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SPOROGENESIS IN POLYPODIACEAE (FILICALES). II. THE GENERA MICROGRAMMA PRESL AND BELVISIA MIRBEL

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SUMMARY

Sporoderm development has been studied in several species of polypodiaceous ferns belonging to the genera *Microgramma* Presl and *Belvisia* Mirbel. Both transmission and scanning electron microscopy have been applied, the latter technique including freeze-fracturing. This has shown a different succession of ontogenetic spore surface patterns in the two genera. For each genus, the ontogenetic series observed is also compared with mature spore surfaces in other congeneric species.

INTRODUCTION

This publication covers the study of sporogenesis in species belonging to the polypodiaceous genera *Microgramma* Presl and *Belvisia* Mirbel. In the first publication in this series, sporogenesis in *Drynaria sparsisora* (Desv.) Moore is described (Van Uffelen, 1990). In a third publication, sporogenesis in several other genera of Polypodiaceae s.s. will be treated (Van Uffelen, in prep.). These publications are the result of a study of spore morphology and the process of sporogenesis in this family, aimed at tracing possible new characters that may help to reconstruct relationships in this group of ferns (see Van Uffelen, 1990). This may also help to explain the astounding variation in mature spore wall morphology as encountered in the family Polypodiaceae s.s. as a whole (Van Uffelen & Hennipman, 1985; Hennipman, 1990).

MATERIAL AND METHODS

Material

The genus *Microgramma* Presl comprises about 15 species and is sometimes treated as part of the large genus *Polypodium* (Hennipman et al., 1990). The species occur in the warmer areas of the American continent (Tryon & Tryon, 1982). *Microgramma lycopodioides* occurs also in Africa, Madagascar, and the Mascarenes.

The genus *Belvisia,* including part of *Lemmaphyllum* Presl (Hennipman et al., 1990; Hovenkamp & Franken, in prep.), comprises 8–10 species and is confined to the tropics of the Old World.

When selecting species of Polypodiaceae for this study on sporogenesis, two criteria were applied. Not only should the species selected represent all variation in ma-

ture spores and sporogenesis as found in the family, they should also be regularly and abundantly fertile, with many different stages of development present at a time. Both *Microgramma ciliata* (Willd.) Alston and *Belvisia mucronata* (Fée) Copel. were suitable as they represent different tribes or subfamilies of the Polypodiaceae (Hennipman et al., 1990) and show quite different series of patterns during outer exospore formation. They were available in the botanic gardens of the universities of Leiden and Utrecht, and were easy to sample.

Microgramma ciliata, recorded from the tropics of South America, has small dimorphic fronds, the fertile ones with round sori arranged in two rows. *Belvisia mucronata* is known from the tropics of South East Asia and the Pacific. The sporangia are densely set along the midrib of the narrowed apical part of the frond, the spike.

Mature spores of other species belonging to *Microgramma* or *Belvisia* have been studied with the SEM (see Table 1): *Microgramma heterophylla* (L.) Wherry, *M. lycopodioides* (L.) Copel. and *M. persicariifolia* (Schrad.) Presl, and *Belvisia novoguineensis* (Ros.) Copel., *B. platyrhynchos* (Kunze) Copel., and *Lemmaphyllum abbreviatum* (Fée) C. Chr., which is to be transferred to *Belvisia* (Hovenkamp & Franken, in prep.). The spores were either taken from herbarium specimens or from plants grown in the botanic gardens of the universities of Leiden or Utrecht (for methods, see Van Uffelen & Hennipman, 1985).

Methods

The sequence of different exospore patterns found in different species is difficult to discern and describe from two-dimensional micrographs as made with the TEM. Therefore, as in *Drynaria sparsisora,* the freeze fracturing technique developed in London by Blackmore & Barnes (1985) has been applied to sporogenesis in *Microgramma ciliata* and *Belvisia mucronata.* It involves the freeze fracturing of whole sori, followed by study with the SEM of the fractured sporangia in different stages of development. In both species the development of the spore wall has also been studied with the TEM. Samples have been treated for study with the transmission electron microscope (TEM) and the scanning electron microscope (SEM) as described for *Drynaria sparsisora* (Van Uffelen, 1990), unless stated otherwise.

For SEM fixation, spikes of *Belvisia mucronata* were cut into transverse slices of about 2 mm thick (Plate V-1, 2). Slices from the base of the spike, where it is broadest, were cut in half longitudinally. The resulting pieces all have one transversal freeze fractured face and one transversally cut face, and some also have a longitudinally cut face. It is difficult to assess which one of the transversal faces is the result of freeze fracturing, and which is not.

Of one other species, *Belvisia platyrhynchos,* mature spores and immature spores have been studied with the SEM. Sporangia of one specimen (see Table 1) were fixed in paraformaldehyde in the usual way (Van Uffelen, 1990), followed by critical point drying. Even without applying freeze fracturing, so many damaged sporangia with immature spores were found in the samples that a series of micrographs covering outer exospore development could be constructed and compared with the series as found in *Belvisia mucronata.* This surprising result shows that it is possible to study

series of surface patterns during spore wall development without the time- and chemical-consuming procedure of freeze fracturing. The quality of the images is almost as good as after the more complicated freeze fracturing treatment. However, this simplified technique can only be applied in species where the sporangia are exceptionally fragile, and at the same time retain enough of their contents for study after breaking.

Germinating spores of both *Microgramma ciliata* and *Belvisia mucronata* were studied after cultivating mature spores in a liquid medium until germination occurred, subsequent fixation in 4% paraformaldehyde, and critical point drying.

Table 1. Specimens studied.

Species	Origin of material	Sd	Sm	Sg	Td	Tm
M. ciliata	LEI 19699	+	+	+	+	+
M. heterophylla	Wright 799 (L)	–	+	–	–	–
M. lycopodioides	LEI 22401	–	+	–	–	–
M. persicariifolia	Martius 384 (L)	–	+	–	–	–
L. abbreviatum	Cadire 97 (P)	–	+	–	–	–
L. abbreviatum	Gaudichaud s.n. (P)	–	+	–	–	–
B. mucronata	LEI 21836	–	+	–	+	+
B. mucronata	LEI batch 957	+	+	+	–	–
B. novoguineensis	Van Royen 11600 (L)	–	+	–	–	–
B. platyrhynchos	86GR00027 U	+	+	–	–	–

Sd = SEM study of spore development
Sm = SEM study of mature spores
Sg = SEM study of germinating spores
Td = TEM study of spore development
Tm = TEM study of mature spores

LEI = Botanic garden of Leiden University
U = Botanic garden of Utrecht University
(L) = Herbarium Leiden
(P) = Herbarium Paris

The temporal order of micrographs

The micrographs representing spore surface patterns during outer exospore development in *Microgramma ciliata, Belvisia mucronata,* and *B. platyrhynchos* are presented as different series in their supposedly correct order, based on the author's interpretation. Their order has been established using the following criteria (see also Van Uffelen, 1990, on *Drynaria sparsisora*):

— spore length, which increases with the age of the spore; however, there is much overlap between stages;
— height of the part of the laesural fold not yet covered with exospore material;
— exospore thickness, although it does not always seem to increase linearly with time, and although exospore material may reduce in thickness by settling after its deposition;

— experience gained by the author while studying the process in other related species; however, this may lead to some circularity in argument when using the process of spore wall development in assessing relationships between species or other taxa.

As already mentioned with respect to *Drynaria sparsisora,* sporangium size is no reliable criterion as it is difficult if not impossible to infer the ontogenetic stage from it: sporangium size does not increase in a linear way with time, and sporangia may not be fractured through their greatest diameter.

In the species studied here, stages change gradually into each other, and separate stages are more difficult to describe than in *Drynaria sparsisora.* On the other hand, these gradual changes from one pattern into another have facilitated ordering the micrographs into a representation of the ontogeny of spore wall formation. In the presentation of results, each series of micrographs is thought to represent a true sequence in time, and is treated as such.

Terminology

In the spores included in this study, and in many other fern spores (Lugardon, 1978a, b), most of the spore wall material is added from outside the spore by a superposition of material in a series of usually different patterns. These patterns are caused by local differences in wall thickness, be it on a large (e.g., verrucae) or on a small scale (e.g., in a scabrate surface), and have elements that are, in cross section, usually continuous with the rest of the exospore. It makes sense to name a pattern by its most prominent features, which are usually protruding as well as eye-catching. However, it is sometimes difficult to choose between depressions and elevations as the factor best characterizing a pattern. For instance, in mature spores of *Belvisia,* one may choose between a term based on depressions (grooves) and one based on protrusions (ridges).

In *Belvisia,* the patterns found during outer exospore formation are best described by depressions ('pits', see Van Uffelen, 1990) in the surface, which is thus called foveolate. When rows of these pits form grooves, these grooves form the main element of the surface pattern. In mature spores the ridges are the most prominent and so the most easily described features. Therefore, in this genus the series of terms describing subsequent patterns change from depression- to elevation-based.

The various patterns occurring during sporogenesis in *Microgramma ciliata* are best described by their most prominent features, the units or warts.

