view. The equatorial diameter is 31–



42 μm and the polar diameter is 24–32 μm. In equatorial view, the proximal face is convex and the distal face is hemispheric. The laesurae are 12.1–21.3 μm long and in some spores they are bifurcated near the equator.

Observed with SEM, the exospore is papillate–tuberculate. The papillae and tubercles are low and small. They are fused to one another. The laesurae have the same ornamentation as the surface (Plate II, 12–15). The perispore is not discernible under SEM.

Isolated or grouped spheroids, associated in masses are fused to the spore surface (Plate II, 15).

3.1.6. *Melpomene peruviana (Plate II, 16–19)*

The spores are trilete, globose with a circular outline in polar view. The equatorial diameter is 51–83 μm and the polar diameter is 53–64 μm. In equatorial view, the proximal face is convex and the distal face is hemispheric. The laesurae are 25–42 μm long and they reach the equator.

Observed with SEM, the exospore is tuberculate. The tubercles are irregular in shape and size. They are frequently fused to one another (Plate II, 16–19). The perispore is not discernible under SEM. Puncta, i.e. small depressions on the tubercle surface, can be seen, and some spheroids, single or fused to one another, are fused to the surface (Plate II, 16–19).

3.2. Ultrastructure

3.2.1. *Grammitis (Plate III))*

When analysed with TEM, the exospore in section is 0.6–1.4 μm thick. It comprises two layers, an inner one, which is thin (ca. 70 nm in thickness), and an outer layer that forms the bulk of the wall. The latter forms the elements of the ornamentation that is composed primarily of verrucae along with gemmae and some tubercles, which are very irregular in form and size.

The exospore outer layer has a number of narrow, roughly radial channels with dark content (Plate III, 22, 28) that are especially abundant near and on both sides of laesurae (Plate III, 20). Particularly in the propitiously oriented (i.e. exactly, or so, transversely cut) exospore sections that clearly show the inner layer, small cavities with a similarly dark content are visible in the innermost area of the outer layer (Plate III, 20, 21, 22, 28), forming a sort of edge to the inner exospore layer. Moreover, the exospore outer layer often shows dark spots which usually are very small and scattered throughout the layer except its outermost part, and which correspond to particles embedded within that layer (Plate III, 21, 22, 25–28). Rounded elements similar in contrast to the exospore inner layer form a mass extending more or less widely within and below the basal area of the apertural slit (Plate III, 20).

Plate III. Spores of *Grammitis* with TEM.

20–22: *Grammitis magellanica*.

20. Section through a laesura. The apertural fold of the exospore shows lateral and apical thickened areas that correspond to the verrucae that can be seen in Plate I, fig. 1. Channels with dark content are associated with the laesura (arrows). Numerous small, slightly contrasted rounded elements are visible within and below the basal part of the exospore fold (arrowhead). Scale bars 0.5 μm

21–22. Sections through the sporoderm. The outer exospore layer (Eo) shows cavities with a dark content arranged near the inner layer (arrowheads) and irregularly distributed dark particles. A radial channel with a dark content is distinguishable (double arrow). The perispore has sparse concentrations of dense material (P). Scale bar: 0.25 μm

23–28: *Grammitis poeppigiana*.

23–24. Sections through the sporoderm. Spheroids with features similar to those of the exospore outer layer are fused one another (fig. 23, black arrow), to the exospore (fig. 24, star) or linked to the spore surface through the perispore (white arrows). They have variable shape and size. In fig. 23, under the exospore the pseudoendospore (Pe) and the endospore (En) are evident. The boundary between both layers as well as their thickness are irregular. Scale bars: 1 μm.

25. Section through a glomerule that is closely fused with the exospore and is formed by fusion of several globules as it can be seen in the surface in Plate I, fig. 11. Scale bar: 0.5 μm.

26. Section through the sporoderm. The pseudoendospore (Pe) is strongly contrasted. A globule is evident (g). It is fused to an element of the ornamentation. Scale bar: 0.5 μm.

27. Section of a glomerule fused to a verruca of the exospore. The outer exospore layer (Eo) shows some cavities with a dark content in its innermost region (arrowhead) and numerous dark particles. The perispore (P) is detached from the exospore in some places. The irregular pseudoendospore is evident at the inner part (Pe). Scale bar: 0.5 μm.

