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Megaspore wall in Lycophyta–ultrastructure and function

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Abstract

New contributions on megaspore walls in the genus *Selaginella* are presented. The study was carried out with electron microscopy (SEM and TEM), light microscopy including phase and differential interference contrast, fluorescence and confocal microscopy. The observations were based on mature material from herbarium specimens and on fresh material in different stages of development. The results refer to:
(1) Units: They are coiled rods. Two types have been recognized, one with a central axis and the other without an axis.
(2) Patterns of ultrastructure: Two patterns have been distinguished, ordered and slack, according to the type of unit with which they are built.
(3) Gaps: They are located within the wall between units, in places where some features change and in the middle and inner parts of the exospore.
(4) Exospore ultrastructure in young stages: Two layers have been recognized. Both of them are built by coiled rods.
(5) Connections between the tapetum and the plasma membrane of the growing megaspore: During megaspore development there are highly organized structures called wicks. They run across the exospore between units.
(6) Mineral deposits within the wall: Deposits of sulphur and potassium in addition to silicon have been detected by X-ray microanalysis.

1. Introduction

Many authors have worked on both fossilised and recent megaspore walls of *Selaginella* Palisot de Beauvois. Some of them have tried to define and model the extremely complex thick wall. However, new interpretations can be made as a result of the use of other equipment and treatments, especially those for TEM, which allow greater and more accurate analysis of the ultrastructure, the chemical composition and the function of megaspore walls.

These new contributions are initial results obtained at the Department of Botany, University of Stockholm in Sweden, in collaboration with Dr. John Rowley.

Our aims are:

(1) to model the megaspore wall ultrastructure in order to know how it is built in the genus *Selaginella*;

(2) to understand the wall substructure (the first polysaccharide template);

(3) to study both micro- and megaspore development.

Basic units in the megaspore wall of *Selaginella*, identified by the light microscope and differential stains were defined by Martens (1960a,b; Stainiér, 1965, 1967).

Few authors have tried to define the basic units in *Selaginella* exospore based on either TEM or SEM studies, or both. Afzelius et al. (1954) were the first to define, with TEM, the basic units and megaspore wall ultrastructure in the genus. Pettitt (1966), with TEM, described the ultrastructural

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features that characterize the megaspore wall of *Selaginella* and *Isoetes L.* He was the first to establish the ultrastructural details to allow differentiation of *Isoetes* from *Selaginella.* Several authors used this seminal work to assign the dispersed fossil material to either *Selaginella* or *Isoetes* within Lycophyta. Kovach (1989) quantified ultrastructural aspects of the megaspore wall and applied statistical methods to investigate inter- and intra-generic variations within lycopod megaspores.

Taylor and Taylor (1987) and Hemsley et al. (1992) studied fossil megaspores with TEM and defined their basic units. They made comparisons with similar extant material.

Some authors particularly studied the ultrastructural patterns in extant species of the genus with electron microscopy (TEM and SEM), for example Kempf (1970), Morbelli (1977), Tryon and Lugardon (1978, 1991), Tryon and Tryon (1982), Minaki (1984), Bajpai and Maheshwari (1986), and Taylor (1991).

Many authors paid attention to different steps in the megaspore development, for example Heinsen (1894), Fitting (1900), Campbell (1895, 1902), Denke (1909), Pettitt (1966, 1971), Sievers and Buchen (1970), Sievers (1971), Buchen and Sievers (1978a,b, 1981), Taylor (1991), and Morbelli and Rowley (1993).

The presence and distribution of silicon features on the megaspore wall were studied by Tryon and Lugardon (1978) and referred to by many others as one aspect of a general study.

2. Materials and methods

The studies were carried out using scanning (SEM) and transmission (TEM) electron microscopy and light microscopy, including phase and differential interference contrast, fluorescence and confocal microscopy.

We worked on mature megaspores, material from Argentina (8 species), and fresh material from a greenhouse (2 species), *S. argentea* (Walich) Spring and *S. kraussiana* (Kuntze) A. Brown. The material from Argentina belongs to the following species: *S. convoluta* (Walker and Arnott) Spring, *S. marginata* (Humboldt and Bonpland) Spring, *S. microphylla* (Humboldt, Bonpland and Kunth) Spring, *S. muscosa* Spring, *S. novae-hollandiae* (Swartz) Spring, *S. peruviana* (Milde) Hieronymus, *S. sellowii* Hieronymus, and *S. sulcata* (Desvaux) Spring ex Martius.

We used alcian blue, ruthenium red and lanthanum nitrate in combination with glutaraldehyde and osmium tetroxide for the fixation procedure.

Fresh megasporangia of *S. kraussiana* and *S. argentea* from the greenhouse at the Departament of Botany, Stockholm University were fixed with 1% lanthanum nitrate (LN) and 1% glutaraldehyde (GA) in 0.1 phosphate buffer (pH 7.4, 24 h, 20°C). The megasporangia were drained, then transferred without washing to 1% lanthanum nitrate and 1% osmium tetroxide (Os). After 1 h the Os–LN mixture was decanted down to about 0.1 ml and dehydration with acetone was started. One drop of 30% acetone was added to the Os–LN mixture every 3 min until the mixture was equilibrated with 30% acetone. The 30% acetone with Os and LN residues was decanted and replaced by 30% acetone. This procedure was repeated with 50, 80 and 100% acetone; then we decanted and replaced the megaspore material three times with dry acetone before infiltration with Spurr's epoxy resin mixture.

We applied some selective stains on thin sections for TEM, in order to get more contrast. They were: 1% phosphotungstic acid (PTA) in 10% chromic acid; 5% PTA in 10% acetone, 1% periodic acid (PA) followed by 0.1% PTA in 10% chromic acid and PA to thiocarbohydrazide to silver proteinate (Thiéry sequence).

With the common, non-specific stains, like uranyl acetate and lead citrate, all the elements as well as the main features contrasted. Therefore, many details were masked and information missed.

