

## ARTICULO INVITADO

## SPORE WALL STRUCTURE IN *SELAGINELLA* (LYCOPHYTA) SPECIES GROWING IN ARGENTINA

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**Summary:** The mega and microspore wall structures of eight species of *Selaginella* that grow in Argentina have been studied. Rod-shaped units are the basic structural components of the megaspore wall. Two main patterns of unit arrangement have been recognized, these are ordered and unordered. The unordered pattern is the same throughout the wall while in the ordered pattern the ordered part is always a major part of the wall but there are unordered portions located both inward and outward. The megaspores of *S. marginata* (H. & B. ex Willd) Spring and *S. sulcata* (Desv.) Spring ex Martius have the ordered pattern. *S. convoluta* (Walk.Arn.) Spring, *S. microphylla* (Kunth) Spring, *S. muscosa* Spring, *S. novae-hollandiae* (Sw.) Spring, *S. peruviana* (Milde) Hieron. and *S. sellowii* Hieron. are unordered. Megaspore wall characters shared by these species are: the circular shape of the units in section, the inward extension of the spaces between structural units, the presence of small gaps (mini-gaps) throughout the wall and the continuity of the conduits throughout the wall. The common features that pertain to the innermost portion of the exospore are: lack of its separation in the proximal zone, its detachment over the rest of the exospore and development of a space (the major gap) between the two parts of the exospore, and the presence on the surfaces of the gap of spheres of variable size. X-Ray Microanalysis has demonstrated the presence of Silica and traces of Al, Ca, S and K on the surface and between the structural units in megaspore exospores of *S. convoluta* and *S. marginata*. Except for *S. convoluta*, microspores of these species were separated from the tetrad condition. The microspores of these species have ornamentation that varies from granules and spines to alveoli. The ornamentation and exospore thickness are different on proximal and distal faces on six of the species studied. Microspores of *S. marginata* and *S. sulcata*, however, have similar ornamentation on both polar surfaces. Dry microspores of *S. peruviana* and *S. sellowii* have an exospore that is pleated on the proximal face and in section they also have a separation between portions of the exospore as in megaspores. Similarities have been found in the surface coating of mega and microspores in four of the species studied. The coating in these cases is composed of rodlets about 50 nm in diameter with a central low dense core 10 nm in diameter.

**Key Words:** *Selaginella*, microspores, megaspores, ultrastructure, Argentina.

**Resumen:** Estructura de la pared en esporas de especies de *Selaginella* (Lycophyta) que crecen en Argentina. Se estudiaron las estructuras de las paredes de mega- y microsporas de ocho especies de *Selaginella* que crecen en Argentina. Los componentes estructurales básicos de la pared de las megasporas son unidades en forma de barra. Se reconocieron dos patrones principales de disposición de las unidades, a saber: ordenado y no ordenado. El patrón no ordenado es uniforme a través de toda la pared, mientras que en el patrón ordenado la parte ordenada es siempre la mayor parte de la pared pero hay porciones no ordenadas ubicadas hacia dentro y hacia fuera de la misma. Las megasporas de *S. marginata* (H. & B. ex Willd) Spring y *S. sulcata* (Desv.) Spring ex Martius tienen patrón ordenado. *S. convoluta* (Walk.Arn.) Spring, *S. microphylla* (Kunth) Spring, *S. muscosa* Spring, *S. novae-hollandiae* (Sw.) Spring, *S. peruviana* (Milde) Hieron. y *S. sellowii* Hieron. son no ordenadas. Estas especies comparten ciertos caracteres de la pared de las megasporas, a saber: la forma circular de las unidades estructurales en sección, el agrandamiento de los espacios entre unidades estructurales hacia el interior, la presencia de espacios pequeños y la continuidad de los conductos a través de toda la pared. Las estructuras comunes que pertenecen a la parte más interna del exosporio son: falta de su separación en la zona proximal, su desprendimiento en el resto del exosporio y el desarrollo de un espacio (el espacio mayor) entre las dos partes del exosporio, y la presencia sobre las superficies de este espacio de esferas de tamaño variable. El microanálisis con Rayos X ha demostrado la presencia de Sílice y trazas de Al, Ca, S y K sobre la superficie y entre las unidades estructurales en los exosporios de las megasporas de *S. convoluta* y *S. marginata*. Con excepción de *S. convoluta*, las microsporas de estas

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especies se dispersan en forma aislada. Las microsporas de estas especies tienen ornamentación que varía desde gránulos y espinas a alvéolos. La ornamentación y el espesor del exosporio son diferentes sobre las caras proximal y distal en seis de las especies estudiadas. Las microsporas de *S. marginata* y *S. sulcata* tienen sin embargo ornamentación similar en ambas superficies polares. Las microsporas secas de *S. peruviana* y *S. sellowii* tienen un exosporio plegado en la cara proximal y en sección también poseen una separación entre porciones del exosporio como ocurre en las megasporas. Se han hallado similitudes en la cubierta superficial de mega y microsporas en cuatro de las especies estudiadas. La cubierta en esos casos está compuesta de varillitas de alrededor de 50 nm de diámetro con una parte central de baja densidad de 10 nm de diámetro.

**Palabras Clave:** *Selaginella*, microsporas, megasporas, ultraestructura, Argentina.

## INTRODUCTION

The present study is aimed to gather together all the information obtained about micro and mega spores of *Selaginella* species that grow in Argentina. We wanted to give detailed information obtained with light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), of high resolution and confocal microscopy apart of those published by Morbelli (1977) about the same species.

Eight species of *Selaginella* grow in Argentina. They are distributed in the Northwest, Northeast and center of the country. The southern boundary is South East of Buenos Aires Province ca. 39° of Latitude. They occur in very different environments and show remarkable ecological adaptations. Some species like *S. marginata* and *S. muscosa*, grow in wet environments while *S. convoluta* lives in the driest parts of Northwestern Argentina.

Morbelli (l.c.) described the mega and microspores with LM and SEM. The author analyzed the megaspore wall ultrastructure and classified the species in two groups. Morbelli & Rowley (1993) studied the megaspore wall of *S. convoluta* from Argentina with TEM and described tubular bodies of silica between structural units in the inner part of the exospore. The authors referred those tubules as fragmented remnants of large active structures during megaspore development stabilized by silica at a mature stage. Morbelli (1995) described the structural units found in *Selaginella* species from Argentina and recognized two main types of structural superunits according to the spatial orientations of basic rod shaped elements. The author also cited gaps and mineral deposits in megaspore exospores. Morbelli & Rowley (1996) studied the megaspore wall ultrastructure of *S. convoluta* and *S. marginata* from Argentina with LM, TEM and confocal microscopy. Those species were taken as the two structural ex-

tremes found in material of that region. The authors interpreted the tridimensional organization of the exospore as a continuous system of interconnected conduits and gave a structural interpretation of superunits arrangement in *S. marginata* based on tomographic records.

These units are arranged in unordered and/or ordered configurations as illustrated by, for example, Kempf (1970), Tryon & Lugardon (1991) and Morbelli (1995). Morbelli & Rowley (1993) have found in one species that strands "wicks", extend from tapetal cells into and through the entire exospore to the protoplast. Morbelli & Rowley (1996) found, using confocal microscopy, that there is a region between coiled and complicated inter positioning of units which is continuous, therefore a conduit. The conduit idea was anyway evident in one species from the routing of wicks, (Morbelli & Rowley, 1993).

Since there is a considerable controversy between different authors about the interpretations of megaspore wall ultrastructure, microspores in *Selaginella* seem not to be so attractive. Although Morbelli (1977) found that microspores were more useful for systematics than megasporas in *Selaginella* species from Argentina.