RESULTS

Microgramma Presl

Microgramma ciliata (Willd.) Alston

Fronds, sori, and sporangia

The sori are situated in two rows, one on each side of the main vein of the frond. The sporangia of one sorus are implanted on a small semispherical cushion which is up to c. 0.5 mm in diameter, intermingled with many hairs which are up to more than

1 mm long and are usually longer than the sporangia (Plate III-6). On freeze-fractured pieces of frond this cushion is not always visible, as the plane of fracturing may lie beside it. More than 50 sporangia of different stages may be found in one sorus. Sporangia are up to c. 0.3 mm long; their stalk is about as long as the sporangium.

Sporogenesis

In *Microgramma ciliata* the first part of outer exospore formation consists of the deposition of small sporopollenin lumps over a smooth surface. After this, the thin exospore layer gets covered with contiguous warts ('units') which are round to angular in outline, irregularly sized (in all three dimensions), c. 0.4 to 2 μm in diameter, and rounded and almost smooth at the top (Plate I-1). The wall is still rather thin, c. 0.2 μm in cross section (Plate I-1). The spores are c. 20 μm long. Some are partly covered with tapetal material.

While the overall exospore thickness increases (Plate I-3: c. 0.5 μm thick between the warts), the pattern remains the same (Plate I-2), with contiguous units that are irregular in size and outline. However, they are more uniform in height. The laesural fold is not covered with much exospore material or embedded in the exospore layer, and is at the most 3 μm high (Plate I-2). The spores are now c. 28 μm long.

Granular material is deposited over and between the warts, so that the warts gradually become less prominent, with granular material in between, and with a slightly scabrate surface (Plate I-4). The laesural fold is not yet completely embedded in exospore material and is c. 2 μm high (Plate I-5). The spores are now c. 32 μm long.

Deposition of granular material continues (Plate I-6, 7). This does not result in a conspicuous thickening of the exospore, which is about 0.4 μm thick between the units. The top of the warts has become slightly more flattened, but the warts still have the same outline, and have a similar fine surface structure. Small channel openings are present on and between warts (Plate I-7). The laesural fold still protrudes about 2 μm from the exospore surface. The spores are c. 36 μm long.

The deposition of finer exospore material causes the surface of the warts to lose its granular character (Plate II-1). The warts become more prominent, and their tops more irregular in shape. The laesura is also covered with a similar pattern (Plate II-2). Small fragments of material are present, which are continuous with the spore surface. The spore surface lies clear from the spore coat or tapetum. A few spherical bodies of up to 2 μm in diameter were found to be attached to the spore surface. The spores are now c. 40 μm long.

When spore length has again increased to 49 μm, the warts have become higher and their tops more irregular (Plate II-4). The exospore is now 1–2 μm thick (Plate II-3). By the end of exospore formation, the tops of the warts have become very irregular indeed (Plate II-6).

In the mature exospore, the outer layer is completely homogeneous, showing a few thin channels around the laesura and perpendicular to the rest of the exospore surface, sometimes ending in a fairly wide opening on the surface between warts (Plate II-5).

Perispore formation starts with the appearance on the exospore surface of a double membrane (Plate III-1) which is sometimes divided into overlapping fragments (Plate III-2). In the tapetum many 'blobs' of electron dense material, c. 0.1 μm in diameter,

are present (Plate III-3). They are subsequently deposited on the membranes (Plate III-1), thus forming the thin perispore layer. Part of this process can also be inferred from Plate III-5. Each spine consists of a tapering bundle of thin rods (Plate III-4). It is not clear whether the spines are assembled lying in the tapetum, or in their final position on the perispore surface. In some mature spores of this species the perispore is covered with a thin amorphous layer, although the perispore layer seems to be complete and finished.

Mature spores (Plate IV-1, 2)

Mature spores are monolete, concavo-convex to biconvex, rather shortly bean-shaped in lateral view (c. 49 µm long and 39 µm high, and c. 37 µm wide). The laesura is c. 23 µm long.

The exospore surface is verrucate-colliculate (that is, with warts covering the whole surface, see Van Uffelen & Hennipman, 1985). The warts are c. 2–5 µm in diameter, flattened, in outline irregular and round to oblong, their surface again irregularly verrucate. Spherical bodies are rarely present. The warts that cover the area around the laesura are sometimes less prominent (Plate IV-3). The inner exospore layer is c. 0.05 µm thick, the outer layer is 0.5 µm thick between the warts, these adding up to 0.5 µm to its thickness. All layers are slightly thicker near the laesura. The perispore overlying this verrucate-colliculate pattern is rather thin and smooth, bearing minute spines (echinulae) which are about 0.5 µm long. The laesura may also be covered with the verrucate exospore pattern and with perisporal spines.

Plate IV-4 shows a mature perispore. The perisporal surface of the warts and the space between them is smoother than the exospore surface in the last stages of outer exospore formation. The outline of the mature exospore surface is still traceable through the thin perispore layer with its spines.

Plate IV-5 shows an opened spore from which the prothallium has fallen out, and of which the inside and section of the spore wall and laesura are well visible.

Other species of Microgramma: *mature spores*

Microgramma heterophylla (L.) Wherry (Plate IV-6)

The spores are about plano-convex, c. 49 µm long, 33 µm high, and 37 µm wide. Their surface is verrucate, the warts usually round and c. 2 µm in diameter, sometimes longish and up to 4 µm long, with steep sides and a rounded or flattened top, c. 1.5 µm high. The warts lie quite close together, but do not touch. The spore surface between the warts is smooth to slightly verrucate. The laesura is a prominent, c. 32 µm long bar, without any large warts. Around the laesura the larger warts are also lacking. The perispore is thin and smooth, closely adhering to the exospore surface, without any further ornamentation.

Microgramma lycopodioides (L.) Copel. (Plate IV-7)

The spores are c. 50 µm long and 32 µm high. The exospore surface is entirely covered with low, slightly pointed or rounded warts. The warts are round, c. 3–5 µm in diameter and 1 µm high. The laesura is a prominent, c. 25 µm long bar without any ornamentation. The area around the laesura is less pronouncedly verrucate. The peri-

spore is thin, closely adhering to the exospore, and bears scattered minute echinae of less than 1 μm long.

Microgramma persicariifolia (Schrad.) Presl (Plate IV-8).

The spores are c. 43 μm long, c. 34 μm high, and 31 μm wide. The exospore surface consists of colliculate warts. The warts are round, up to 3 μm in diameter, and c. 1.5 μm high, with a rounded or slightly pointed top, smaller and lower in the area around the laesura. The laesura is a prominent, c. 25 μm long bar with only the slightest ornamentation. The exospore is c. 1 μm thick, smooth on the inside. The perispore is smooth and thin, adhering to the exospore surface and without any ornamentation.

Belvisia Mirbel

Belvisia mucronata (Fée) Copel.

Fronds, sori, and sporangia

The fertile part of a frond of *Belvisia mucronata* consists of an apical, long, thin, folded structure ('spike') densely set with sporangia in many different stages of development with paraphyses in between, which cover the sporangia when young. Sporangia become visible only when they are almost mature; usually there are also some younger, shorter stalked sporangia present underneath.

The spores of *Belvisia*, both young and old, are not always retained in the broken sporangia as they are in *Drynaria sparsisora* and *Microgramma ciliata*. Therefore they are more difficult to study in situ. Furthermore, after having been observed for a first time, they are more difficult to retrace for further study.

Sporogenesis

The surface of young spores that are already bean-shaped and have a distinct laesural fold, is almost entirely smooth. Very low and small round lumps of exospore material are deposited on this surface. These lumps are up to 0.4 μm in diameter. The spore is then about 30 μm long, the laesural fold rather short (c. 13 μm long) and c. 2.5 μm high (Plate V-3). TEM micrographs show that the outer exospore is much thicker on the proximal side (c. 0.4 μm thick) than on the distal side (0.1–0.2 μm thick) (Plate V-4).

This granulate pattern is overlaid by a finely rugulate pattern. The rugulae are contiguous, covering all of the spore surface, separated only by tiny holes and short fine grooves; they are 0.2–0.3 μm broad, therefore on approximately the same scale as the lumps that cover slightly younger spores (Plate V-5, 6). The spores are now over 30 μm long. The laesural fold is maximally 1.3 μm high, so that a considerable part of the fold is already embedded in exospore material.

On this rather even surface more of the same rugulate surface is deposited. Gradually, shallow but conspicuous depressions on the surface become visible. They are round, slightly less than 1 μm in diameter, and are for the greater part scattered over the spore surface, while only a few of them touch each other (Plate V-7, 8).

As more exospore material is added, the depressions or 'pits' become deeper, but hardly more numerous. Some start touching each other as they become wider (c. 0.4 μm in diameter). During this process the surface looses its finely rugulate character.

The fine grooves disappear, but the small holes that were visible between the rugulae are still clearly there. These are less than 0.1 μm in diameter and probably correspond to small channels traversing the exospore layer. Such channels are also numerous in mature spores.

The pits increase slightly in number and become larger (c. 1 μm in diameter) and deeper, so that the edges, which were fairly distinct at the beginning of pit formation, are now beginning to slope. The spores are now c. 40 μm long, the laesural fold entirely embedded in exospore material. Some of the pits are fusing, giving a first indication of the pattern of grooves and ridges characteristic of the mature exospore (Plate VI-1, 2). On TEM, the pits and the smooth surface in between are well visible. All pits show about the same depth, so they must have originated at the same time. The typical layering of the thick mature exospore begins to show already (see Plate VIII-1). The exospore is about 1 μm thick on the distal side and almost 2 μm thick near the laesura (Plate VI-3). The pits become deeper and start fusing in greater numbers. The laesura also bears a pattern resembling that of the rest of the spore surface (Plate VI-4, 5). The spore surface between the fusing pits is rather smooth, apart from the still numerous and clearly visible small holes.