The micrographs were taken with a Zeiss Electron Microscope EM-10 A.

The study with SEM was done at the Electron Microscopy Unit of The Natural History Museum, London, with a Hitachi S800 field emission scanning electron microscope, in collaboration with Donald Claugher. We analyzed dry samples from herbarium specimens and fresh samples from a greenhouse (Department of Botany, Stockholm University). The latter were fixed with 1% GA in

Phosphate buffer + 1% LN, then transferred to phosphate buffer to be disected. Then the material was mounted on SEM stubs and coated with gold.

We also studied the number and position of bright dots in a sequence of optical sections using confocal microscopy. The material was stained with fluorochroms like Basic Fucsin, Primulin and Coriphosphine. This analysis gave us some valuable information about the relationship between superunits (e.g. frequency of superimpositions and junctions).

For X-ray microanalysis (XRMA) sections of 0.1–0.2 μm thickness were cut from epoxy embedded megaspores of *S. convoluta* that had been acetolysed. After that, the sections were coated with evaporated carbon and analyzed using a JEOL 1200 CX electron microscope equipped with a Tracor 5200 X-ray microanalyser.

3. Results

3.1 Units

The megaspore wall (exospore) in all cases is composed of rod-shaped elements, circular in section. They are curved and coiled making loops. According to the frequency of coiling and the orientation of the loops, two main types of units can be recognized: one with a hypothetical central axis and the other having no axis.

Units with a hypothetical central axis (Plate I, A, a)

In this type of unit the rods are coiled, making several loops arranged in a helical system. The loops are very closely located, one on top of the other. All of them have the same diameter and orientation. The centres of the loops are superimposed on the same axis. We can therefore recognize a theoretical central axis which goes through the centres of the loops. Evidence of coiling can be recognized in typical notches from a lateral view.

Units without axes (Plate I, B, b)

In this type of unit the rods are curved and coiled, making loops from time to time. Both the loop size and orientation are variable, the distance between the loops in the same unit is also variable. The units look as if they have undergone stress.

According to the unit type, two main patterns of ultrastructure in the genus *Selaginella* can be recognized: ordered and slack. (Plate I, A, B; Plate III, A, D)

In the ordered type the units are arranged in groups with the same orientation and they change direction many times in a regular way, in the thickness of the wall. Of the material studied, this was found only in *S. marginata* (Plate I, A; Plate III, A), *S. sulcata* and *S. kraussiana.*

An interesting observation was made with SEM. The ordered type can be split into sheets. Each sheet has units in parallel array. (Plate III, B).

When this type is present it always shares the structure with the slack type in two or three different levels. The material under study has a pattern with two levels: ordered in the outer part and slack in the inner one. (Plate III, A, C) The slack pattern, which is also referred to as reticulate, lax, spongy, labyrinthine or grid-like, constitutes the structure by itself and several levels (zones or strata) within the same pattern could be recognized. It does not split into sheets. (Plate I, B; Plate II, D; Plate III, D). Ordered or slack types can be distinguished according to differences in the features of the rods within the wall thickness. The variable features are rod diameter, frequency of coiling and junctions. These variations could be important at species level.

3.2 Lacuna or gaps

Using fluorescence microscopy, we have seen holes on the inner exospore surface in mature megaspores sectioned in half. Narrow spaces and lacunae were also detected in thin sections with TEM, in different species with different ultrastructural patterns.

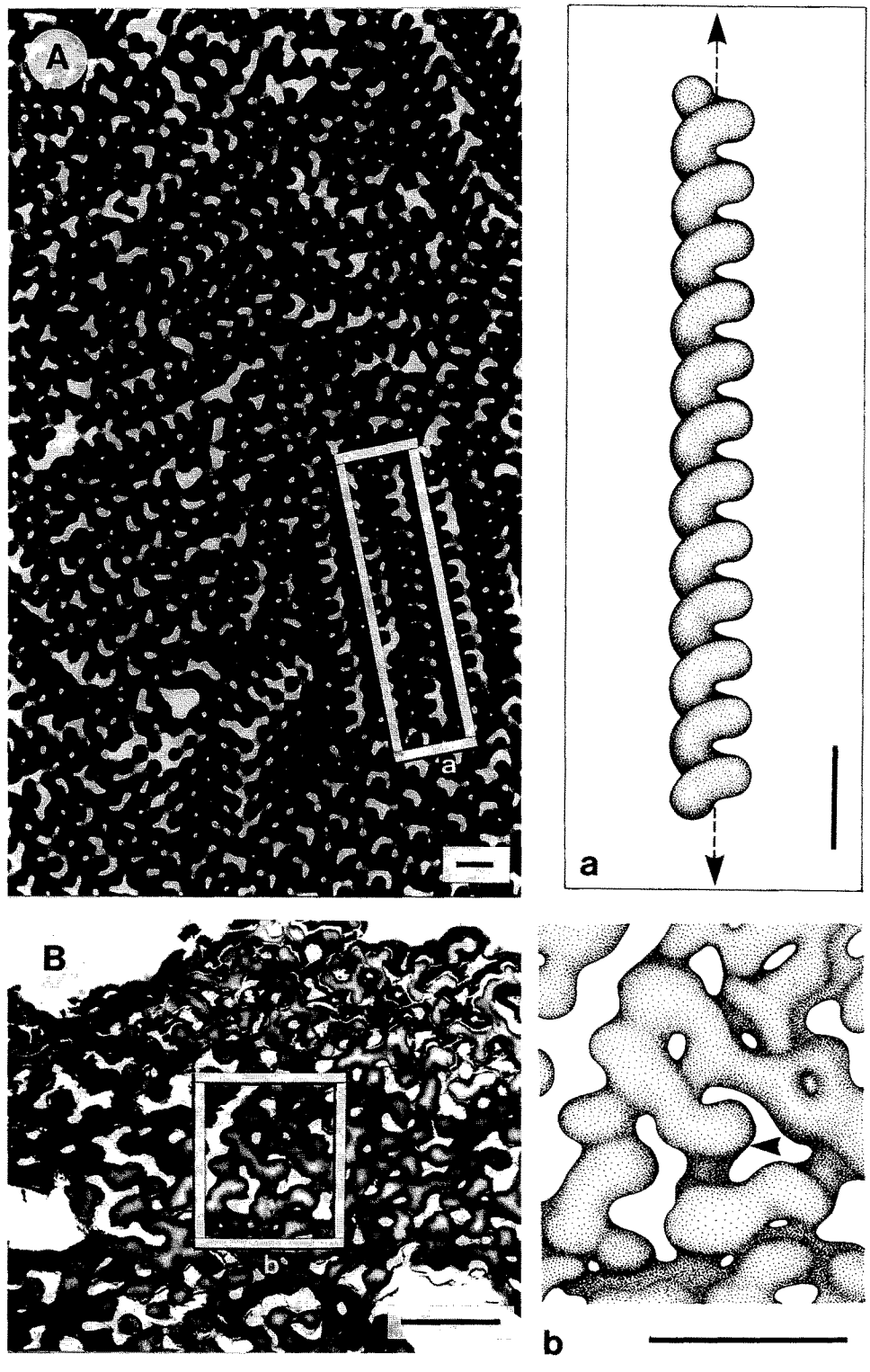
According to their location the gaps could be referred to as:

(1) Narrow spaces between units.

(2) Gaps at different levels. (Plate II, A–C). These gaps may or may not be related to changes in the orientation of the units. This is evident in the so-called ordered type (e.g. *S. marginata*; Plate II, A). Another example is the case of *S. muscosa* (Plate II, B, C).

(3) Interconnected big gaps. They are located

PLATE I



in the middle and/or near the inner exospore surface (Plate II, D) in places where some features change, between different zones or strata. In most cases they are interconnected.

3.3 *Wall ultrastucture in the young stages*

In the young stages of development, the megaspores of *S. argentea* showed only one wall of sporopollenin, in which it is possible to differentiate an outer and an inner zone of similar thickness. Both of them are detached at the equator and distal pole (Plate IV, A).

Both zones or layers have slack ultrastructural patterns. The inner zone has a wider mesh with rods of smaller diameter, mainly tangentially oriented. At this stage we saw in the locular space, between the tapetal cells and the growing megaspores, highly organized structures traversing spaces of about 20 μm to over 88 μm (Plate IV, B). Morbelli and Rowley (1993) named them "wicks".

These wicks ranged 27–(40.7)–55 nm in diameter. They show a variety of orientations in the loculus, but they are in parallel arrays near the megaspore wall. They enter the exospore and split into subunits (Plate IV, C). They extend through the wall to the plasma membrane of the megaspore cytoplasm.

Using the reaction PTA in 10% chromic acid pH ~1 either one or several central spots and the entire wick profile became dark. Using a reaction 5% phosphotungstic acid in 10% acetone, the wicks were more contrasted in both their surface and the core.

3.4 *Mineral deposits within the wall*

We have detected tubular structures, of 30–40 nm in diameter, between units, in the middle and inner parts of mature exospore of *S. convoluta* (Plate IV, D). Tubules resist the acetolysis procedure of Gunnar Erdtman (Erdtman, 1960).

They have a central core of about 7 nm in diameter. Both the outer surface and core show oblique striations. In many regions they appear to be broken into small fragments.

The results of X-ray microanalysis indicate that the tubules contain aluminum, sulfur and calcium in addition to silicon.

4. Discussion and conclusions

(1) The basic units in *Selaginella* megaspore wall are rods. They are coiled, forming loops. some have a theoretical central axis, others do not (Plate I, A, a, B, b). This characteristic is limited to the genus *Selaginella* within the Lycophyta.

In our interpretation there are two ultrastructural patterns, defined by their different arrangements and combinations, as well as secondary variations in the features of the rods (diameter, degree of compactness, etc.). So far it has been considered that different patterns have different types of units. Considering rods as basic units, we agree with the earliest observations made by Afzelius et al. in the 1950s using TEM. They considered the megaspore wall as a three-dimensional network of rounded bars.

Kempf (1970), using TEM, defined the units of the main part of the wall as threads. Also, Minaki (1984) stated that fundamentally the features of the exospore were the same: "Basically they are three dimensional bars".

The units in the so-called ordered ultrastructure were also defined by several authors as sheets (Taylor, 1988), plates (Taylor and Taylor, 1987, 1988), lamellae (Taylor, 1988), hexagons (Tryon and Lugardon, 1978), points or granules (Martens, 1960a,b; Stainiér, 1965, 1967), particules (Minaki, 1984; Hemsley et al., 1992). Kedves (1990) quoted helical subunits as a typical feature of a *Selaginella* megaspore wall.