The seminal work of Pettitt (1966) established the typical megaspore exospore structure for both *Selaginella* and *Isoetes* within the Lycophyta based in TEM studies and gave the clue to differentiate through the exospore structure their megasporas both actual and fossilized material. Reeve (1935) and Tryon (1949) studied the spores of *Selaginella* species that grow in North America. Tryon (l.c.) studied the megasporas of thirty-two species that grow in North America and North of Mexico with stereo microscopy. Hellwig (1969) described and drawn the spores of the heterophyllous *Selaginella* of Mexico and Central America.

Nasu & Seto (1980, 1986 a, b) studied the mega and microspores of 24 species of *Selaginella* from

Japan. Liu Bao-doug, Bao Wen-mini & Aur Chih-wen (1990) studied the general morphology of the microspores of 28 species and the megaspores of 26 species from China, analyzed the systematics of the actual groups and made a new proposal on the basis of the spores ornamentation.

Works about megaspore wall ultrastructure in *Selaginella* with electron microscopy that include species growing in certain areas or large number of species from different areas are those of Minaki (1984) who described the megaspores of 38 species and grouped them into four morphological types based on megaspore wall ultrastructure as seen with SEM. Bajpai & Maheshwari (1986) studied the megaspores of five species of *Selaginella* from India with SEM. They defined the structural units of the exospore as rod shaped and randomly arranged and showed megaspores sectioned in half with an exospore and a detached mesospore. Stadford (1991) studied the micro and megaspores, with LM and SEM, of five species of *Selaginella* that occur in Northwest Europe and described their megaspore wall structure in section.

Tryon & Lugardon (1978) studied the megaspore wall ultrastructure with SEM, TEM and mineral deposits with X-ray microanalysis in some species of *Selaginella*.

Taylor (1989) studied the ultrastructure of the megaspore wall in 19 species of *Selaginella* with TEM, divided the species in three main types according to their megaspore wall ultrastructure, and offered three-dimensional models according to his interpretations.

A synthesis of previous reports about the shape of structural units reported for a *Selaginella* megaspore wall could be found in Morbelli (1977, 1995) and Taylor (1989).

Hemsley *et al.* (1992) have considered the basic units in ordered types of megaspore exospore as spheres. Hemsley *et al.* (1994) offered a colloidal model of construction and interpreted the iridescence of the middle stratum of the megaspore exospore in some recent and fossil *Selaginella* or material with sellaginelloid affinity.

Knox (1950) studied the microspores of 350 species of *Selaginella* with LM. Aspects of microspore wall ultrastructure and differentiation during development were studied by Robert (1970, 1971 a, b, 1972) and Lugardon (1990). The microspore wall ultrastructure has been studied by Lugardon (1972, 1976, 1978, 1990) and Tryon & Lugardon (1991). Stainer (1966)

and Tryon & Lugardon (1978) analyzed comparatively the fine structure and organization of mega and microspores in some *Selaginella* species.

## MATERIAL AND METHODS

Studied material:

### *S. convoluta*

PARAGUAY. Locality not specified, Fiebrig 4099 (SI); Cerro Pelado, near Paraguari, Balansa 3293 (BAF); Cerro Perón, near Paraguari, Balansa 1116 (BAF).

### *S. marginata*

URUGUAY. Colonia, Cabrera 13.620 (LP)  
ARGENTINA. Prov. de Buenos Aires, Cabrera 2080 (LP); Cabrera 1595 (LP). Anderson 6798 (LP).

### *S. microphylla*

ARGENTINA. Prov. de Tucumán, Legname s/nro. 15/05/1964 (LIL, LP); Idem, Sota 1078 (LP). Tafi, Barkley s/n 65 April 14 (LIL, LP). Prov. de Córdoba, Gamarro 70 (LP); Castellanos 20 (BA); Prov. de San Luis, F. Pastore 88 (BAF).

### *S. muscosa*

ARGENTINA. Prov. de Misiones, Arroyo Marques, Morbelli, Amat & Giles s/n<sup>o</sup>, 1/04/1993 (LP); Prov. de Entre Rios, Burkart & Troncoso 27.601 (LP); Prov. de Buenos Aires, Bachman s/n., 27/11/1949 (LP).

### *S. novae-hollandiae*

ARGENTINA. Prov. de Salta, Pierotti 1145 (SI); Idem, de la Sota s/n<sup>o</sup> (LP).

### *S. peruviana*

ARGENTINA. Prov. de Jujuy, Cabrera *et al.* 15.505 (LP). Prov. de La Rioja, Morello 5254 (LP); Prov. de San Luis, Pastore 89 (BAF).

### *S. sellowii*

ARGENTINA. Prov. de Jujuy, Burkart & Troncoso 11.197 (LP, SI); Prov. de Córdoba, Nicora 2744 (SI); Hassel (BA 51795); Bernardello s/n<sup>o</sup> (LP).

*S. sulcata*

PARAGUAY. *Salto Guairá*, Rojas 4093 (LP).

ARGENTINA. *Prov de Misiones*, Iguazú Falls, Arení Suaiz 631 (LP); ; Idem, Morbelli & Amat s/n<sup>o</sup>, 20/03/1994 (LP); Schinini & Fernández 6119 (LP); Capurro 340; Krapovickas *et al.* 18.346; San Javier, Cabrera, Corte & Gebhard 324 (LP); Sota, Biancolini, DeFerrari & Roller 6109 (LP).

The observations were based on mature micro and megaspores collected both in the field and taken from herbarium specimens.

For light microscopy semi-thin to thick (1 to 15  $\mu$ m) sections were examined unstained or after contrasting with toluidine blue 0 (pH 4.5) or with 0.1 % basic fuchsin in Calberla's solution (EtOH, water and Glycerin). Sequences of optical sections were studied using confocal microscopy. A confocal laser (PM-voltage 707, 23.5 W) scanning microscope PHOIBOS - 1000 was used to record thick sections of *S. convoluta* and *S. marginata* stained with the fluorochroms basic fuchsin, primulin and coriophosphine. For TEM the megaspores were placed in 1% Osmium tetroxide in water (1 h, 20°C). After washing megaspores were embedded in agar to facilitate handling without damage to the megaspore surface, the agar was then cut into small cubes and dehydrated in an acetone series.

The material was embedded in Spurr's hard mixture. Fresh microspores of *S. sellowii*, *S. muscosa* and *S. novae-hollandiae* were fixed with RR in GA and Phosphate buffer (pH 7.4, 20°C, one hour).

Many of the methods we have used are well-recognized reactions with cytochemical implications. Our application of a rather great many tests and their controls was provoked by a wish to find reactions contrasting wall substructures differentially.

Thin sections were stained in the following ways:

- 1) 2% Uranyl acetate followed by 0.2% Lead citrate (UA-Pb), (Venable & Coggeshall, 1965).
- 2) 0.1% Phosphotungstic acid (PTA) in 10 % Chromic acid (PTA - C) (Rambourg *et al.*, 1969).
- 3) 1% Periodic acid (PA) to 1% Phosphotungstic acid (PTA) in 10% Chromic acid (PA - PTA - C) Roland *et al.* 1972.
- 4) Periodic acid to Thiocarbonylhydrazide (PA-TCH).
- 5) Thiocarbonylhydrazide followed by Silver proteinate (TCH-Sp).
- 6) 5% Phosphotungstic acid in 10 % Acetone

(PTA - Ac). (Marinozzi, 1968; Mayo & Cocking, 1969).

7) Phosphotungstic acid in 100 % Methanol (PTA - Me).

8) 1% Silver proteinate (Sp) in water.

The above cytochemical methods (nos. 2 to 5) were used to improve contrast.

Megaspores of *S. convoluta* were acetolized (GEA) followed by OsO<sub>4</sub> or oxidation by potassium permanganate or acetone.