28. The exospore shows two layers: a thin inner layer (Ei) that appears slightly less dark than the other in this section, and an outer layer (Eo) that forms the elements of the ornamentation. The inner layer is outwardly bordered by cavities roughly lined up tangentially above its surface (lower arrowheads). Smaller spots corresponding to dark particles are distributed within the outer exospore layer (upper arrowhead) except in its outermost region. A radial channel with a dark content is also evident (double arrow). The perispore (P) is scantly visible. Scale bar: 0.25 μm.

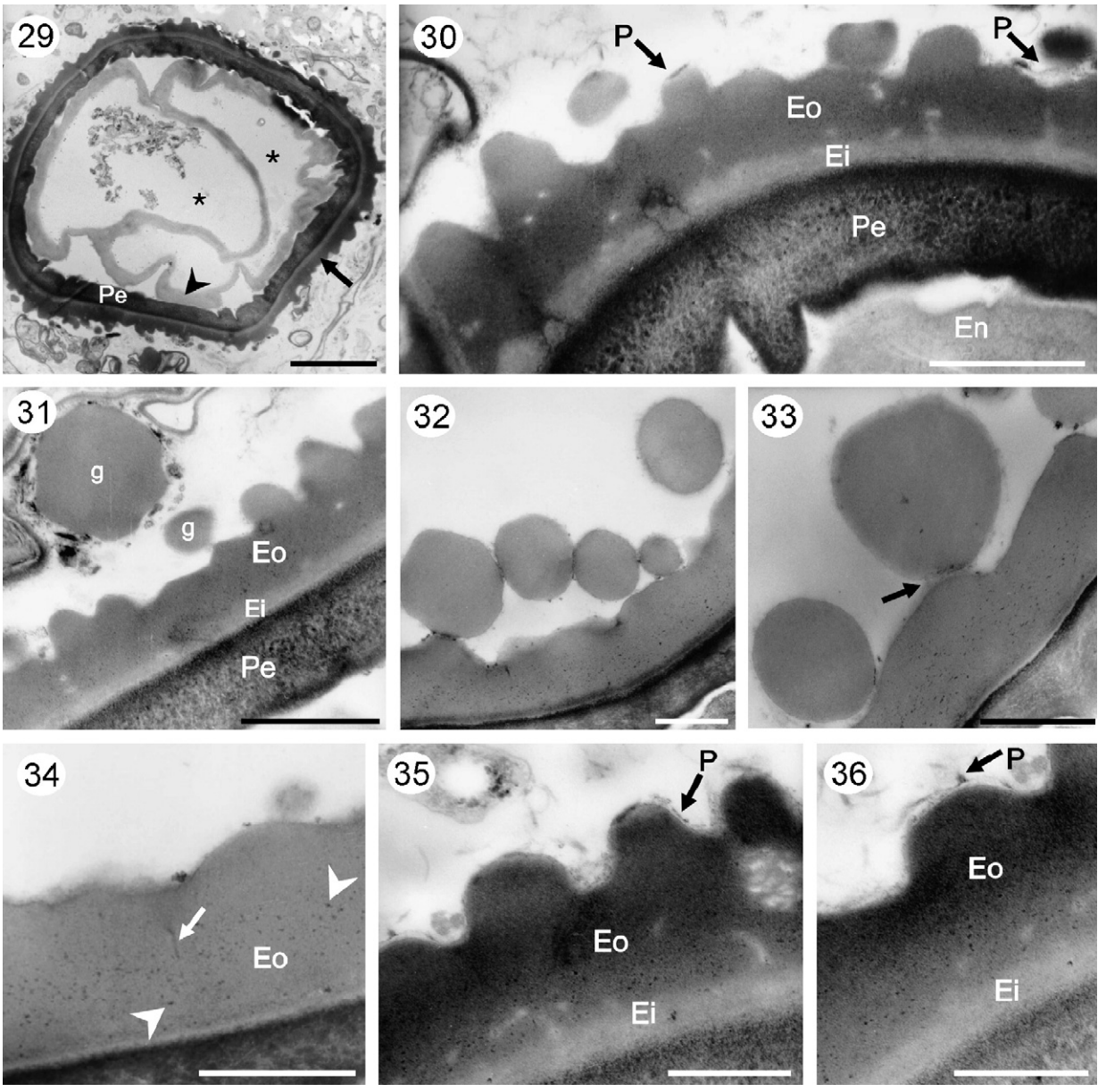


Plate IV. Spores of *Lellingeria tungurahue* with TEM.