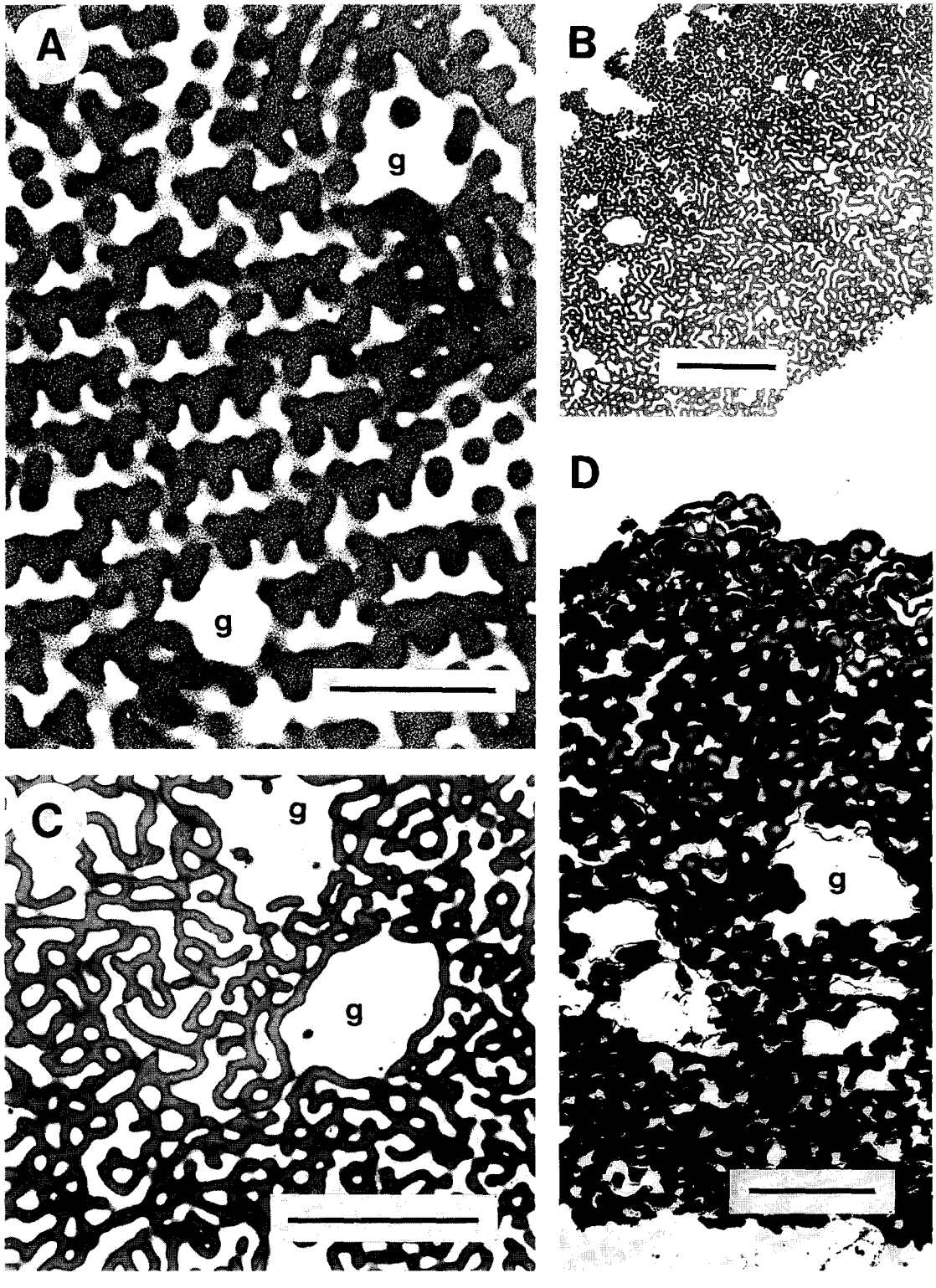
PLATE I

Basic units in the megaspore wall of *Selaginella* (TEM).

A, a. Unit with a hypothetical central axis in *S. marginata*. Scale bar = 1 μm. Argentina, Buenos Aires, Cabrera 1595 (LP).

B, b. Unit without axis in *S. convoluta*. Scale bar = 3 μm. Paraguay, Paraguarí, Balansa 1116 (BAF).

PLATE II



Since the pioneering work of Pettitt (1966) it was largely emphasized that unit orientation and, consequently, spaces between units within the wall were the most important features for differentiating the wall ultrastructure at the generic level within Lycophyta.

In our opinion, the main, distinctive and clear character of differentiation is the type of basic unit. Thus the differences in spatial organization of basic units and their arrangement in superunits following determinate patterns are the primary consideration and the spaces between them are of secondary importance.

Secondary features, such as spaces and wall thickness, are very much influenced by the degree of megaspore development. It is well known that there is a tendency in *Selaginella* to reduce the number of viable megaspores per sporangium (see Lyon, 1901; Goebel, 1910; Duerden, 1929; Tryon, A.F., 1949; Tryon, R.M., 1955; Horner and Arnott, 1963; Hellwig, 1969; Robert, 1972; Morbelli, 1977; de la Sota and Morbelli, 1981; Tryon and Tryon, 1982). This tendency determines that four, three or two members of each tetrad develop different sizes ranging from small to gigantic. The thickness of the wall of a megaspore depends on the degree of development of the megaspore. For the above reasons, I conclude that spaces are not a reliable characteristic and must be used with extreme caution, and not as a main feature. Spaces could be considered as a reference to define ultrastructural changes (zones, levels or strata) within a typical pattern.

The differential development of the four members within the same tetrad - distinctive of *Selaginella* - has been interpreted by Pettitt (1970) as a way of reducing in the number of viable megaspores, from four to one, which is distinctive for plants with a seed habit.

(2) According to our interpretations, in highly patterned parts of the wall, the units may be arranged in superunits. This implies a spatial arrangement in groups or bunches of units.

(3) Based on the type of unit that constitutes the walls, two main patterns of ultrastructure within the genus *Selaginella* can be recognized, ordered and slack. (Plate I, A, b; Plate III, A, D). If the ultrastructure is interpreted as proposed here, the megaspore wall patterns found in both fossil and recent material of *Selaginella* are essentially the same. No new types have been recorded since the Paleozoic.

Nevertheless, differences could be recognized according to whether or not both ordered and slack types are combined or not. Variations in rod features and coiling determine different types of ultrastructure which could be of value at species level. The slack pattern has been found in Lycophyta since the Carboniferous and the ordered one since the Cretaceous.

The similarities in wall ultrastructure of fossil and recent *Selaginella* megaspores were analyzed by Tryon (1985). According to my interpretation, there has been a remarkable retention of both slack and ordered wall structures for a long time. I agree with A. Tryon that this stability of features provides evidence of "stasis".

The persistence of this character enables us to assign some dispersed fossil megaspores to Lycophyta.