Oxidation using potassium permanganate greatly increases stainability of exines and other plant and animal material (Rowley *et al.*, 1981, Soloff, 1973). Staining with uranyl acetate followed by lead citrate is greatly facilitated following this oxidation. For SEM most specimens had been dried either as herbarium specimens or working collections. The epoxy embedded specimens were freed of the plastic with Sodium methoxide (see Skvarla *et al.*, 1988) then prepared for SEM. The methods used for SEM are noted in the illustration descriptions. The electron microscopes used were a Zeiss 10A TEM and Cambridge Stereoscan 600 SEM (in the Department of Botany, Stockholm University, Sweden) and a Hitachi S800 FESEM (in EM - Unit, Department of Mineralogy, The Natural History Museum, London, England).

For X - ray microanalysis (XRMA) sections 0.1-0.2  $\mu$ m thick were cut from epoxy-embedded megaspores of *S. convoluta* and *S. marginata*. The spores of *S. convoluta* had been acetolized as described above. Following evaporation of a carbon layer onto the sections, these were analyzed using a JEOL 1200 CX electron microscope equipped with a Tracor 5200 X - ray microanalysis. Analysis conditions were as follows: 100 kV, nominal beam current, 77 $\mu$ A; magnification, x2500; spot 4; analysis urea, approximately 1 mm in diameter; tilt angle, 35°; 0.10 and 0-20 keV; 10 eV/channel.

## RESULTS

### Megaspores

#### Ornamentation

In the studied species the ornamentation can be similar or differentiated on the two polar faces. Similar sculpture in both faces is characteristic for *S. marginata* (Fig. 9 J-L).

*S. peruviana* (Fig. 25 A, B) and *S. sulcata* (Fig. 36 A, B) which are reticulated and for *S. sellowii* (Fig. 28 A - C; Fig. 29, A, B) which is rugulated. In *S. marginata* and *S. sulcata* there are differences in the reticulum between proximal and distal poles. In both cases muri are lower proximally than on the distal pole. In *S. marginata* the proximal reticulum is incomplete and the muri closest to the proximal pole have free ends (Fig. 9 K, L).

In *S. marginata* and *S. sulcata* the characteristics of the reticulum differ from one another. Muri in *S. marginata* are laminar and sinuous and the lumina contain echinulae, papillae and rugulae (Fig. 9 M, N). While in *S. sulcata* the muri are rather low, solid and slightly sinuous; they are conic in section and the lumina polygonal with a smooth or a faintly granular surface (Fig. 36 B, D).

There are cases in which the ornamentation is different in both polar surfaces. In *S. convoluta* (Fig. 3 A) normally it is proximally echinulate and capitate distally (Fig. 1 B, C; Fig. 3 D, E, H). In *S. microphylla* (Fig. 10 E - I; Fig. 11 A - D) the ornamentation is slightly reticulated proximally but markedly reticulated distally. The megaspores of *S. muscosa* (Fig. 13 A - G) are verrucated proximally and reticulated distally (Fig. 13 A-C) or completely reticulated (Fig. 13 H - M) according to the specimen, while those of *S. novae-hollandiae* (Fig. 21 A; Fig. 22 C - E) are capitate - rugulate proximally and rugulate to slightly reticulated distally.

A differentiation can be observed in the equator by alignment and or enlargement of the structural elements. Such a feature (curvature) is evident in *S. microphylla* (Fig. 11 B), *S. muscosa* (Fig. 13 I, J) and *S. peruviana* (Fig. 25 B) but is scarcely evident in *S. sellowii* (Fig. 28 B; Fig. 29 A, B). This line can be located at the equator or slightly toward the proximal pole as in *S. marginata* and in *S. sulcata* (Fig. 36 B).

The megaspore surface in all the species is perforated: *S. convoluta* (Fig. 1 C); *S. muscosa* (Fig. 13 B, C, E); *S. novae-hollandiae* (Fig. 22 A - E; Fig. 23 B - D); *S. peruviana* (Fig. 25 C, E, F); *S. sellowii* (Fig. 28 C - D; Fig. 29 C - F; Fig. 30 B); *S. sulcata* (Fig. 40 A). It is sometimes reticulated - perforated as in *S. novae-hollandiae* (Fig. 22 B - D) and the distal face surface in *S. muscosa* (Fig. 13 B, C, K, M).

#### Megaspore wall in section

Rods are the structural units in all the studied species. The units are circular in cross section. Rods of adjacent subunits interdigitate at different levels

forming a three dimensional labyrinth. Two main types of superunit arrangement have been recognized in the studied species. Superunits may be helically curved and banded without an ordered orientation.

In *S. marginata* (Fig. 4 A, B; Fig. 5 C - E; Fig. 6 A - C; Fig. 7 A, C; Fig. 8 A - D) and *S. sulcata* (Fig. 36 C, D; Fig. 37 A, B; Fig. 38 A, B; Fig. 40 A - D) the exospore has a highly oriented structure. The super units axes are oriented parallel. In this ordered distribution each axial conduit is embraced by five or six helical units that interdigitate (Fig. 6 C; Fig. 8 C, D; Fig. 37 A). The ordered part of the exospore constitutes three quarters of the total thickness. In those cases there are groups of superunits that change directions irregularly. (*S. marginata*, Fig. 6 A - C; Fig. 8 B). There are local expansions ("minigaps") in places in which the superunit orientation changes direction (Fig. 5 C - E; Fig. 6 C). The outer portion of the ordered wall shows a weakness according to parallel planes. There are transitional zones (*S. marginata*, Fig. 7, A, C), where it is possible to follow a superunit change through loss of axial orientation through to disorganization. Thus a loose pattern results having unoriented units *S. marginata* (Fig. 5 E; Fig. 7 A - C). In *S. marginata* at this level (Fig. 7 A, B, D) the spaces between units are wide and most of them seem to have been stretched because they are smaller in diameter than those of the ordered zone. The units are also flattened in some orientations.

*S. marginata* and *S. sulcata* have the largest megaspores and the thickest exospores (Table 1).

In *S. convoluta* (Fig. 3 C, D), *S. microphylla* (Fig. 12 B, C), *S. muscosa* (Fig. 14 B, E; Fig. 15 A - D), *S. novae-hollandiae* (Fig. 21 D, E; Fig. 22 A, B; Fig. 23 A; Fig. 24 A, B), *S. peruviana* (Fig. 25 E, F; Fig. 26 A) and *S. sellowii* (Fig. 30 A - D; Fig. 32 A) the megaspore exospore is formed by rods not following an ordered orientation.

*Rod diameter.* In general the diameter of the rods structural units decreases both outwards and inwards.

#### Mini-gaps (= lacunae), their presence and location

Spaces of a larger size than those between units are always present throughout the exospore and are common features in a *Selaginella* megaspore wall. We refer to those spaces as mini-gaps. These mini gaps are not specifically located since in the studied species they have been found at different levels, with different orientations and sizes.

**Table 1.** Megaspore and microspore sizes in *Selaginella* species from Argentina.

Species	Megaspores		Microspores	
	diameter	wall	diameter	wall
<i>S. convoluta</i>	166 – 500 $\mu\text{m}$	18.9 $\mu\text{m}$	25.2 - 42.3 $\mu\text{m}$	6.3 $\mu\text{m}$
<i>S. marginata</i>	450 – 720 $\mu\text{m}$	44.0 $\mu\text{m}$	21.6 - 39.6 $\mu\text{m}$	4.1 $\mu\text{m}$
<i>S. microphylla</i>	187 – 270 $\mu\text{m}$	18.0 $\mu\text{m}$	27.0 - 38.7 $\mu\text{m}$	1.8 $\mu\text{m}$ proximal 4.1 $\mu\text{m}$ distal
<i>S. muscosa</i>	220 – 350 $\mu\text{m}$	14.5 $\mu\text{m}$	22.5 - 37.8 $\mu\text{m}$	0.6 $\mu\text{m}$ proximal 5.8 $\mu\text{m}$ distal
<i>S. novae-hollandiae</i>	160 – 280 $\mu\text{m}$	10.4 $\mu\text{m}$	25.2 - 42.3 $\mu\text{m}$	1.8 $\mu\text{m}$
<i>S. peruviana</i>	170 – 400 $\mu\text{m}$	18.1 $\mu\text{m}$	29.7 - 58.5 $\mu\text{m}$	2.5 $\mu\text{m}$
<i>S. sellowii</i>	130 – 430 $\mu\text{m}$	10 - 16 $\mu\text{m}$	29.7 - 52.2 $\mu\text{m}$	3.0 $\mu\text{m}$
<i>S. sulcata</i>	230 – 720 $\mu\text{m}$	24.8 $\mu\text{m}$	17.1 - 38.7 $\mu\text{m}$	5.0 $\mu\text{m}$