Later on the pits are fusing to such an extent that it becomes difficult to distinguish individual pits. The pits, as far as they can still be counted, do not increase in number any more. In this pattern of ridges and grooves the surface between the grooves is becoming smoother, the small holes becoming less numerous (Plate VI-6, 7). The pattern of tortuous, irregular ridges with grooves in between is now firmly established.

The fairly smooth surface of the ridges and the laesura (see Plate VII-2) is becoming more and more verrucate. The spore surface becomes distinctly knobby (Plate VII-1). At first, the warts do not stand much apart from the rest of the surface, later on some of them are recognizable as separate warts (Plate VII-3, 4). The spore surface is finely striate (Plate VII-4). On the mature exospore surface, the numerous warts deposited on the ridges are so conspicuous that in this species it has become difficult to trace the underlying pattern of ridges and grooves (Plate VII-5).

As the perispore consists of a thin layer lying close to the exospore surface, on SEM micrographs the perispore is not visible except where it peels off. Therefore, it is best studied with the aid of TEM. On TEM the first indication of perispore formation is the appearance of thin double membranes (Plate VII-6) which develop into multilayered membrane structures (Plate VII-6). They appear to be deposited by the tapetum (Plate VII-8). Blobs of tapetal material of about 0.1 μm in diameter are also found (Plate VII-7).

Mature spores (Plate VIII-1–3)

Mature spores are bean-shaped, plano-convex to slightly biconvex, c. 51 μm long, 35 μm high, and 33 μm wide. The irregularly shaped, tortuous ridges are c. 0.25 μm wide and more conspicuous than the grooves. Their surface is also irregular, and densely set with warts of varying size that are around 0.25 μm in diameter. The 'grooves' often consist of a series of pits. The laesura is a prominent bar which is c. 21 μm long and covered with the same ornamentation as the ridges. The perispore is an extremely thin layer, closely adhering to the exospore surface.

Belvisia platyrhynchos (Kunze) Copel.

Sporogenesis

Of this species immature spores in different stages of development have been found after paraformaldehyde fixation and critical point drying without freeze fracturing. In most aspects the series of patterns are similar, but there are some significant differences, despite the similarity between mature spores of *B. mucronata* and *B. platyrhynchos*.

After the stage in which the young spore has a smooth surface with small lumps of c. 0.33 μm in diameter (spore length about 36 μm, Plate IX-1), the spore surface does not show a finely rugulate pattern as in *B. mucronata,* but is almost entirely smooth. The spore is already more than 40 μm long (Plate IX-2), but the laesural fold is not yet entirely embedded in exospore material: more than 1 μm is still uncovered.

During a further deposition of exospore material, shallow pits become visible in a smooth surface instead of in a rugulate surface as in *B. mucronata.* At first only a few pits are present on a spore (Plate IX-3), mainly on the distal side, and varying in size from 0.5 to 2 μm in diameter. However, as they get larger and deeper, they also get more numerous (Plate IX-4). The first pits occur when the spore is already quite long (about 50 μm), but when the laesural fold is not yet entirely covered with exospore material.

As in *B. mucronata,* the pits get larger (about 2 μm in diameter) and deeper, and start fusing into the usual pattern of grooves, separated by ridges (Plate IX-5). Gradually, the surface develops into that of the mature spore, as described for *B. mucronata* (Plate IX-6, 7). It differs from that of *B. mucronata* in that the grooves and ridges are on a slightly smaller scale, and are eventually covered with even more warts.

Mature spores (Plate IX-8)

The spores are bean-shaped, concavo-convex to plano-convex, c. 60 μm long. Their surface pattern consists of small, irregular, rounded warts, c. 1–3 μm in diameter, arranged along a pattern of ridges. The laesura is small, c. 14 μm long, inconspicuous, and also bears some warts.

Other species in Belvisia

All other species studied in the genus have basically the same exospore pattern. They all have a thin perispore, which is not visible on SEM micrographs.

Lemmaphyllum abbreviatum (Fée) C. Chr. (Plate VIII-5)

The spores are slightly concavo-convex to plano-convex, about 48 μm long, 36 μm wide. The ridges on the surface are more prominent than the grooves. The ridges are around 0.25 μm wide (sometimes up to 0.5 μm) and irregular as to surface and form, with grooves and deeper pits between them. The laesura is about 14 μm long, distinct, devoid of much surface ornamentation.

Belvisia novoguineensis (Ros.) Copel. (Plate VIII-4)

The spores are approximately plano-convex, about 53 µm long, 40 µm high, and 44 µm wide. The exospore surface pattern is smaller in all dimensions than in the other species studied, so that the spores are almost smooth in outline. The 0.1–0.2 µm wide ridges stand out from a smoother surface, albeit with smallish pits, than in the other species. The ridge surface is sparsely set with small warts of up to 0.1 µm in diameter. The laesura is a prominent rounded bar of about 16 µm long, without much ornamentation.

DISCUSSION

Van Uffelen (1986) distinguished six main stages during sporogenesis in *Drynaria sparsisora,* mainly based on the study of micrographs made with the TEM:

1) presence of spore mother cells (smc's)
2) meiosis
3) formation of the spore plasmalemma
4) formation of the inner exospore (ie)
5) formation of the outer exospore (oe)
6) formation of the perispore

In Van Uffelen (1990), where sporogenesis in *Drynaria sparsisora* is described in detail, stage 5 – the formation of the outer exospore – has been divided into different substages, based on the different surface patterns found during the development of this layer in this fern as seen on SEM micrographs.

In the species of which sporogenesis is described in the present paper, the stages 1–4 are similar to those of *Drynaria sparsisora,* therefore their description has not been included here. During the first part of outer exospore formation (stage 5) the differences in spore wall formation between these species begin to show after the deposition of small sporopollenin lumps on a smooth surface. As already mentioned in the section on material and methods, the change from one pattern into another occurs so gradually in the species treated here that a subdivision of stage 5 is not practicable. In the three species studied, the onset of stage 6, the formation of the perispore, is indicated by the appearance of double membranes, followed by a multimembrane layer of sometimes overlapping lamellae. In all these species, similar blobs of tapetal material are involved in perispore formation.

The genus Microgramma

Although in both *Drynaria sparsisora* (Van Uffelen, 1990) and *Microgramma ciliata* outer exospore formation starts with the deposition of small lumps of sporopollenin on a smooth surface, followed by the deposition of neatly defined units of roughly similar size and shape, the formation of the rest of the outer exospore layer is rather different. While in *Drynaria sparsisora* outer exospore formation is characterized by a succession of dramatically different patterns, on which a clear subdivision of this stage can be based, in *Microgramma ciliata* the patterns that succeed each other are not strikingly different, and change gradually from one into another.

In *Microgramma ciliata,* increase in spore length seems to occur by stretching of the developing spore wall rather than by intercalary growth. There are two arguments for this supposition:

— The considerable increase in spore length during the first stages of outer exospore formation, from 20 to 36 µm (see series Plate I-1 – Plate I-2 – Plate I-4 – Plate I-6), occurs without any increase in the number of units covering the spore surface. Therefore, the increase in spore length apparently results from the stretching of existing elements.

— Despite the addition of new granular material (see Plate I-4 and Plate I-6, 7) exospore thickness does not increase; this is in contrast with the length of the spore, which increases from 32 to 36 µm. However, one part of this effect may be due to the setting of the sporopollenin after its deposition, and another part to a not unusual variation in spore length between different spores.

Increase in spore length by stretching is also supposed to occur in *Drynaria sparsisora* (Van Uffelen, 1990), where the increase in unit size was partly attributed to spore wall stretching (and partly to a change in surface pattern). This may be a universal trend in the process of exospore growth in ferns, indicating that the material of which the exospore consists remains quite elastic for a long time.

An amorphous layer covering the perispore has also been found in some spores of *Pyrrosia* and other Polypodiaceae. It is not clear whether it consists of remnants of tapetum, which are deposited on the mature perispore when the sporangium opens rather early, before the transition from the liquid to the gaseous phase is completed. If the sporangium opens only when its contents have reached the gaseous phase, these tapetal remnants are deposited either as a fine granulate layer, or as a much thinner one, and mainly on the inside of the sporangial wall.

Assuming that the perispore is deposited centrifugally, i.e., the spines are only formed after the basal layer of the perispore has been finished, then the occasionally observed amorphous layer covering the perispore can only be explained by an abnormal, later deposition of tapetal residues.

In most recent publications concerning the family Polypodiaceae s.s., the genera *Drynaria* and *Microgramma* are placed in different tribes of the subfamily Polypodioideae (Drynarieae and Polypodieae, respectively: Hennipman et al., 1990), or even in different subfamilies (Drynarioideae and Polypodioideae: Hennipman, 1990). The differences in outer exospore formation support a not very close relationship between these genera.

Within the genus *Microgramma* (Tryon & Tryon, 1982), or, alternatively, the Microgramma-group in *Polypodium,* there is some, but not a striking degree of variation in mature spore surfaces (see above, and Tryon & Lugardon, 1991): all c. 14 species have a thin perispore adhering closely to the exospore surface, which is always discernible in mature spores; in some species the perispore bears minute echinae; the mature exospore surface bears similar warts of more or less irregular appearance, except for *M. megalophylla* (Desv.) Sota which has large spores with a shallowly rugate surface.