29. Section of a spore. It can be seen the exospore (arrow), the pseudoendospore (Pe), and the endospore (arrowhead) connected to the wall developed between two gametophyte cells (black asterisks). Scale bar: 4 μm.
30. The endospore (En) is less dark than the pseudoendospore (Pe). The exospore has two layers: an inner one, thin (Ei) and an outer one, darker, that forms the ornamentation (Eo). The perispore (P) is scantly visible. Scale bar: 1 μm.
31. Section through the sporoderm. The pseudoendospore (Pe) and both layers of the exospore (Ei, Eo) are evident. Some spheroids can be seen (g). Scale bar: 1 μm
32. Several spheroids with features similar to those of the outer layer of the exospore can be seen. They are linked together and to the exospore through the almost indistinct perispore. Scale bar: 0.5 μm.
33. Some globules are attached to the exospore through their perispores (arrow). Scale bar: 0.5 μm.
34. The outer exospore layer (Eo) shows numerous dark particles (arrowheads) except in its outer area. Part of a radial channel (arrow) with a dark content can be seen within that layer. Scale bar: 0.5 μm.
35, 36. In both sections the boundary between the inner (Ei) and outer (Eo) exospore layers, as well as that between inner layer and pseudoendospore, are blurred because the sections are slightly oblique. The outer layer (Eo) forms the elements of the ornamentation (tubercles and papillae). The perispore is thin and has sparse concentrations of dark materials (P). Scale bar: 0.5 μm.

The perispore is ca. 30 nm thick and its thickness varies according to the area of the spore analysed. It is darker than the exospore and shows scattered irregular amounts of denser material (Plate III, 21, 22). It can be detached from the exospore in places (Plate III, 27).

Spheroids of 0.6–1.4 μm in diameter are on the surface, single or fused to one another, wrapped in a perispore layer comparable to that covering the exospore. They can be fused to the exospore forming glomerulae. These spheroids in section have appearance and contrast similar to those of the exospore outer layer, and many of them show dark spots like those scattered in that layer (Plate III, 23–27). In sections of some spores a pseudoendospore is evident next to the inner part of the exospore. It is more or less dark and shows an irregular thickness (Plate III, 23–27).

3.3. *Lellingeria tungurahue (Plate IV)*

Observed with TEM, the exospore appears 0.4–0.6 μm thick, tuberculate–papillate, to verrucose. The exospore is two-layered, the inner layer is thin and the outer one, which is usually darker in the investigated spores, forms the elements of the ornamentation. In that outer layer, some radial channels and cavities (Plate IV, 34) and dark particles plentifully distributed save near the outer surface (Plate IV, 31–36) can be observed, while the boundary between both layers and the cavities adjoining outwardly this boundary are almost indistinct because of somewhat oblique sections.

The perispore is ca. 20 nm thick, fastened to the exospore and it has occasional spaced concentrations of darker material (Plate IV, 35–36).

On the outer surface, spheroids of 0.2–1 μm in diameter are evident. They are single or fused to one another and/or with the exospore (Plate IV, 31–33). These spheroids have a similar structure and contrast as the exospore.

The dark pseudoendospore and the endospore are evident (Plate IV, 29–31) in these germinated spores.

3.4. *Melpomene peruviana (Plate V)*

When analysed with TEM, the exospore is 0.6–1.24 μm thick and double–layered. In the investigated specimens, the thin inner layer, is generally less dark than the outer layer that forms the elements of the ornamentation. These elements are tubercles and gemmae (Plate V, 39) that are partially fused to one another (Plate V, 41).

According to the plane of sectioning, some radial channels are evident outside the apertural area (Plate V, 40) while rather abundant channels are associated with the laesurae (Plate V, 37). Some cavities with a dark content are tangentially aligned above the outer surface of the inner layer (Plate V, 40). Dark particles are present in the outer layer, although apparently in rather reduced number (Plate V, 38–41). Within the basal part of the apertural fold and below this area, it can be seen a mass of packed, irregularly rounded elements showing the same contrast as the exospore inner layer (Plate V, 37)

The perispore is less than 270 nm thick. Its thickness varies according to the spore areas. It is darker than the exospore and has isolated concentrations of dense material (Plate V, 39–41).

On the spore surface, there are spheroids of 0.2–0.9 μm in diameter, showing features similar to those of the exospore outer layer. These spheroids are single or fused together or with the exospore, and may be linked one another and/or with the spores through strands made up of perisporial material (Plate V, 37, 38).

The pseudoendospore is darker than the adjacent material (Plate V, 38, 41).