In *S. convoluta* (Fig. 3 C, D) mini-gaps are mainly concentrated in the inner zone of the exospore and have their main axis tangentially oriented. In *S. marginata* (Fig. 4 A, B; Fig. 5 E; Fig. 6 A, C) and *S. sulcata* (Fig. 38 A; Fig. 39 A) mini-gaps are in the ordered zone in places where units change direction and also between two different unit arrangements like in the ordered-unordered transition (Figs. 4 A; 5 B, E; 6 A; 7 A, C; 8 A; 37 B; 38 A - B; 39 A). In *S. microphylla* the mini-gaps are distributed at random (Fig. 12 B). In *S. muscosa* (Fig. 15 B, C) the mini-gaps are mainly concentrated in the outer stratum of the exospore. In *S. peruviana* they are mainly located in the inner part of the wall (Fig. 26 A).

#### Spaces between structural units

Regardless the pattern-type in which the superunits are arranged the spaces between exospore units are continuous conduits. The tomographical observations (Morbelli & Rowley, 1996) in both ordered (*S. marginata*) and unordered patterns (*S. convoluta*, Fig. 11) showed those spaces between units are continuous throughout the wall. Spaces between units, however, are broader inward (*S. convoluta* Fig. 3 C, D; *S. marginata* Fig. 5 E, Fig. 6 A, Fig. 7 A - C; Fig. 8 A; *S. microphylla* Fig. 12 A; *S. muscosa* Fig. 15 A, B, D; *S. novae-hollandiae* Fig. 22 A, B, Fig. 24 A; *S. peruviana* Fig. 25 E, F, Fig. 26 A; *S. sellowii* Fig. 30 A, Fig. 31 C; *S. sulcata* Fig. 36 B, D, Fig. 38 A, Fig. 39 A).

#### Spheres in the major gap region

Spherical bodies were found on both surfaces of the gap remnant in six of the eight species studied. These were *S. microphylla*, *S. muscosa* (Fig. 14 B,

C, F), *S. novae-hollandiae* (Fig. 23 A; Fig. 24 B), *S. peruviana* (Fig. 25 G), *S. sellowii* (Fig. 30 C - D; Fig. 31 A-E; Fig. 32 A) and *S. sulcata*. The spheres are not restricted to the major gap surfaces but also in levels next to both surfaces (Fig. 31 E).

#### Presence of minerals

The mature *Selaginella* megaspore wall of the studied species usually had a coating of silica and other minerals. This coating is mainly located on the outer surface but it follows the irregularities of the exospore (*S. convoluta*, Fig. 3 C - E; *S. marginata* Fig. 6 A, Fig. 9 O; *S. microphylla* Fig. 12 A, C; *S. muscosa* Fig. 13 E, F; Fig. 14 D, E; *S. novae-hollandiae* Fig. 22 B, E; Fig. 24 A; *S. peruviana* Fig. 25 C, E; *S. sellowii* Fig. 29 D, E; Fig. 30 A; *S. sulcata* Fig. 36 D; Fig. 37 A; Fig. 38 B). As there are great differences in surface color (brown, light brown to yellowish and white) between megaspores of a single specimen thus the amount of silica on the exospore surface may vary between spores of a specimen and between spores of different specimens. Minerals also penetrate the exospore at levels immediately below of surface (*S. marginata* Fig. 5 C, D). Silica covers and penetrates the large processes that constitute the sculpture (*S. convoluta* Fig. 3 D-E). The presence and amount of minerals decrease toward the inner part of the exospore.

There is a thick layer of silica on the surface in *S. marginata* (Fig. 6 A, Fig. 9 O). It is also present within the non ordered pattern that constitutes the ornamentation (Fig. 4 A, B). In megaspores of *S. microphylla* (Fig. 12 A, C). Silica fills the spaces between units within the muri.

In thick sections of *S. muscosa* and *S. novae-hollandiae* it is possible to appreciate the difference in structure and contrast between the sporopolleninous exospore and the silica on its surface. In Fig. 19 B the projections have a stem of sporopollenin covered by a tridimensional reticule of silica. Both, structure and composition of this outer stratum are different from the exospore.

Rodlets of silica of 30 to 40 nm in diameter were found between structural elements of the wall in the inner exospore of *S. convoluta* (Morbelli & Rowley, 1993). They were resistant to the acetolysis procedure of Erdtman (1960). They have a central core about 7 nm in diameter. There are striations on the outer surface and the core of these rodlets. The areas with rodlets give very high x-ray microanalysis signals for silicon and lower signals for Al, S, K and Ca.

We found strong differences in surface (= ornamentation) in megaspores with silica and without it. In *S. convoluta* (Fig. 2 G, I), *S. muscosa* (Fig. 13 B, C, F, G), *S. novae-hollandiae* (Fig. 22 B-E; Fig. 23 B-D), *S. peruviana* (Fig. 25 C, E) and *S. sellowii* (Fig. 29 C-E), both surfaces, that of the exospore and that of the silica coating differ greatly but both are perforated.

#### Surface coating on micro and megaspores

There are rodlets having the same size and shape in the siliceous coatings we show here for megaspores of *S. microphylla* (Fig. 12 A) and microspores of *S. muscosa* (Fig. 17 A-D; Fig. 18 A), *S. novae-hollandiae* (Fig. 20 A-E); *S. sellowii* (Fig. 35 A, B) and *S. sulcata* (Fig. 42 A, B). We showed the similarity of size and morphology between the siliceous rods of *S. convoluta* megaspores and wicks of *S. argentea* (Morbelli & Rowley, 1993: Figs. 6 and 9).

#### The innermost part of the exospore

A detached narrow part of the exospore is always present in mature megaspores at the inner part in equatorial and distal regions. In the studied species the general feature of this part of the exospore is a thin, compact, more or less continuous innermost stratum and rods in an unordered arrangement, with irregular margins. *S. convoluta* (Fig. 2 F, G, I; Fig. 3 A, B, F, H), *S. marginata* (Fig. 5 A), *S. muscosa* (Fig. 14 A, B; Fig. 19 A); *S. novae-hollandiae* (Fig. 21, C-E; Fig. 22 A; Fig. 24 B); *S. peruviana* (Fig. 25 D), *S. sellowii* (Fig. 30 C-D; Fig. 32 A), *S. sulcata* (Fig. 36 A).

#### Endospore

We had details of the endospore in *S. convoluta* (Fig. 3 B), *S. marginata* (Fig. 6A), *S. sellowii* (Fig. 30 C, D; Fig. 32 A) and *S. sulcata* (Fig. 36 D). In (Fig. 30 C, D) two zones are distinguishable; the boundary between them is darkly contrasted. The nature of this layer is fibrillar. Several differentially contrasted strata can be distinguished.

#### Microspores

In seven of the Argentinean species the microspore tetrad separates into monads but in *S. convoluta* the tetrad condition is retained (Fig. 1 A; Fig. 2 A).

The equatorial diameter of pleated microspores (from *S. peruviana* and *S. sellowii*) ranges from 29.7 to 58.2  $\mu\text{m}$  while in the others it ranges from 17.0 to 42.3  $\mu\text{m}$ .