(4) The slack ultrastructure constitutes the wall by itself (Plate III, D). The ordered ultrastructure is always mixed or combined with the slack one (Plate III, A, C). This organization seems to be the same for fossil and recent material. Mixed patterns, like the ordered one, were reported to be present in recent material by Martens (1960b), Stainiér (1965, 1967), Kempf (1970), Morbelli (1977), Tryon and Lugardon (1978, 1991), Minaki

PLATE II

System of gaps (*g*) in *Selaginella* megaspore wall (TEM).

A. Gaps in the ordered pattern of *S. marginata*. Scale bar = 2 μm. Argentina, Buenos Aires, Cabrera 1595(LP).

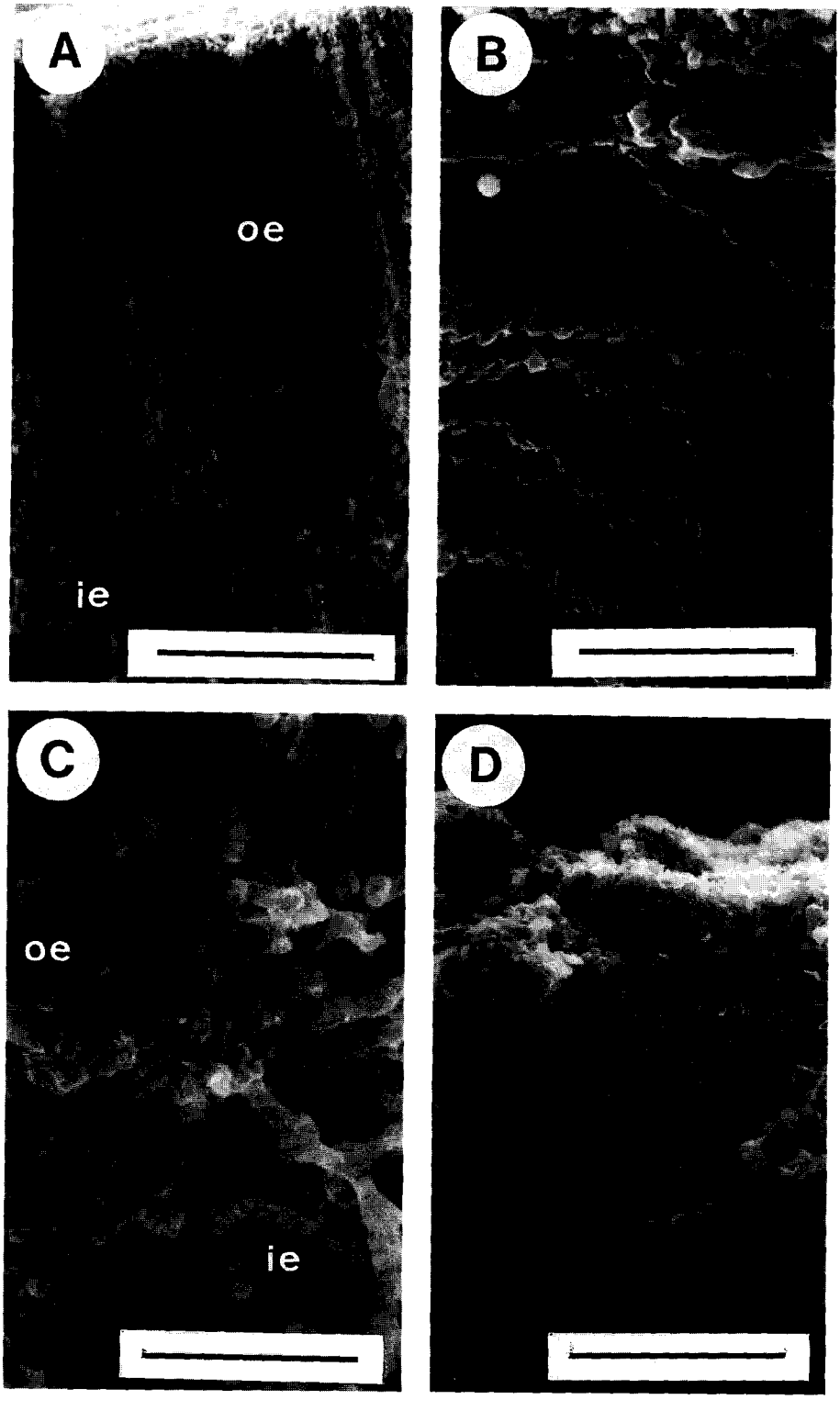
B, C. Gaps at different levels in *S. muscosa*. Argentina, Buenos Aires, Bachman s/nro. (LP).

B. General view. Scale bar = 10 μm.

C. Detail. Scale bar = 5 μm.

D. Large gaps in the middle part of the exospore of *S. convoluta*. Scale bar = 5 μm. Paraguay, Paraguarí, Balansa 1116 (BAF).

PLATE III



(1984), Taylor and Taylor (1988), and Hemsley et al. (1992).

When the ordered pattern is present, it is located in the middle or outer areas of the exospore. There is a gradual transitional zone where the units change their arrangement from helical to having no axis (e.g. *S. marginata*; Plate III, C).

Kempf (1970) established mono-, bi- and trizonal types to describe variations in both ordered and slack patterns. Tryon and Lugardon (1978) called these variations "strata".

(5) The fact that the ordered type of exospore can be detached in sheets, when observed with SEM, implies that the attachment is stronger between units of the same plane than between those of different planes (Plate III, B).

(6) Lacunae or gaps are here considered as a typical feature within a *Selaginella* megaspore wall. I think that they belong to a three-dimensional system of interconnected spaces and channels (Plate II).

Lacunae were described by Taylor and Taylor (1988) for fossilized material of Cretaceous age and interpreted as a result of expansion or local removal of wall material.