As is the case in *Microgramma ciliata,* the first differences in wall deposition between *Drynaria* and *Belvisia* became visible during outer exospore formation. In both *Drynaria sparsisora* and *Belvisia mucronata* the granulate stage (smooth-with-small-lumps) is overlaid by a finely rugulate pattern. After this, in *Drynaria sparsisora* the surface is covered with units, while in *Belvisia* shallow wide depressions appear, a development not found in either *Drynaria sparsisora* or *Microgramma ciliata.* As in *Microgramma ciliata,* clear-cut substages cannot be recognized during further outer exospore development because of the gradual change in pattern.

The mechanism underlying the formation of the pits that are so characteristic for outer exospore formation in *Belvisia* is still unclear. During the very first appearance of shallow depressions (Plate V-7), nothing that may have guided their formation is apparent, either in the tapetum or on the spore surface. Unfortunately, this is the case with most features of spore wall ornamentation, except for the laesural fold, of which the position is fixed by the position of the young spore in the tetrad. However, the mechanism of formation of these depressions can be speculated upon. I prefer to postulate the easiest process: the spore surface is marked in an as yet unknown way, which indicates places where further exospore material will not be deposited. In this way depressions, pits, and grooves are located on parts of the spore wall where in a certain phase of the deposition of exospore material no more material is deposited. The way in which these places are left open must be governed by the spore surface at the onset of pit formation, and probably not by some attribute of the material deposited in between the depressions (see Van Uffelen, 1991). A mechanism needing both a marker system and a complicated enzyme system governing the local dissolving of exospore material in order to 'scoop out' depressions in an already existing layer, is far more complicated.

The lack of a rugulate stage between the smooth-with-small-lumps-stage and the smooth-with-deepening-pits-stage in the series reconstructed for outer exospore formaton in *Belvisia platyrhynchos* may result from the fact that it was not present in the broken sporangia I studied, or that it does not occur at all in this species. In both possible cases it indicates that there are two independent processes at work: pit formation (found in both species) and, apart from that, the change from a smooth-with-small-lumps-stage via a rugulate surface to a smooth surface as found in at least one of the species, *Belvisia mucronata.*

In *B. platyrhynchos,* spore lengthening occurs early with respect to exospore deposition processes, such as pit formation and the covering of the laesural fold. All this suggests a fairly loosely timed occurrence of independent processes of spore growth and exospore deposition, which may vary between related species and still result in similar mature spore surfaces. One has also to take into account that mature *B. platyrhynchos* spores are usually larger than those of *B. mucronata* (c. 60 μm and c. 51 μm, respectively).

In the genus *Belvisia,* which comprises 8–10 species (Hovenkamp & Franken, in prep.), all species have basically the same exospore surface, and they all have a similar thin perispore without any ornament (Tryon & Lugardon, 1991). Sporangia in several species of *Belvisia* tend to shed their spores before they are ripe; in all of

these species (e.g., *B. platyrhynchos*) immature spores with similar pitted patterns were found. This has led to the conclusion that in *Belvisia* similar series of patterns have led to similar mature exospore surfaces. It also shows the necessity to check whether even spontaneously shed spores are truly mature.

When observing immature spores as if they were mature, the conclusion that neoteny may be common among species of *Belvisia* is obvious. However, this is not necessarily so. Neoteny may only have occurred affecting a very late stage of outer exospore formation, as the ridges in some species carry many warts (e.g., *B. mucronata*), while in other species not so many warts are found on the mature spore surface (e.g., *B. novoguineensis*). The occurrence of neoteny depends on whether the 'warty' situation as found in *B. mucronata* is plesiomorphic with respect to 'non-warty' or the reverse. Evidently, in *B. platyrhynchos*, where in mature spores the ridges are densely set with irregular warts (Plate IX-8), this warty pattern is formed later, and may be seen as an addition to the ontogenetic series, leading to the mature spore surface as found in some other species of *Belvisia*, e.g., *B. novoguineensis*.

In the related genera *Drymotaenium*, *Lemmaphyllum*, and *Lepisorus* (including *Paragramma*), which together with the genus *Belvisia* form the tribe Lepisoreae (Hennipman et al., 1990), similar rugate mature exospore surfaces are found (Tryon & Lugardon, 1991). However, in some species of *Lemmaphyllum* and *Lepisorus* the mature exospore surface is verrucate or tuberculate, reminiscent of mature surfaces in *Microgramma* – to make things more complicated, the spores of the one deviating species in the genus *Microgramma*, *M. megalophylla*, are shallowly rugate. However, in establishing relationships, not only the series of surface patterns during outer exospore development, which are rather different in *Microgramma ciliata* on the one hand and several species of *Belvisia* on the other, are of value, but also mature exospore ultrastructure as seen on TEM is important. Hennipman (1990) described the *Belvisia* type of exospore ultrastructure and characterized it by its extreme thickness, its verrucate, fissured or cerebriform surface, and by its abundant microchannels and tangential bands on cross section. He indicated that this type of exospore is typical for and confined to the tribe Lepisoreae. In his Table 2.2, however, the *Belvisia* type of perispore is also listed for the Microsorinae and Polypodiinae, to which last one the Microgramma group belongs. However, the exospore in the species of *Microgramma* studied here with the TEM indicate no similarity in ultrastructure with the *Belvisia* type.

CONCLUSIONS

In all species of Polypodiaceae s.s. studied up to now, the process of sporogenesis concurs with the series of stages described in Van Uffelen (1986). But there are large differences in the way of outer exospore formation on the level of genera or groups of genera. Therefore, it is impossible to divide outer exospore formation in sub-stages that may be generally applied to all species in this family.

In the species where increase in spore length could be related to changes in other spore features, such as exospore thickness, embedding of the laesural fold in exospore material, and changes in spore surface patterning, spore length apparently increases by stretching of the existing spore wall material, and not by intercalary growth.

Comparison of outer exospore formation in Belvisia mucronata with the same process in *B. platyrhynchos* has shown that during outer exospore ontogeny, different sequences of processes are at work at the same time, but more or less independent of each other.

Despite differences in perispore morphology, membranes and characteristic blobs of material of tapetal origin are involved in the process of perispore formation in all species of the genera studied so far.

Similarities in the process of outer exospore formation correspond with a similar mature exospore ultrastructure and surface, as is the case in the tribe Lepisorinae.

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LEGENDS OF PLATES I–IX
(pages 532–540)

Plate I: *Microgramma ciliata*, sporogenesis (1)

1: SEM – young spores covered with contiguous warts, which are rounded and almost smooth at the top; × 3150.
2: SEM – lateral view of a spore with a surface pattern similar to that found in younger spores (Plate I-1); × 1850.
3: SEM – cross section of a spore from the same sporangium, with a substantially thicker exospore than the spores on Plate I-1; the laesural fold (arrow) is not yet covered with exospore material; × 7000.
4: SEM – proximal detail of a spore on which granular material is found on and between the warts; × 7000.
5: SEM – detail of a laesural fold, from the same sporangium; some exospore material (arrow) has been deposited near the base of the laesural fold; × 8000.
6: SEM – approximate lateral view of a spore in a slightly later stage, showing further deposition of granular material, and a large number of small holes on and between the warts; × 1350.
7: SEM – detail of Plate I-6; × 5000.

Plate II: *Microgramma ciliata*, sporogenesis (2)

1: SEM – a spore with warts of which the surface has lost its granular character; × 1600.
2: SEM – detail of a spore from the same sporangium; the laesura is also covered with a similar pattern; × 8000.
3: SEM – cross section of a spore of which the warts have become higher, and their tops more irregular; the exospore is 1–2 μm thick; × 4500.
4: SEM – surface view of a spore from the same sporangium; × 8000.
5: TEM – cross section showing a few narrow channels around the laesura, perpendicular to the exospore surface, sometimes ending in fairly wide openings on the surface (arrow); × 10,000; oe = outer exospore.
6: SEM – surface view of a spore at the end of exospore formation; the tops of the warts have become quite irregular; × 3300.

Plate III: *Microgramma ciliata*, sporogenesis (3)

1: TEM – start of perispore formation: a double membrane (arrow) appears over the exospore surface; × 40,000.
2: TEM – the double membrane is sometimes divided into overlapping fragments (arrow); × 10,000.
3: TEM – the tapetum contains many 'blobs' of electron dense material; × 10,000; w = sporangium wall.
4: TEM – a spine, attached to the basal perispore layer; each spine consists of a tapering bundle of thin rods; × 40,000; oe = outer exospore.
5: SEM – part of perispore formation as shown with TEM is also visible on this SEM micrograph, a lateral view of a spore; × 1500.
6: SEM – cross section of a freeze-fractured sorus; × 19.

Plate IV: Mature spores of different species of *Microgramma*

1: SEM – *M. ciliata:* proximal view; × 1000.
2: SEM – *M. ciliata:* lateral view; × 1000.
3: SEM – *M. ciliata:* mature spore with a peeling perispore, showing perispore thickness and structure; × 1200.
4: SEM – *M. ciliata:* detail of the perispore surface; × 6500.
5: SEM – *M. ciliata:* a germinated spore; the inside and section of the spore wall and laesura are clearly visible; × 1000.
6: SEM – *M. heterophylla:* lateral view; × 1050.
7: SEM – *M. lycopodioides:* proximal/lateral view; × 950.
8: SEM – *M. persicariifolia:* lateral view; × 1100.