#### Ornamentation

Microspores of some species have different ornamentation in both polar faces as in *S. convoluta* (Fig. 1 A; Fig. 2 A-E), *S. microphylla* (Fig. 10 A-D), *S. muscosa* (Fig. 16 A-F; Fig. 17 B; Fig. 19 C) and *S. novae-hollandiae* (Fig. 20 B; Fig. 43 A). In these species the exospore thickness is different at either pole (Table 1).

The distal exospore in *S. convoluta* consists of alveoli (Fig. 1 A; Fig. 2 A-E). Some of them show a thinner part on the distal side, which may become a perforation (Fig. 1 A; Fig. 2 C, D). Several globules of different sizes with the same exospore contrast are attached to these alveoli. There are also globules, in a few cases, on the internal surface of the alveoli. Alveoli of large size have several internal partitions. The inner exospore is perforated (Fig. 2 C). The exospore at the proximal pole is thin and ornamentation is granular (Fig. 1 A; Fig. 2 E). There are bridges of the distal alveolated exospore between adjacent microspores in a tetrad (Fig. 1 A; Fig. 2 A). The bridge is a more or less continuous alveolus with internal partitions (Fig. 2 B).

In *S. microphylla* (Fig. 10 A-D) the microspores have gemmae, truncated spines, cones and capitated processes distally. The proximal surface is verrucate in the central region. In *S. muscosa* (Fig. 16 A-F; Fig. 17 B, D; Fig. 18 A) the spores are verrucated proximally and distally they have truncated cones (Fig. 16 B), capitated processes (Fig. 16 E) and/or long spines

(Fig. 17 B, Fig. 18 A) on a field of verruca. Each distal process is formed by several rods fused distally but splayed toward the base and connecting with the rest of the exospore at separate locations. The distal surface between the cones is pitted.

The ornamentation is similar on both faces in *S. marginata* (Fig. 9 A - I) and *S. sulcata*. In *S. marginata* (Fig. 9 A - I) processes of variable shape form the ornamentation; e.g., some processes show an acuminate tip (Fig. 9 A, B, D, E) while others have, both verrucae and capitate processes (Fig. 9 C, F, G, H, I). As seen at the surface these processes are arranged in a dense pattern with a granular background. The exospore surface has small perforations and is divided by deep grooves (Fig. 9 B, E). In *S. sulcata* the ornamentation is composed of sparse processes having varied size and shape e.g. cones and spines (Fig. 41 A, B; Fig. 42 A, B; Fig. 43 B).

Other species have pleated spores like *S. peruviana* (Fig. 27 A - F) and *S. sellowii* (Fig. 33 A - F) with a granular surface. In those cases the pleats are radially oriented in each proximal facet. It has been found in this study that the "pleated condition" is typical for dry material since material fixed when living shows an expanded proximal face (Fig. 32 C).

#### *Microspore wall in section*

The microspore wall structure was studied with TEM in four of the eight species. They were: *S. muscosa* (Fig. 17 A - D; Fig. 18 A); *S. novae-hollandiae* (Fig. 20 A - E); *S. sellowii* (Fig. 34 A - F; Fig. 35 A - C) and *S. sulcata* (Fig. 41 A, B; Fig. 42 A - B). The exospore in these is composed by one to several strata of different structure according to the species. In some cases the exospore is cavated (laminated) adjacent to the aperture as in *S. novae-hollandiae* (Fig. 20, C). Conduits traversing the wall are present in proximal and distal parts in *S. sulcata* (Fig. 41 A, B). Spores of these species have a thin perispore. In some cases there is a striking similarity in shape and size with some elements produced by the tapetum. There are one to three strata of different electron density in exospores of *S. muscosa*, *S. novae-hollandiae* (Fig. 20 B) and *S. sulcata* (Fig. 41 A, B) microspores.

In *S. muscosa* the microspore exospore is composed of two strata. The inner part of the exospore has a light contrast (Os, Pb and UA) and is the thickest part of the wall. At the proximal region at both sides of the laesura it has large spaces (cavea) and

inward it has lamellations. It has numerous small round cavea scattered throughout the exospore. The cavea are small and more numerous in the long spines that constitute the distal ornamentation. The outer stratum is irregular in thickness, only a few nm thick and the outer surface of this stratum shows striations on the long spines (Fig. 17 D, Fig. 18 A) and has tubular elements.

In *S. novae-hollandiae* the exospore in section has a massive structure. The exospore is thicker distally. The inner part of the exospore is the thickest and is darkly contrasted. There is a continuous stratum on the outer surface (Fig. 20 C - E). This stratum is low in contrast and composed by tubular elements. The tubular elements can be seen in traverse and longitudinal orientations at the exospore surface. When seen longitudinally they show a white line in the center.

In *S. sellowii* the exospore is fibrous and compact. The matrix has elements that are differently contrasted (Fig. 32 B, C; Fig. 34 E, F). There is a separation (= cavea) at equatorial and distal regions between the inner layer of the exospore and the complex two layered outer part of it (Fig. 34 A, B). The outer part of the exospore is the thickest part of the wall (Fig. 34 A, B, C, E, F). Two strata can be recognized structurally in it. The outer stratum (Fig. 35 A, B) has pits and perforations. The inner stratum is the thickest and it is formed by several levels of rods that are circular in section and banded, twisted or interlaced. Both strata are connected by columns of different diameter. The outer part of the exospore is interrupted at the laesura. The inner part of the exospore is continuous and it is next to the outer part only at the proximal pole. The inner part of the exospore protrudes at the laesura (Fig. 34 E).

On the exospore surface there is a continuous stratum (Fig. 34 D - F; Fig. 35 A, B), that is irregular in thickness, constituted by rodlets, with a lighter contrast. This stratum is at the edge of exospore units throughout the wall.

In *S. sulcata* the exospore is one layered in section. The dark outer part of it has projections of different shape (spines, bacula, cones, verruca) and sizes (Fig. 41 A, B). Radial conduits at both sides of each sculptural process are present over the whole surface. Few of them traverse the wall completely but most of them traverse only the outer part of the exospore. There is a complex surface coating that is only a few nm thick.

## DISCUSSION AND CONCLUSIONS

It has been found using several methods that rods were the structural units of megaspores in the eight species studied from Argentina. The same type of units were previously cited by Morbelli (1992, 1995) and Morbelli & Rowley (1996) for some of the species from the same area.

In the studied species we observed that spaces (conduits) within the exospore are continuous. The same feature was also referred to by Morbelli & Rowley (1996) based in observations with confocal microscopy for *S. convoluta* and *S. marginata* from the same region.

According to Morbelli (1992, 1995) helical units are present in outer parts of the exospore of *S. marginata* and *S. sulcata*. This pattern has been described as spongy (Martens, 1960 a, b), disoriented (Stainier, 1965), labyrinthine (Tryon & Lugardon, 1978, 1991), unordered (Taylor, W., 1989, 1991) and slack (Morbelli 1995).

What has been described here as the outer part of the exospore in the case of cavate microspores (*S. peruviana* and *S. sellowii*) has been named by Lugardon (1972) for microspores of *S. selaginoides* as paraexospore. Lugardon (1990) cited separations between microspore wall layers in some species of *Selaginella* and compared these spaces with the characteristic cavations of pollen grains of some Gymnosperms.

We found that the megaspores of *S. convoluta*, *S. muscosa*, *S. novae-hollandiae*, *S. peruviana* and *S. sellowii* have a silicious "perispore" similar to what Robert *et al.* (1973) reported for *Isoetes setacea*. The report of Robert *et al.* was based upon results using X-ray microanalysis.

Rodlets similar to those present on the surface of *S. microphylla* megaspores and throughout the exospore in megaspores of *S. convoluta* and on the surface of *S. muscosa*, *S. novae-hollandie* and *S. sellowii* microspores were cited by Morbelli & Rowley (1993), Morbelli (1993, 1995). Rods on megaspores and microspores are comparable in size with those previously cited by Morbelli & Rowley (1993).