(7) During the young stages of development in *S. argentea*, the megaspore wall is composed of two layers. The inner layer of the exospore is specially built. It is composed, like the outer one, of curved rods making loops (Plate IV, A). The two layers are attached to each other in the apertural area and, to some extent, on both sides of it towards the equator. An inner zone like this was referred to by some authors as "mesospore" and as only being present in immature or abortive spores.

This enigmatic structure and its possible detachment from the outer layer has been a controversial issue since the seminal studies of Fitting (1900), Lyon (1901, 1905) and Campbell (1902). A structure like this was also referred to by Taylor and Taylor (1988) and Archangelsky and Villar de Seoane (1990) concerning fossilized material of the Cretaceous age, assigned to Selaginellales.

In Pettitt's (1966) opinion, the mesospore probably represents the remains of an aborted cytoplasm. He mentioned this structure to be present in some members within the same tetrad. However, as is possible to see in our photographs, it is always present in the young stages, so I assume that there must be some genetic information to build it.

I agree with the observations of Campbell (1902), who noticed that the "mesospore" closely resembles the exospore in appearance in such a way that it could arise by separation of an inner layer from the exospore. According to this interpretation, the fact that both layers have a similar ultrastructure seems to be pointing to this relationsip with respect to the origin of the mesospore.

(8) During development wicks connect tapetum and megaspore cytoplasm (Plate IV, B).

Working on development, Sievers and Buchen (1970) have shown that there is contact between the growing wall and the megaspore cytoplasm. Now we show that the growing wall is in contact with both tapetal cells and megaspore cytoplasm. This indicates the presence of dynamic and active structures.

(9) According to the reactions of the selective stains used, it is concluded that wicks consist of polysaccharide and protein (glycoprotein and/or mucopolysaccharide).

(10) It is considered likely that wicks are built, maintained and supplied by the tapetum because

PLATE III

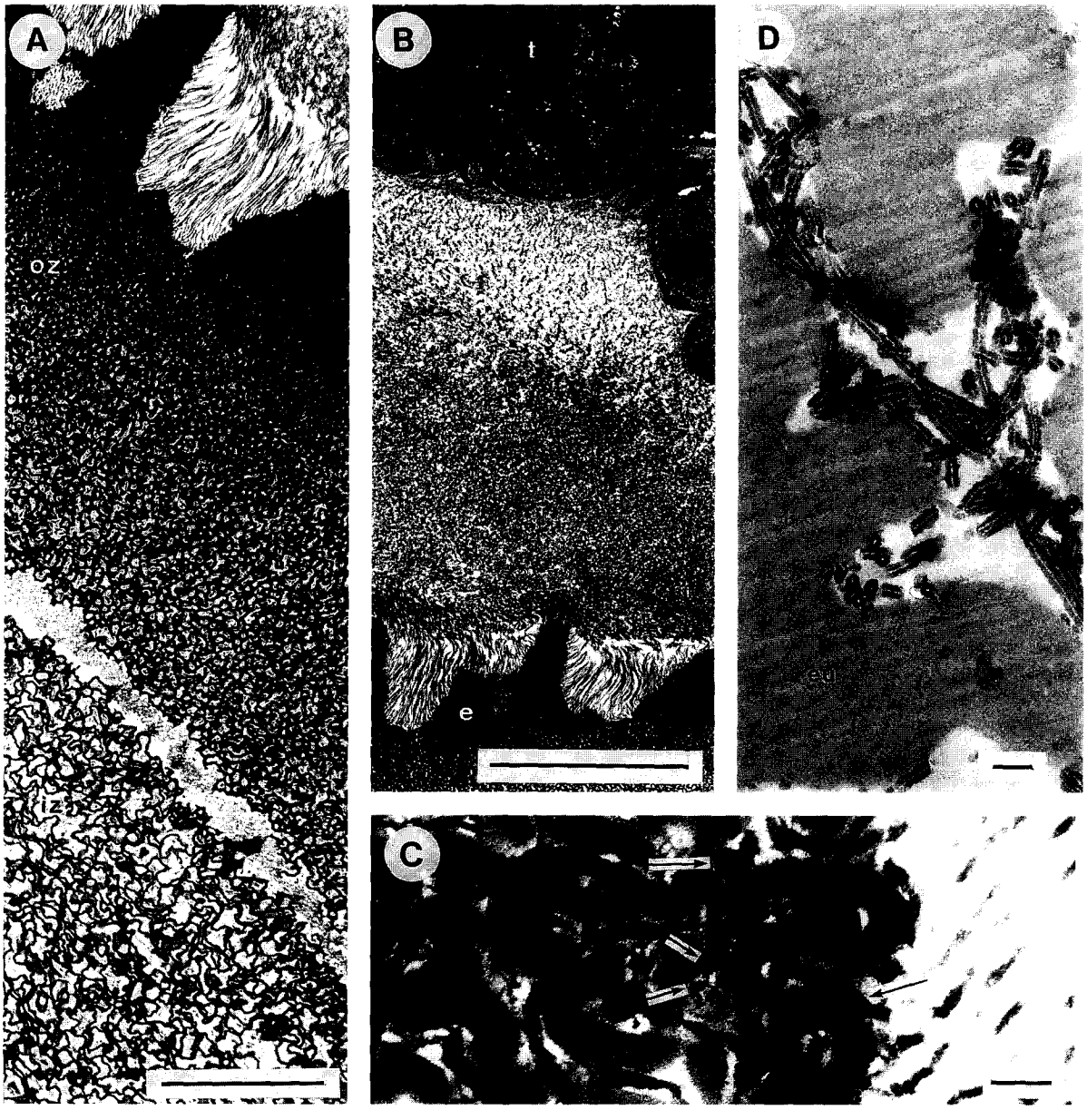
Patterns of ultrastructure in the megaspore wall of *Selaginella* (SEM).

A. Ordered (which in taxa known to me includes both ordered and slack = mixed) pattern in *S. marginata* (*oe* = outer exospore; *ie* = inner exospore). Scale bar = 7.5 μm. Uruguay, Colonia, Cabrera 13.620 (LP).