4. Discussion and conclusions

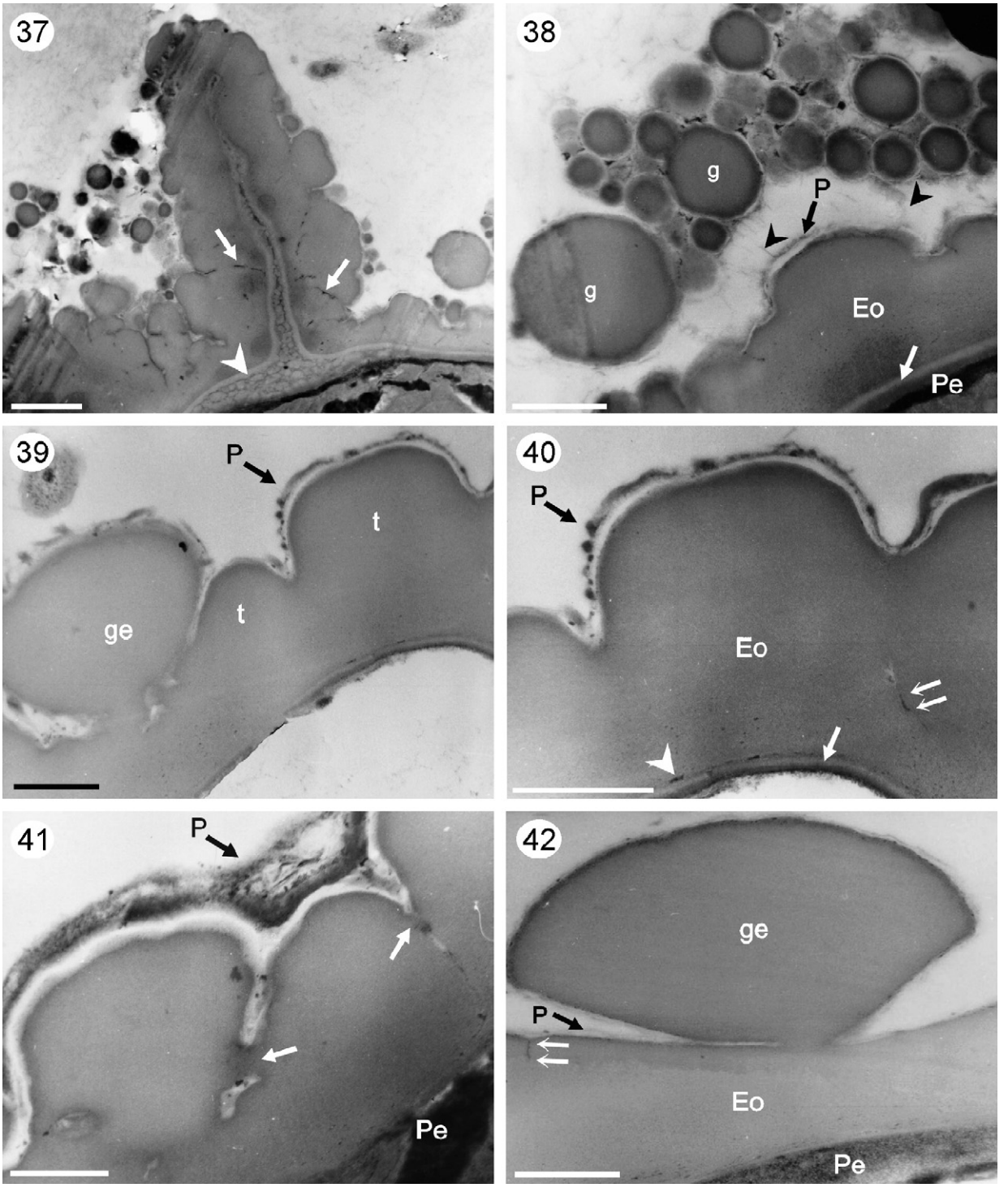
Grammitidaceae together with the Osmundaceae, Hymenophyllaceae some Dryopteridaceae and Polypodiaceae have spores with chlorophyll. These spores are globose and have distinctive thin walls, short viability, and fast germination (Wagner, 1974). Unlike spores devoid of chlorophyll, the spores produced by Grammitidaceae species keep their viability for short periods varying from a few to 45 days (Lloyd and Klekowski, 1970).

The spores with chlorophyll have a high content of water and when they get dry, the walls get depressed (Tryon and Lugardon, 1991).

The specimens analysed here produce spores with tuberculate–papillate or verrucate, in some cases gemmulate, ornamentation. These observations are consistent with those mentioned by Rodríguez Ríos (1995), Wagner (1985), and Tryon and Lugardon (1991) for American specimens. In addition, the ornamentation is similar to Grammitidaceae species from India (Nayar and Devi, 1965), New Zealand (Large and Braggins, 1991) and others regions of the Old World (Tryon and Lugardon, 1991).