We propose that the template for the siliceous accumulations are wicks which we consider to be plasmodesmata equivalents between tapetal cells and megaspores and microspores of *Selaginella*.

We presume that conduits and wick equivalents are common to all *Selaginella* megaspores because of the nutritional requirements of a living system.

In the eight species of *Selaginella* that grow in Argentina we find the following characters.

In megaspores there is in general a distinction in ornamentation between the two poles.

In those cases in which the ornamentation of the megaspores is reticulated over the whole surface the characteristics of the reticulations vary. The muri are lower proximally than distally as in *S. marginata* and *S. sulcata*, and some muri on the proximal surface have free ends. The reticulation varies between spores within the same specimen and between spores of different specimens.

The species that have megaspores with similar ornamentation on the entire surface (like *S. marginata* and *S. sulcata*) also have microspores with the same ornamentation on the entire surface although with fine distinctions. The megaspores of the above species have an ordered exospore pattern.

Megaspores of these species have a zone with unique features that is scarcely evident on or near the equator (=curvature).

In our experience megaspore exospores fixed when living show striking differences in exospore stratification compared with those fixed from dry specimens (herbarium material).

Megaspore surface with a covering of silica looks different from an exospore of the same species without this mineral.

The analysis with SEM allowed us to see that under the thick stratum of beautifully ornamented silica, there is a multiperforated exospore surface.

According to the results of the study with TEM the structure of silica deposition is unlike the structure of the sporopolleninuous organic exospore.

Regardless of the kind of super unit organisation in the Argentinean selaginellas megaspores walls the structural units are rods.

According to our interpretation the mini-gaps are transitory local expansions. Thus the size, shape and location of these mini-gaps are not constant in the megaspore wall of the species under study. Thus, the records we have of gap location are dependent upon the specific stage in which the megaspores were fixed (including drying for herbarium specimens) for study.

While all megaspore walls have the inner part of the exospore split in two parts apparently the pleated microspores (*S. peruviana* and *S. sellowii*) also have that feature. In these cases the outer portion of the exospore is the thickest.

It has been found in this study that the "pleated

condition" in microspores is typical only for dry-material since material fixed when living show an expanded proximal face.

Microspores with different ornamentation in either face have a thin proximal exospore and a thicker distal exospore.