B. Outer exospore in *S. kraussiana*. Detail showing detachment into sheets within the ordered (outer) zone. Scale bar = 3 μm. Cultivated, Kew Garden, London.

C. Transitional zone within the ordered (= mixed) pattern of *S. marginata* (same as A), left side ordered pattern, right side slack pattern (*oe* = outer exospore; *ie* = inner exospore). Scale bar = 2 μm.

D. Slack pattern in *S. novae-hollandiae*. Scale bar = 6 μm. Argentina, Salta, Pierottii 1145 (LP).



A–C. Development in *Selaginella argentea*. Cultivated, Department of Botany, University of Stockholm.

A. Megaspore wall in the young stage (*oz* = outer zone of the wall; *iz* = inner zone of the wall). Scale bar = 50 μm.

B. Wicks in the locular space and entering the megaspore wall (exospore) (*t* = tapetum; *w* = wicks; *e* = exospore). Scale bar = 10 μm.

C. Wicks (arrows) enter the exospore and divide in sub-units (shorter arrows). Scale bar = 100 nm.

D. Mature megaspore wall of *S. convoluta*, with TEM. Wick remnants between exospore units (*eu* = exospore unit; *wr* = wick remnants). Scale bar = 100 nm. Paraguay, Paraguarí, Balansa 1116 (BAF).

[The micrographs in this Plate are figs. 2, 9, 20, 21 in Morbelli and Rowley (1993)].

of their vast extent and the small size of the megaspore cytoplasm until late in the development.

(11) Wicks have a coiled structure that suggests that they are specially built to offer a surface for delivering essential substances during megaspore development (Plate IV, B, C).

(12) The passage of wicks and their subunits through the megaspore wall implies that the wall is a three-dimensional network of interconnected spaces (Plate IV, C). The arrangement of elements in a three dimentional system was suggested by Afzelius et al. (1954) and Minaki (1984). It is therefore possible to consider that the functions of the wall are, first, to protect the cytoplasmic content and, at the same time, to offer an open way for free passage of substances from the tapetum to the megaspore cytoplasm during development.

(13) At maturity, depending on the species, there are tubules between the units of the wall stabilized by minerals (aluminium, sulfur and calcium in addition to silicon (e.g. *S. convoluta* Plate IV, D).

We have interpreted these tubules according to their substructure and size as fragments of wicks, stabilized mainly by silicon, during the maturation of the wall. Morbelli and Rowley (1993) named them "wick remnants". Rod-like elements with a white line in the middle were found by Kempf (1970) in the inner zone of the wall of *S. galeottii*. So the mineral deposits in tubular structures within the wall are present in both slack (*S. convoluta*) and ordered patterns (*S. galeottii* sensu Kempf, 1970).

The existence of tubular units of silicon inside the megaspore wall is likely to be related to the availability of silicon in the soil and the ecological requirements of the species, since tubular deposits were seen only in *S. convoluta*, which has a revivifying habit.

This work is an example of how a better knowledge of sporoderm ultrastructure and chemistry could improve our knowledge of its function. It is necessary to continue with studies, especially about development, in order to understand, as a whole, the morphology and behavior of both female and male gametophytes in heterosporate groups.

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References

Afzelius, B.M., Erdtman, G. and Sjöstrand, F.S., 1954. On the fine structure of the outer part of the spore wall of *Lycopodium clavatum* as revealed by the electron microscope. Sven. Bot. Tidskr., 48: 151–161.

Archangelsky, S. and Villar de Seoane, L., 1990. Morfología y estructura de megasporas cretacicas de Patagonia, República Argentina. Rev. Esp. Micropaleontol., 22(3): 419–450.

Bajpai, U. and Maheshwari, H.K., 1986. SEM study of megaspore sporoderm of some Indian *Selaginella*. Phytomorphology, 36(1/2): 43–51.

Buchen, B. and Sievers, A., 1978a. Megasporogenese von *Selaginella*. I. Ultrastrukturelle und cytochemische Untersuchungen zur Sekreton von Polysacchariden. Protoplasma, 96: 293–317.

Buchen, B. and Sievers, A., 1978b. Megasporogenese von *Selaginella*. II. Ultrastrukturelle und cytochemische Untersuchungen zur Sekreton von Lipiden. Protoplasma, 96: 319–328.

Buchen, B. and Sievers, A., 1981. Sporogenesis and pollen grain formation. In: O. Kiermayer (Editor), Cytomorphogenesis in Plants. Springer, New York, NY, pp. 349–376.

Campbell, D.H., 1895. Structure and development of the Mosses and Ferns. Macmillan, London, pp. 485–504.

Campbell, D.H., 1902 Studies on the gametophyte of *Selaginella*. Ann. Bot. London, 16: 419–428.

Collinson, M.E., 1991. Diversification of modern heterosporous pteridophytes. In: S. Blackmore and S.H. Barnes (Editors), Pollen and Spores. Patterns of Diversification. Syst. Assoc. Spec. Vol. 44. Clarendon, Oxford, pp. 119–167.

De la Sota, E.R. and Morbelli, M.A., 1981. Distribucion de esporangios en estrobilos de las especies sudamericanas de *Selaginella* Pal. Beauv, I. *Selaginella peruviana* (Milde) Hieron y S. sellowii Hieron. Physis (Buenos Aires) C, 40(98): 39–53.

Denke, P., 1909. Sporenentwicklung bei *Selaginella*. Beih Bot. Zentralbl., 12: 182–199.

Duerden, H., 1929. Variations in megaspore numbers in *Selaginella*. Ann. Bot., 43: 451–457.

Erdtman, G., 1960. The acetolysis method. A revised description. Sven. Bot. Tidskr., 39: 561–564.

Fitting, H., 1900. Bau und Entwickelunggageschichte der Makrosporen von *Isoetes* und *Selaginella* und ihre Bedentung fur die kenntniss des wachsthums pfanslicher Zeellmembranen. Bot. Z., 58: 107–156.