Similar characteristics to those observed in our material with respect to form, ornamentation and

presence of spherical deposits on *Grammitis* spores were quoted also by Tryon and Tryon (1982) and generally resembling those produced by *Loxogramme* (Blume) Presl (Polypodiaceae) spores. According to Tryon and Lugardon (1991), Chang (1963) considered *Loxogramme* to be more closely related to Grammitidaceae than to Polypodiaceae based on palynological evidence.



In specimens of *Lellingeria tungurahue* from Salta some spores showed a bifurcated laesura next to the equator. The same characteristic was mentioned by Wagner (1985) for monolete spores in several species of Grammitidaceae from Costa Rica.

Spheroids are frequent on the spore surface of the analysed species. According to their size, location, resistance to the process of acetolysis and ultrastructure, they appeared to be "globules" as defined by Lugardon (1981) and they may be homologous to Ubisch bodies of the Spermatophytes. Similar globules were also reported on the spores in specimens of the related family Polypodiaceae from Argentina (Giudice et al., 2004).

The presence of globules often fused together and/or with the exospore was a characteristic found in the three genera analysed. They were found in a higher density and were more fused in *Grammitis* species. Tryon and Lugardon (1991) emphasize that the abundance of globules and especially their frequent fusion with the exospore are unusual in other ferns and add to the distinctiveness of the family.

The characteristics of the sporoderm ultrastructure such as the presence of channels, cavities and those referred to the pseudoendospore and endospore when they are present in consequence of precocious germination, are similar in all the taxa studied and comparable to those shared by the spores of most leptosporangiate ferns.

The rounded elements observed within and below the lower part of the exospore apertural fold in a spore of *M. peruviana*, and also distinguishable in the laesura section of *G. magellanica*, very likely are made up of material similar to that of the inner layer and are part of the "subapertural mass" common in fern spores (Tryon and Lugardon, 1991). However these elements appear especially abundant in those species (as they seem to be also in several other investigated Grammitidaceae; Lugardon, oral communication), which suggests that the subapertural mass is unusually developed in this family.

The spore size and the exospore ornamentation are variable characters. According to Tryon and Lugardon (1991) a larger spore size could be related in some cases to the endosporic development of the gametophyte.

Based on this analysis, it is verified that there is a morphological and ultrastructural homogeneity in the spores of this group. Such homogeneity suggests that there may be few characters of systematic value within the family.

Nevertheless, the various characteristics recorder here could be useful to differentiate the Grammitidaceae from other families.

Further studies, in which wider geographic areas would be taken into account and a higher number of neotropical taxa would be included, would allow us to clarify whether the morphological homogeneity is constant within the different genera of Grammitidaceae.

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Plate V. Spores of *Melpomene peruviana* with TEM.

37. A section through the sporoderm at the laesura level. The exospore has a rugged outline. Several channels with a dark content are visible on both sides of the laesura (arrows). The inner exospore layer (arrowhead) shows a low contrast, similar to that of the rounded elements packed within and below the basal part of the apertural fold. Scale bar: 1 μm.
38. The outer layer (Eo) is darker than the thin inner layer (arrow). There are numerous spheroids (g) showing the same appearance as the outer layer in section. They are attached together through perisporal material that, moreover, forms strands (arrowheads) linking the cluster of globules with the perispore (P) of the spore. The pseudoendospore (Pe) is darker than the exospore. Scale bar: 0.5 μm.
39. Transverse section of exospore tubercles (t) and a gemma (ge), with the perispore (P) following the outline of these ornamentation elements. Scale bar: 0.5 μm.
40. The outer boundary of the inner exospore layer (arrow) is lined by cavities with a dark content (arrowhead). Part of a radial channel with a dark content is distinguishable (double arrow). The perispore has irregular concentrations of dense materials (P). Scale bar: 0.5 μm.
41. The sections shows two points of fusion between elements of the exospore ornamentation (arrows). The perispore (P) has a variable thickness according to the analysed area. The pseudoendospore (Pe) is darker than the exospore. Scale bar: 0.5 μm.
42. A gemma (ge) formed by the outer exospore layer (Eo) has been tangentially cut so much so that it seems almost completely detached from the exospore. The outer end of a radial channel (double arrow) can be seen under the perispore (P). The pseudoendospore (Pe) is moderately dark in this section. Scale bar: 0.5 μm.

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