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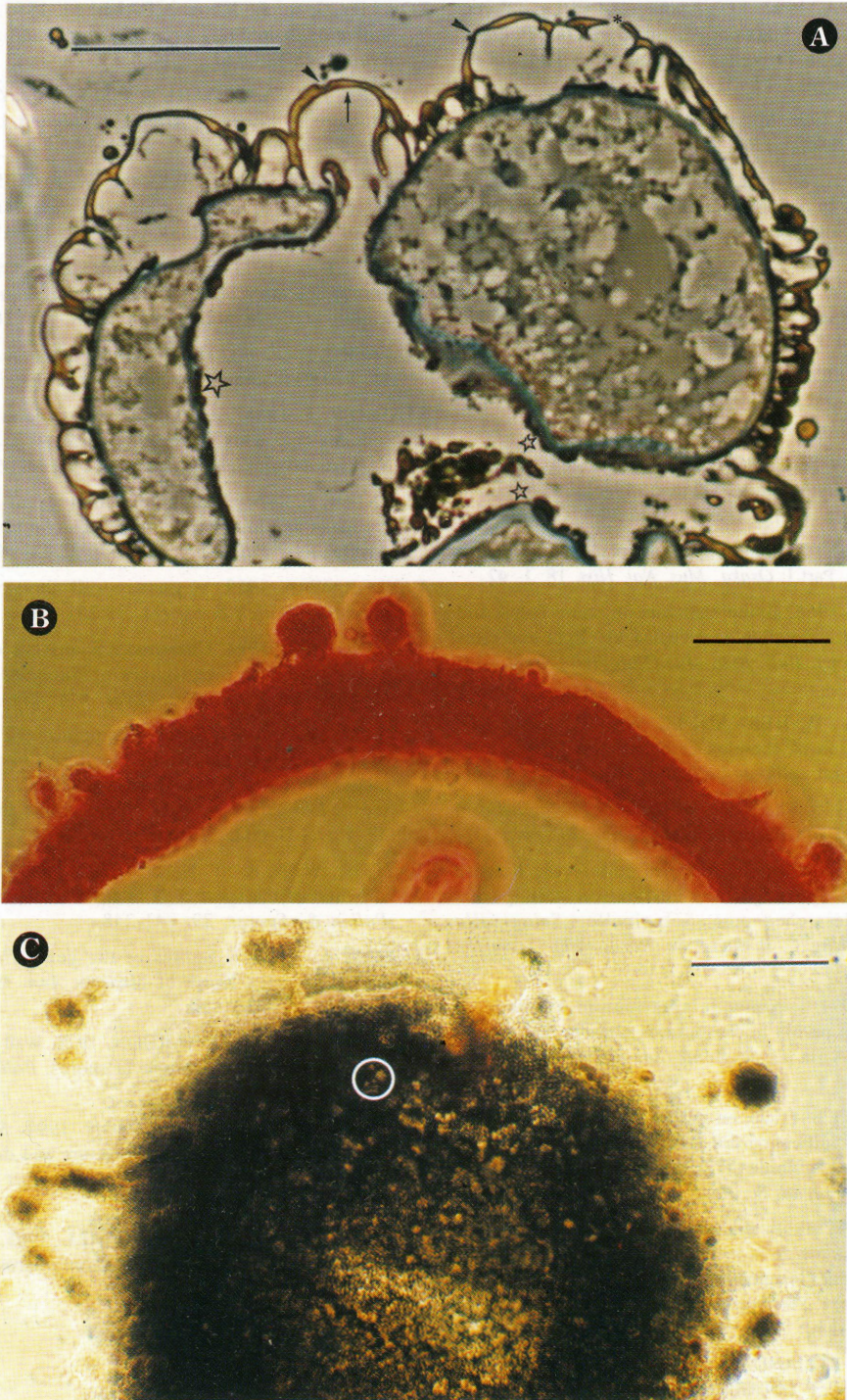
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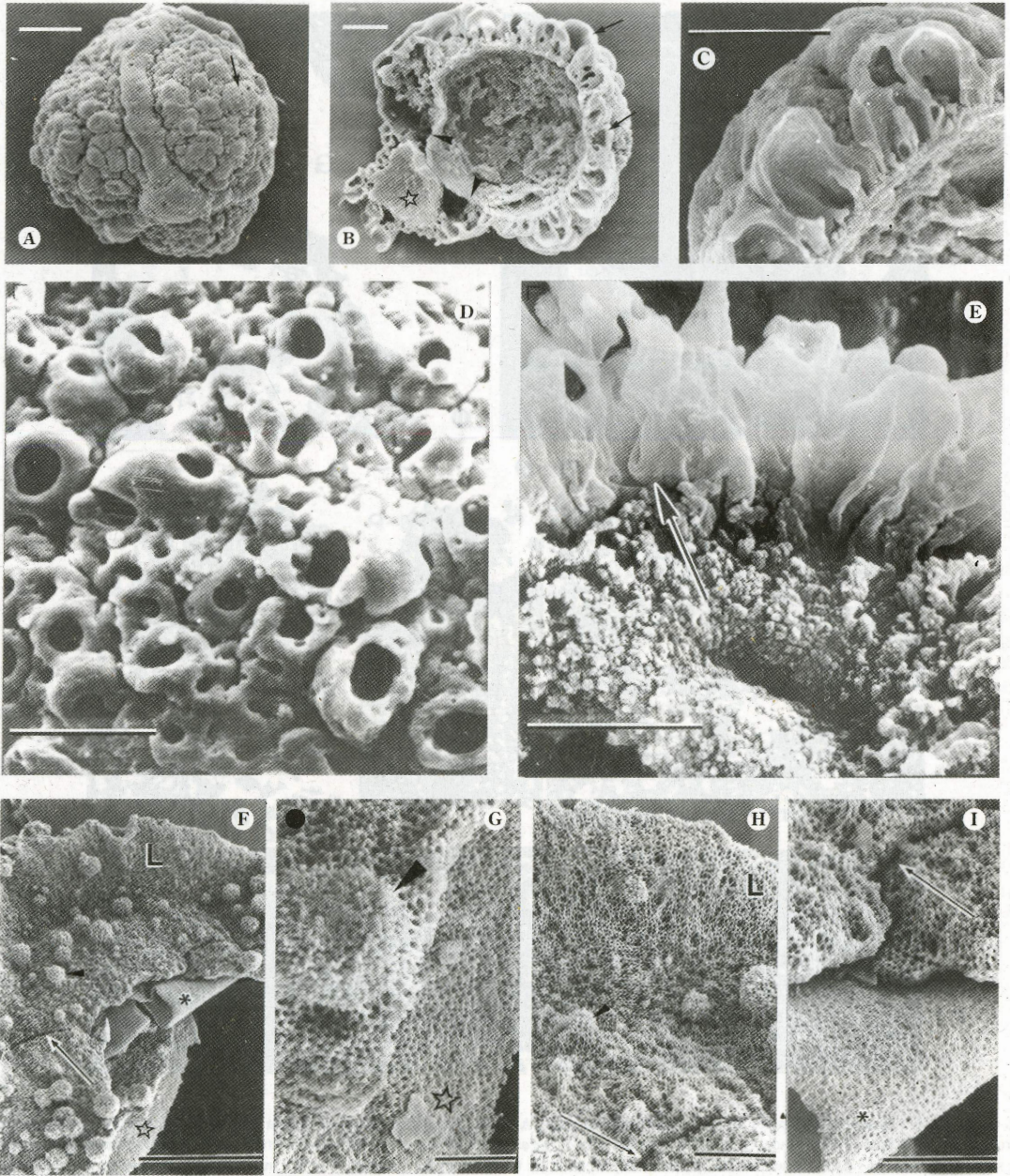
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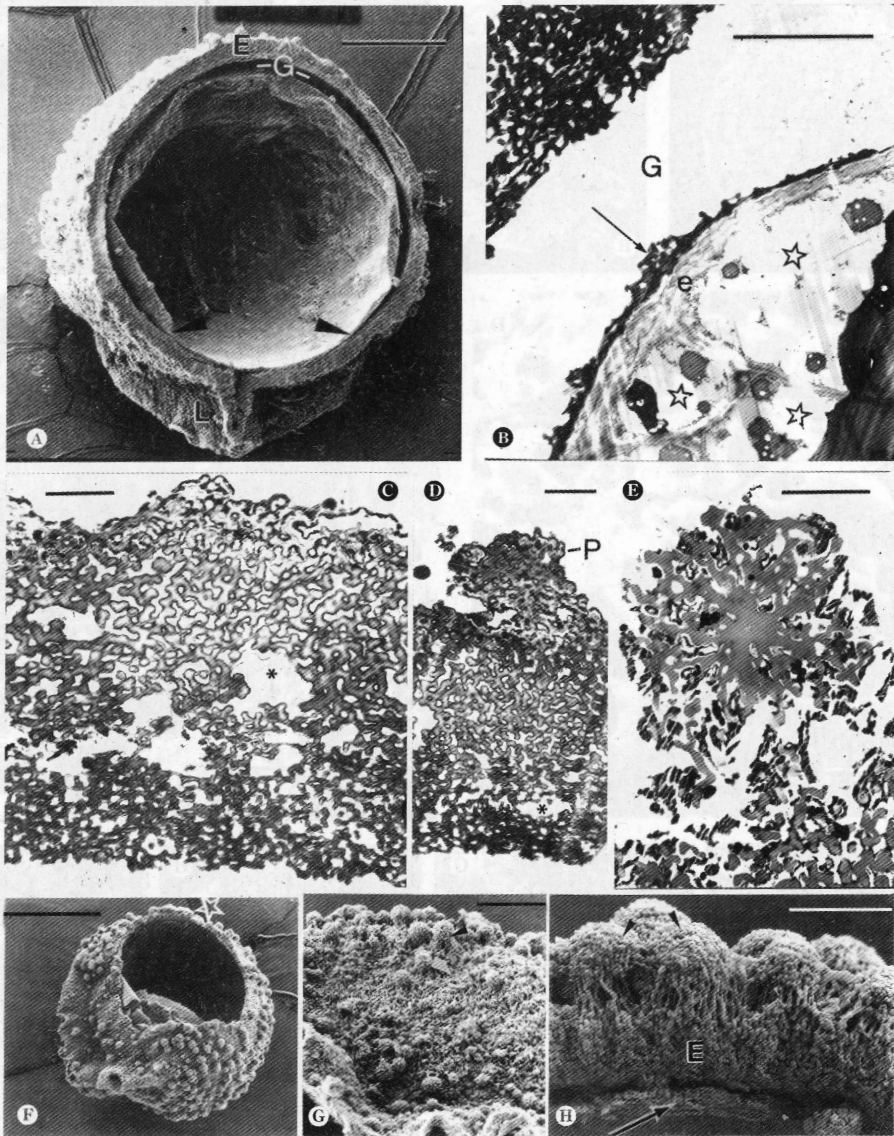
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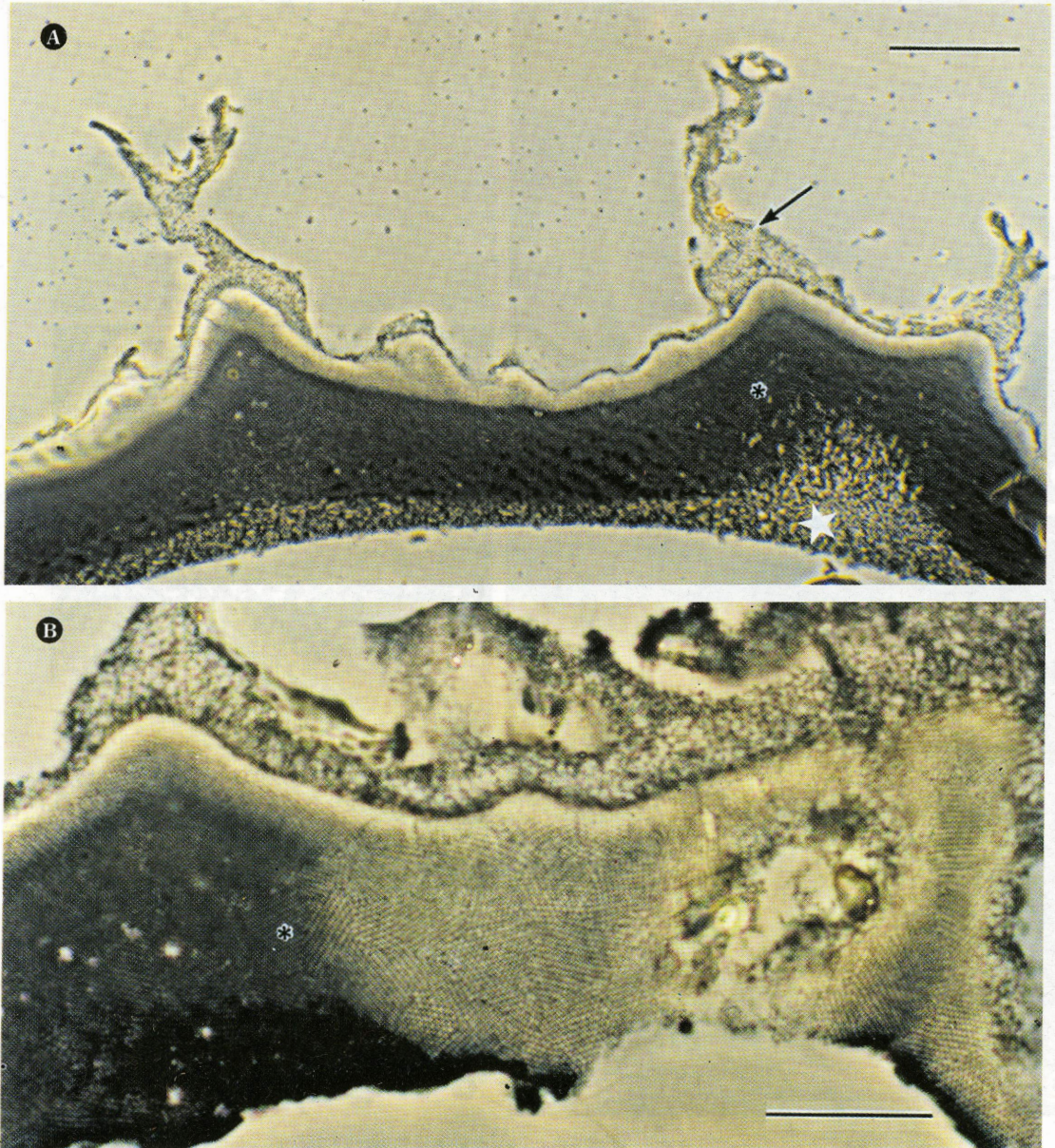
**Fig. 1.** A: LM photomicrograph of a *S. convoluta* microspore tetrad showing distal connections between microspores through an exospore bridge (arrow). The proximal exospore (stars) is thin and without alveoli. Some alveoli have thin walls distally (arrowheads) and some have a perforations (asterisk). Same material as in Figs. 2 B & C. Stain: Toluidine blue. Bar: 50  $\mu$ m. B: and C: LM photomicrographs of sections of *S. convoluta* megaspores showing several of the pila that are characteristic of this species. Fig. 1 B shows pila in transversal section while Fig. 1 C, shows the surface and its pila in tangential section. The small bright spots are part of the conduits (microchannels) that are continuous throughout the exospore. The larger, bright sites (circled) are mini gaps in the exospore. Stain: B, Safranin - O; C, Sudan black - B in 70 % alcohol. Bars: 25  $\mu$ m.