Plate V: *Belvisia mucronata*, sporogenesis (1)

1: SEM – cross section of a sorus; × 17.
2: SEM – detail of the cross section of this sorus; × 90.
3: SEM – young bean-shaped spore; surface with low and small round lumps of exospore material; × 1800.
4: TEM – section of a young spore, showing the laesural fold, and the outer exospore, which is much thicker on the proximal side (c. 0.4 μm) than on the distal side (0.1–0.2 μm); × 4000.
5: SEM – the pattern as shown on Plate V-3 is overlaid by a finely rugulate pattern; × 1650.
6: SEM – detail of Plate V-5; × 3900.
7: SEM – rugulate spore surface showing shallow but conspicuous depressions; × 2300.
8: SEM – detail of Plate V-8; × 5300.

Plate VI: *Belvisia mucronata*, sporogenesis (2)

1: SEM – spore showing a first indication of the grooves plus ridges pattern of the mature exospore; the pits have increased in number, and have become larger and deeper; × 1700.
2: SEM – detail of Plate VI-1; note the numerous small holes (arrow); × 6000.
3: TEM – cross section of a spore of which the exospore is about 1 μm thick on the lateral side, and almost 2 μm thick near the laesura; × 4500.
4: SEM – lateral view of a spore with deeper pits, which start fusing in greater numbers; × 1400.
5: SEM – detail of Plate VI-4: the laesura shows a similar pattern; × 5000.
6: SEM – spore on which it has become difficult to distinguish individual pits; the pattern of ridges and grooves is evident; × 1300.
7: SEM – detail of Plate VI-6; × 5000.

Plate VII: *Belvisia mucronata*, sporogenesis (3)

1: SEM – lateral view of a spore of approximately the same stage as Plate VII-1, with a knobby looking spore surface; × 1250.
2: TEM – cross section of the laesural area; the surface of the ridges, as well as that of the laesura has become more distinctly verrucate; × 6000; oe = outer exospore.
3: SEM – spore surface on which some warts are recognizable as separate warts; × 1450.
4: SEM – detail of Plate VII-3; the spore surface is finely striate; × 6500.
5: SEM – lateral view of a mature exospore; the numerous warts deposited on the ridges are so conspicuous that it has become difficult to trace the underlying pattern of ridges and grooves; × 2800.
6: TEM – cross section of a spore with double membranes and multi-layered membrane structures (arrow) on its surface; × 40,000.
7: TEM – blobs of tapetal material (arrow) involved in perispore formation; × 29,000; w = sporangium wall.
8: TEM – cross section of a spore where the membranes appear to be deposited by the tapetum (t); × 40,000.

Plate VIII: Mature spores of different species of *Belvisia*

1: TEM – *B. mucronata:* cross section of a mature spore; × 2300; note layers in outer exospore (arrow).
2: SEM – *B. mucronata:* proximal view; × 950.
3: SEM – *B. mucronata:* germinated spore, which has opened along the laesura; × 1100.
4: SEM – *B. novoguineensis:* lateral view; × 1000.
5: SEM – *Lemmaphyllum abbreviatum:* lateral/proximal view; × 1000.

Plate IX: *Belvisia platyrhynchos* – sporogenesis and mature spore

1: SEM – lateral view of a young spore; surface smooth with small lumps; × 1500.
2: SEM – proximal/lateral view; spore surface almost entirely smooth; × 1250.
3: SEM – lateral view of a spore with only a few pits; × 1000.
4: SEM – spores with pits that are larger, deeper, and more numerous; × 900.
5: SEM – lateral view of a spore where the pits start fusing into the pattern of grooves and ridges; × 1000.
6: SEM – lateral view of a slightly older, immature spore; × 1150.
7: SEM – proximal view of spore; exospore not yet finished; × 800.
8: SEM – lateral/proximal view of a mature spore; × 850.

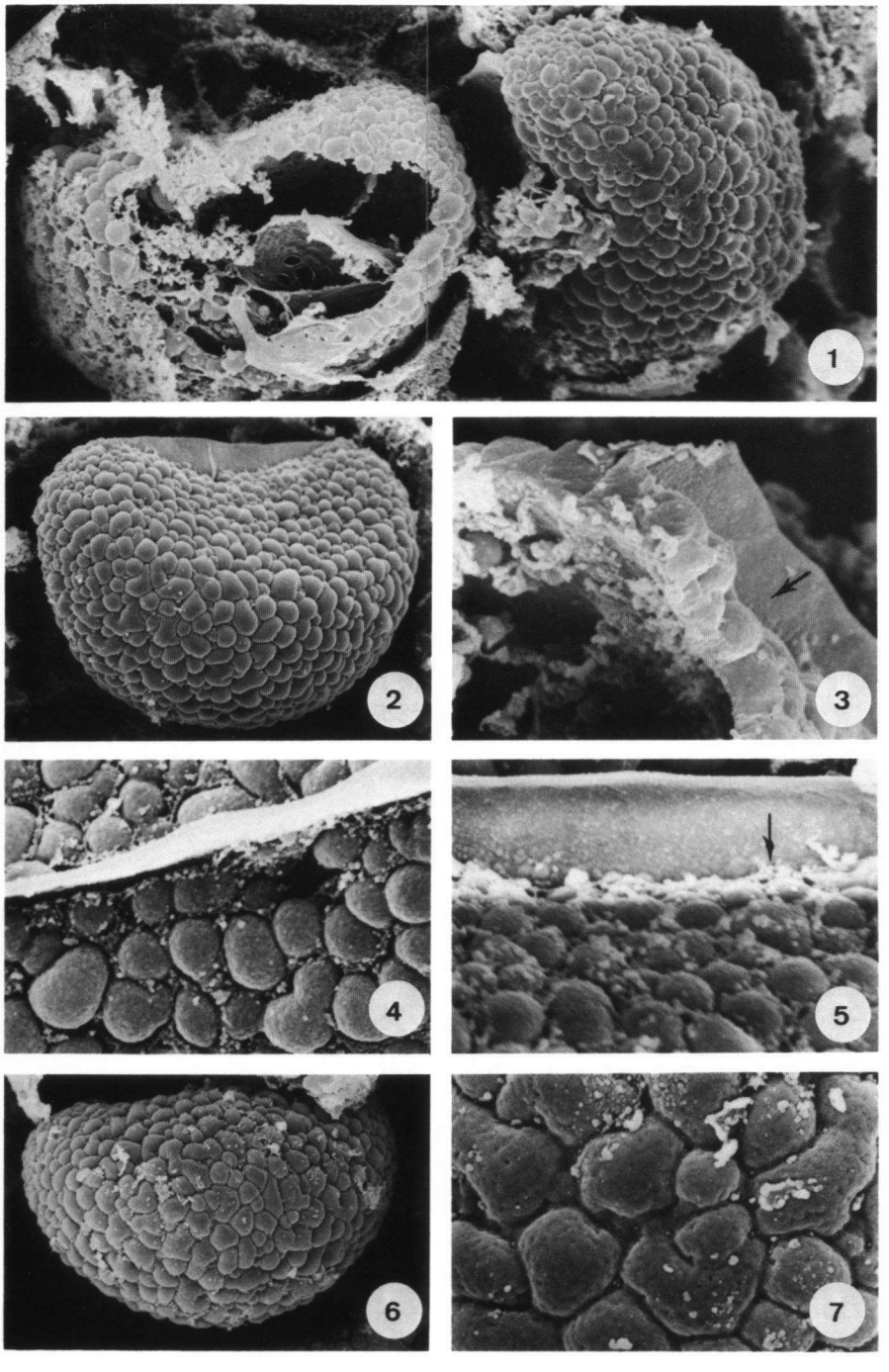
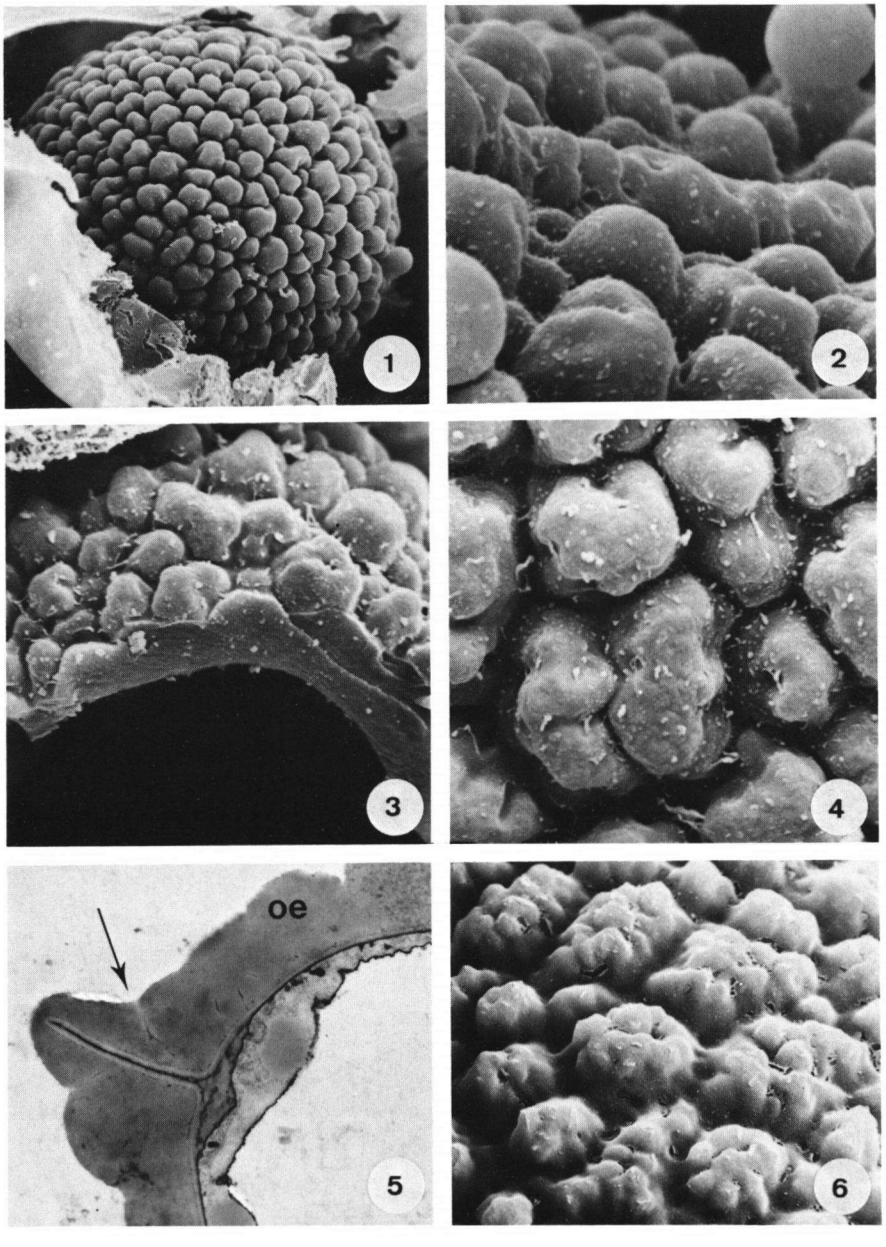