Goebel, K., 1910. Ueber sexuellen Dimorphismus bei Pflanzen. Biol. Zentralblatt., 30: 676.

Heinsen, E., 1894. Die Makrosporen und as weibliche Prohalieum von *Selaginella*, Flora Jena, 78: 466–496.

Hellwig, R.L., 1969. Spores of the heterophyllous *Selaginella* of México and Central América. Ann. Mo. Bot. Gard., 56: 444–464.

Hemsley, A.R., Collinson, M.E. and Brain, A.P.R., 1992. Colloidal crystal-like structure of sporopollenin in the megaspore walls of recent *Selaginella* and similar fossil spores. Bot. J. Linn. Soc., 108: 307–320.

Horner, H.T. and Arnott, H.J., 1963. Sporangial arrangements in North American species of *Selaginella*. Bot. Gaz., 124: 371–383.

Kedves, M., 1990. Quasi-crystalloid basic molecular structure of the sporoderm. Rev. Palaeobot. Palynol., 64: 181–186.

Kempf, E.K., 1970. Elektronnenmikroskopie der sporodermis von megasporen der gattung *Selaginella* (Pteridophyta). Rev. Palaeobot. Palynol., 10: 99–116.

Kovach, W.L., 1989. Quantitative methods for the study of lycopod megaspore ultrastructure. Rev. Palaeobot. Palynol., 57: 233–46.

Lyon, F.M., 1901. A. study of the sporangia and gametophytes of *Selaginella apus* and *Selaginella rupestris*. Bot. Gaz 32: 124–170.

Lyon, F.M., 1905. The spore coats of *Selaginella*. Bot. Gaz., 40: 285–295.

Martens, P., 1960a. Sur une structure microscopique orientee dans la paroi megasporale d'une Selaginelle. C.R. Acad. Sci. Paris, 250: 1599–1602.

Martens, P., 1960b. Nouvelles observations sur la structure des parois megasporales de *Selaginella myosurus* (Sw.) Alston. C.R. Acad. Sci. Paris, 250: 1774–1775.

Minaki, M., 1984. Macrospore morphology and taxonomy of *Selaginella* (Selaginellaceae). Pollen Spores, 26(3/4): 421–480.

Morbelli, M.A., 1977. Esporas de las especies argentinas de *Selaginella*. Obra Cent. Mus. La Plata, 3: 121–150.

Morbelli, M.A. and Rowley, J.R., 1993. Megaspore development in *Selaginella* I."Wicks", their presence, structure and presumed function. Sexual Plant Reprod., 6: 98–107.

Pettitt, J.M., 1966. Exine structures in some fossil and recent spores and pollen as revealed by light and electron microscopy. Bull. Br. Mus. Nat. Hist. Geol., 13(4): 15–257.

Pettitt, J.M., 1970. Heterospory and the origin of the seed habit. Biol. Rev., 45: 401–415.

Pettitt, J.M., 1971. Some ultrastructural aspects of sporoderm formation in Pteridophytes. In: G. Erdtman and P. Sorsa (Editors), Pollen and Spore Morphology and Plant Taxonomy. Pteridophyta. Almqvist & Wiksell, Stockholm, pp. 227–251.

Robert, D., 1972. Quelques particularités de la reproduction chez les Selaginelles. Bull. Soc. Bot. Fr., 119(5/6): 373–382.

Sievers, A., 1971. Contact between the spore cytoplasm and the growing sporoderm of the *Selaginella* megaspore. In: J. Brooks, P.R. Grant, M. Muir, P. van Gijzel and G. Shaw (Editors), Sporopollenin. Academic, London, pp. 654–658.

Sievers, A. and Buchen, B., 1970. Ueber en Feinbau der wachsenden megaspore von *Selaginella*. Protoplasma, 71: 267– 279.

Stainiér, F., 1965. Structure et infraestructure del parois sporales chez deux selaginelles (*Selaginella myosurus* et *S. kraussiana*). Cellule, 65: 219–244.

Stainiér, F., 1967. morphological study of the walls of the mega and microspores of *Selaginella myosurus* (Sw.) Alston. Rev. Palaeobot. Palynol., 3(1/4): 47–50.

Taylor, W.A., 1991. Ultrastructural analysis of sporoderm development in megaspores of *Selaginella galeottii* (Lycophyta). Plant Syst. Evol., 174: 171–182.

Taylor, W.A. and Taylor, T.N., 1987. Subunit construction of the spore wall in fossil and living lycopods. Pollen Spores, 29: 241–248.

Taylor, W.A. and Taylor, T.N., 1988. Ultrastructural analysis of selected Cretaceous megaspores from Argentina. J. Micropalaeontol., 7(1): 73–87.

Tryon, A.F., 1949. Spores of the genus *Selaginella* in North America, North of Mexico. Ann. Mo. Bot. Gard., 36: 413–431.

Tryon, A.F., 1985. Stasis, diversity and function in spores based on an electron microscope survey of the Pteridophyta. In: S. Blackmore and I.K. Ferguson (Editors), Pollen and Spores: Form and Function. Linn. Soc. Symp. Ser., 12. Academic, London, pp. 233–249.

Tryon, A.F. and Lugardon, B., 1978. Wall structure and mineral content in *Selaginella* spores. Pollen et Spores, 20(3): 315–340.

Tryon, A.F. and Lugardon, B., 1991. Spores of the Pteridophyta. Surface, Wall Structure and Diversity Based on Electron Microscope Studies. Springer, New York, NY, 648 pp.

Tryon, R.M., 1955. *Selaginella rupestris* and its allies. Ann. Mo. Bot. Gard., 36: 413–431.

Tryon, R.M. and Tryon, A.F., 1982. Ferns and Allied Plants, With Special Reference to Tropical America. Springer, New York, NY, 858 pp.