Plate I (legend on page 529)



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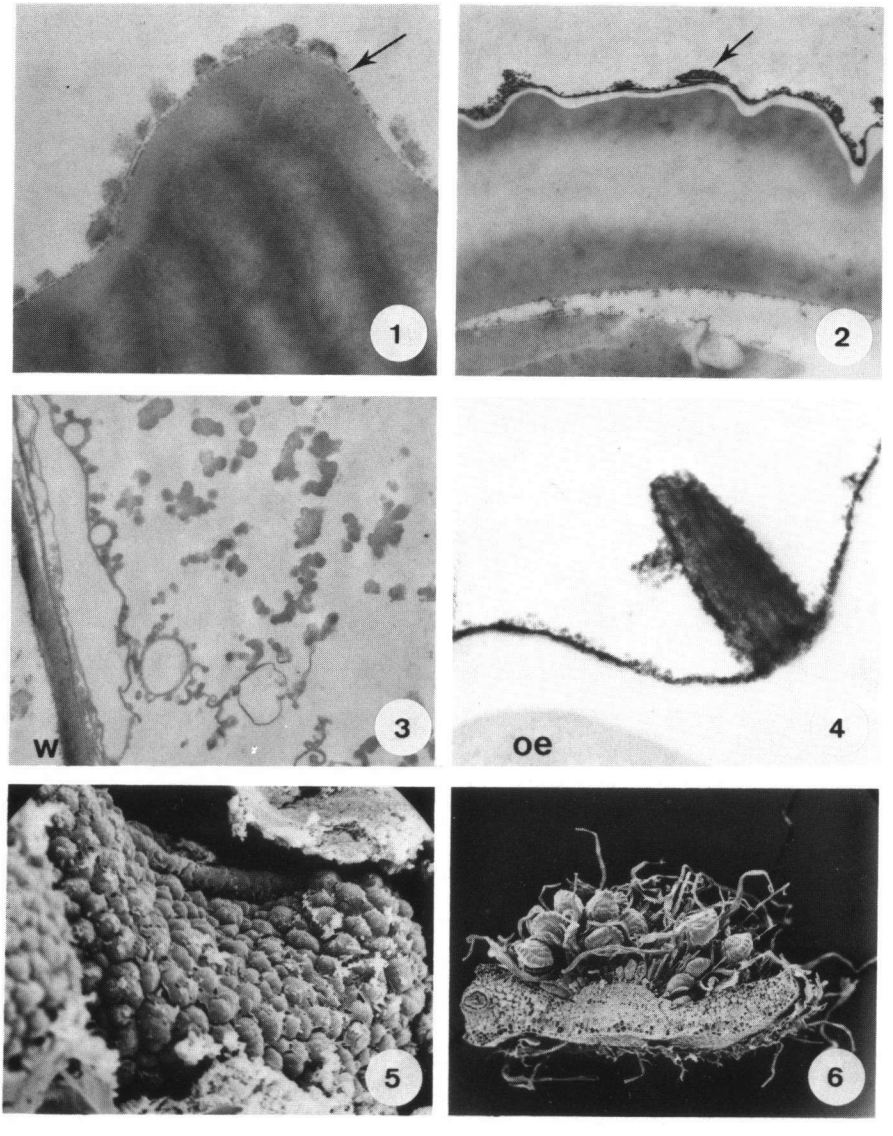

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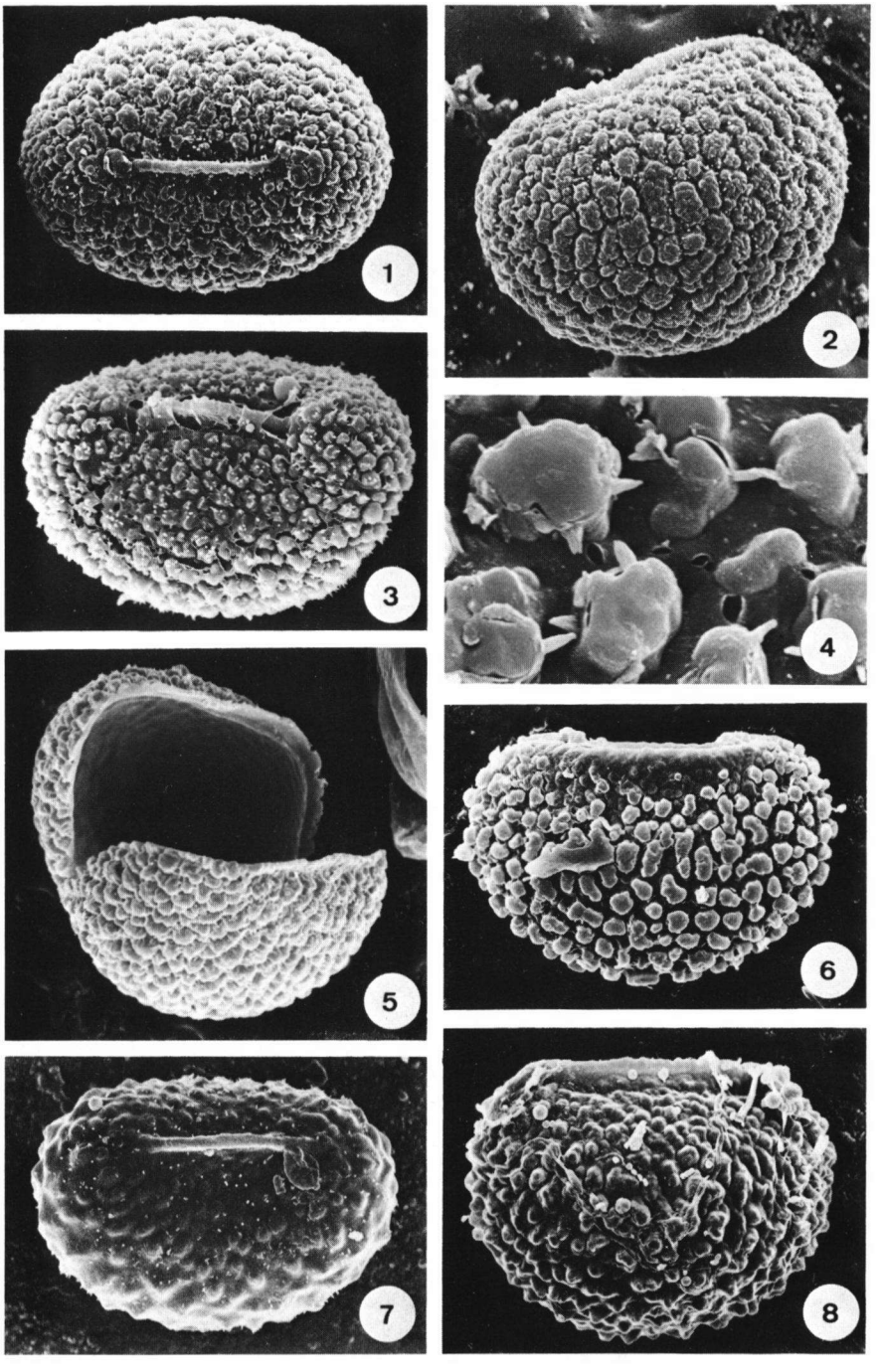

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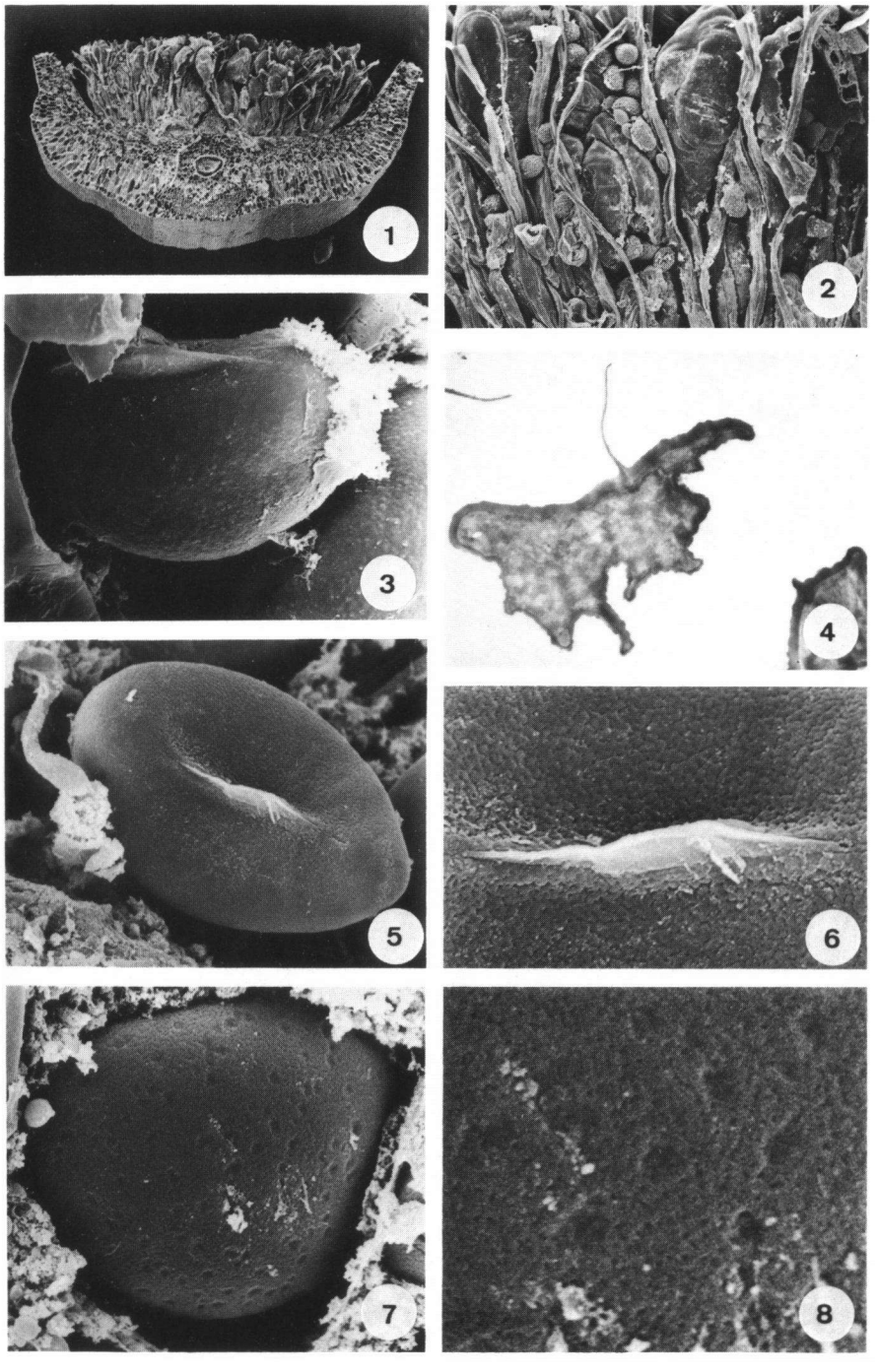

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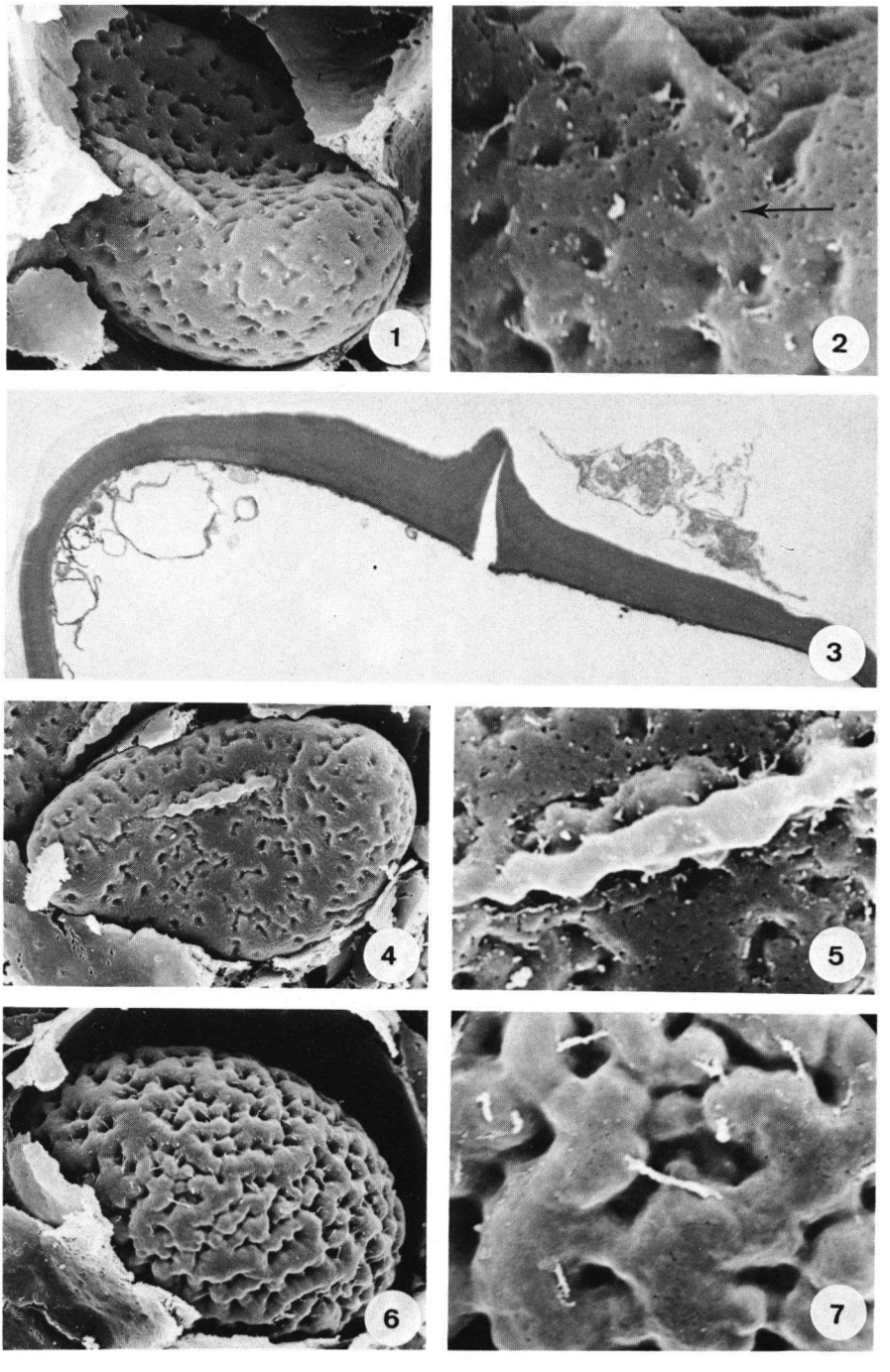

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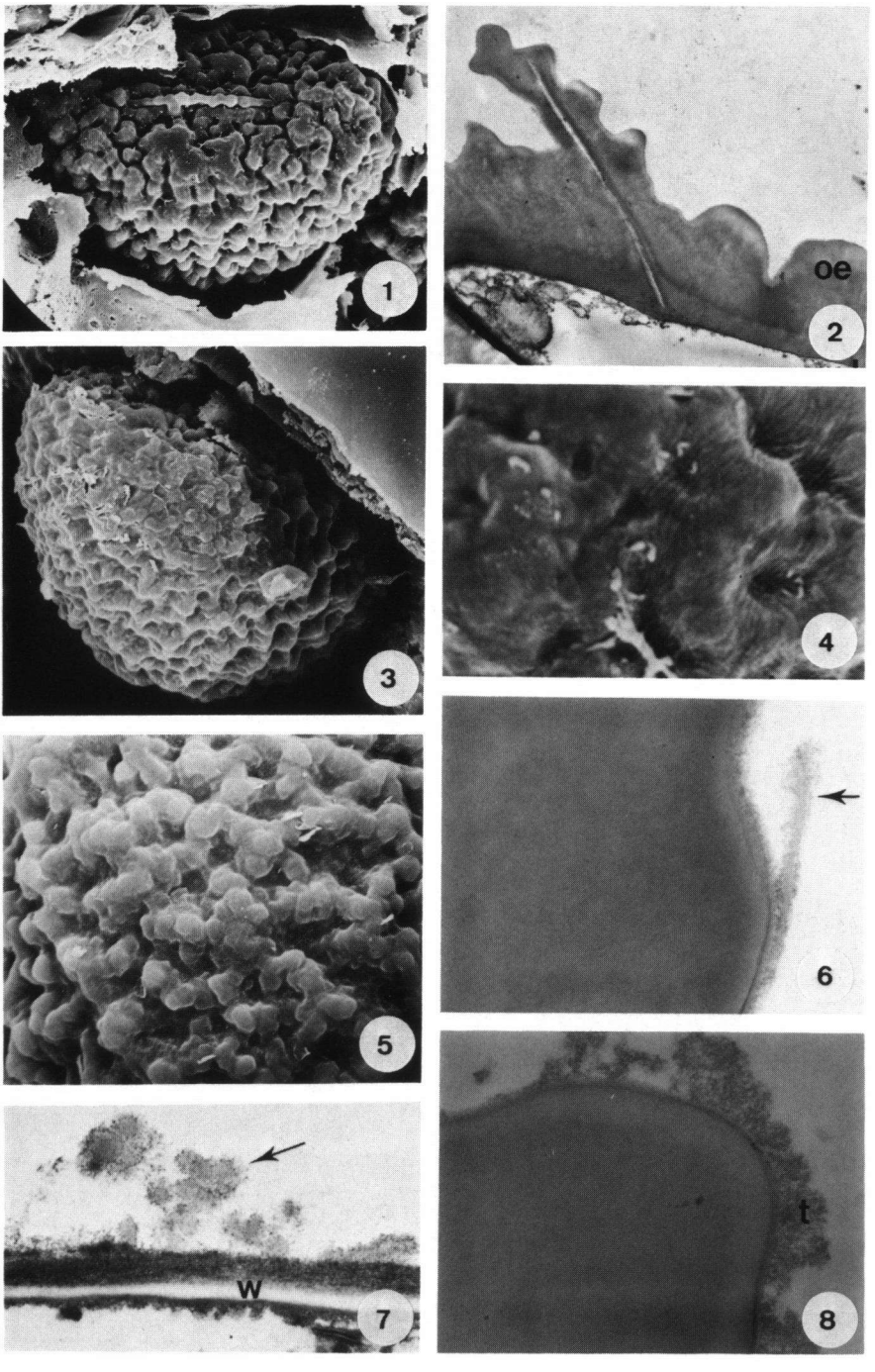

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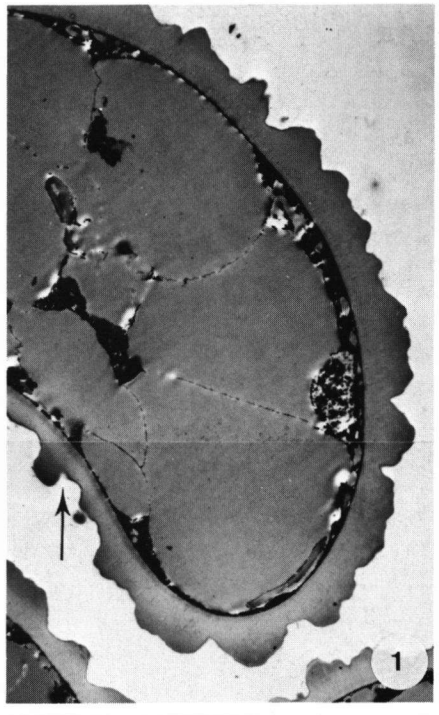

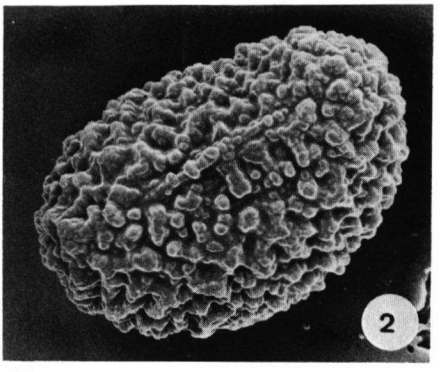

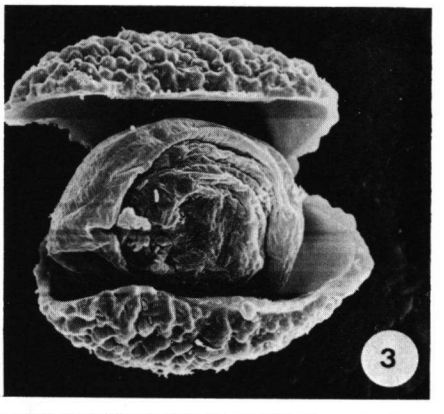

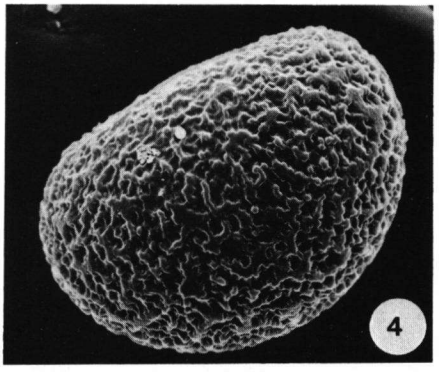

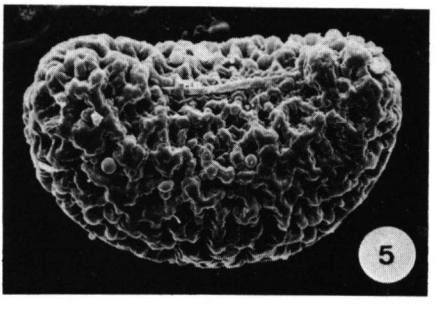

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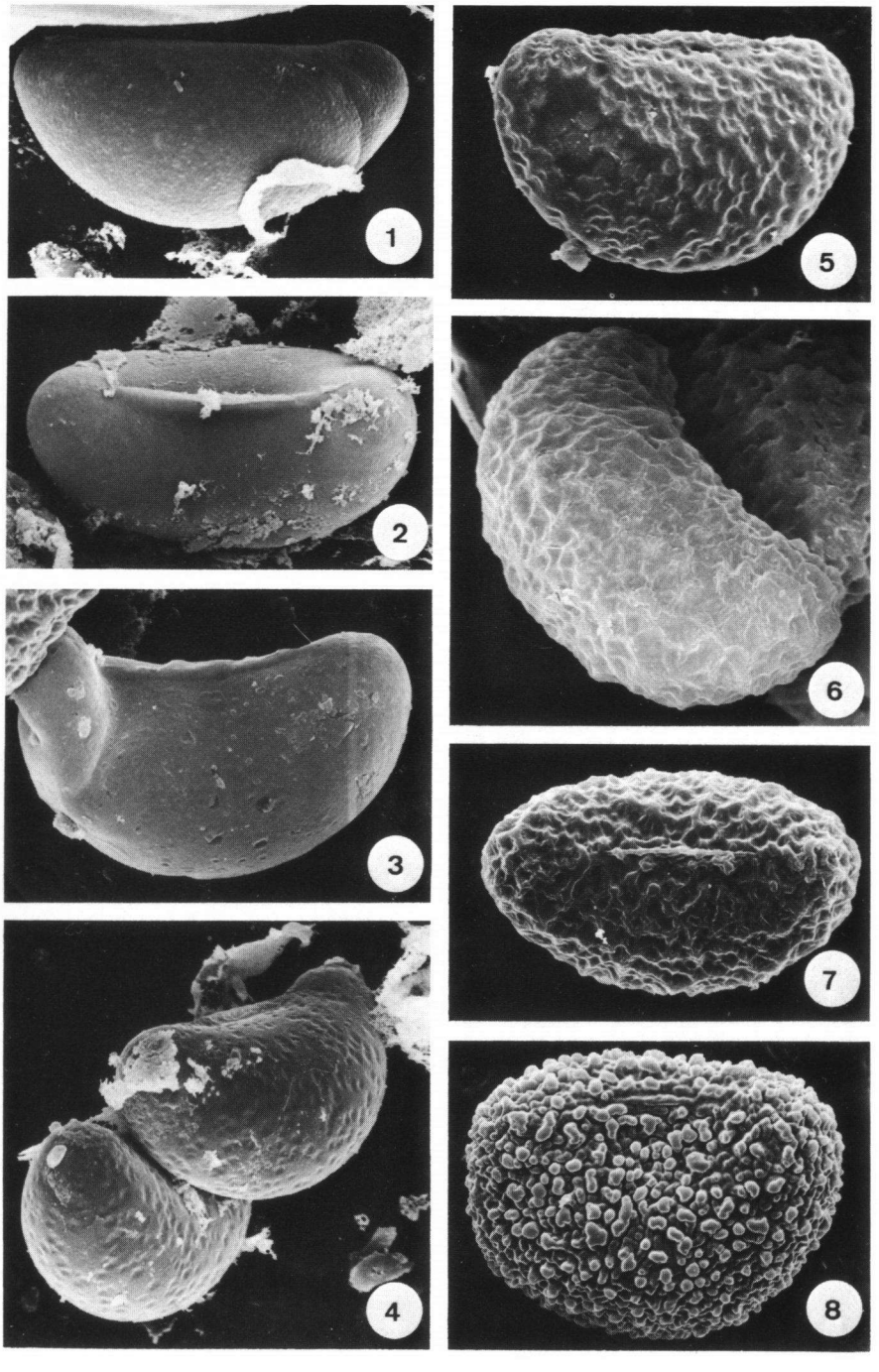

Plate IX (legend on page 531)