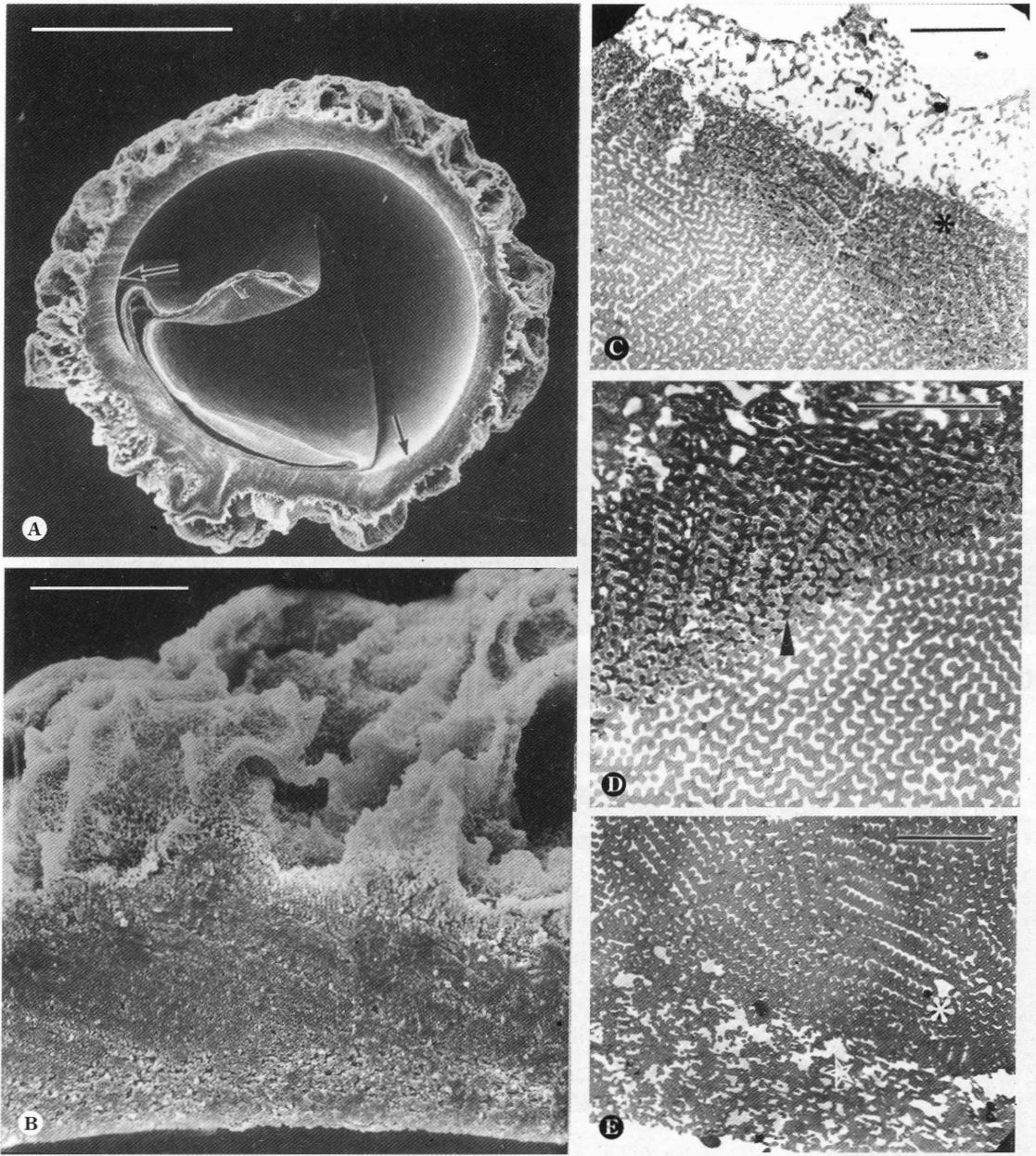
**Fig. 2.** A - E: SEM micrographs of *S. convoluta* microspores. **A:** Microspore tetrad showing distal exospore alveolate (arrow) and bridges between microspores. Bar: 20  $\mu$ m. **B and C:** A sectioned isolated microspore. The proximal part is marked by arrowheads. There is a small part of the adjacent spore (star) at the lower left. The distal and equatorial parts of the exospore consist of alveoli (arrows) of different sizes and a thin basal layer that forms the proximal exospore (between arrowheads). Walls of adjacent alveoli are partly fused. Note the difference between distal and proximal exospore. Bars: 20  $\mu$ m. **D:** Distal surface of a microspore showing that some alveoli have large perforations at their distal edge. Bar: 5  $\mu$ m. **E:** The figure shows the granulate proximal surface of a microspore. Alveoli arise at the equator (arrow). Bar: 5  $\mu$ m. **F - I:** SEM micrographs of *S. convoluta* megaspores. **F:** The proximal surface close to the laesura (L). There are pila (arrowheads), as in **G, H** but scattered in each proximal facet. The exospore is broken and the inner part of the exospore is exposed (asterisk). Bar: 100  $\mu$ m. **G:** The figure shows a spot of the same megaspore in **F**. The surface of the outer part of the exospore is on the left and the surface of the inner part of the exospore (star) is on the right. The perforated nature of the surface is evident. Bar: 10  $\mu$ m. **H:** The figure shows a detail of the exospore surface next to the laesura (L). The whole surface is perforated. Bar: 25  $\mu$ m. **I:** This is a detail of the same spore in **F** that shows the difference between the surfaces of the outer (arrow) and inner (asterisk) parts of the exospore. Bar: 20  $\mu$ m.



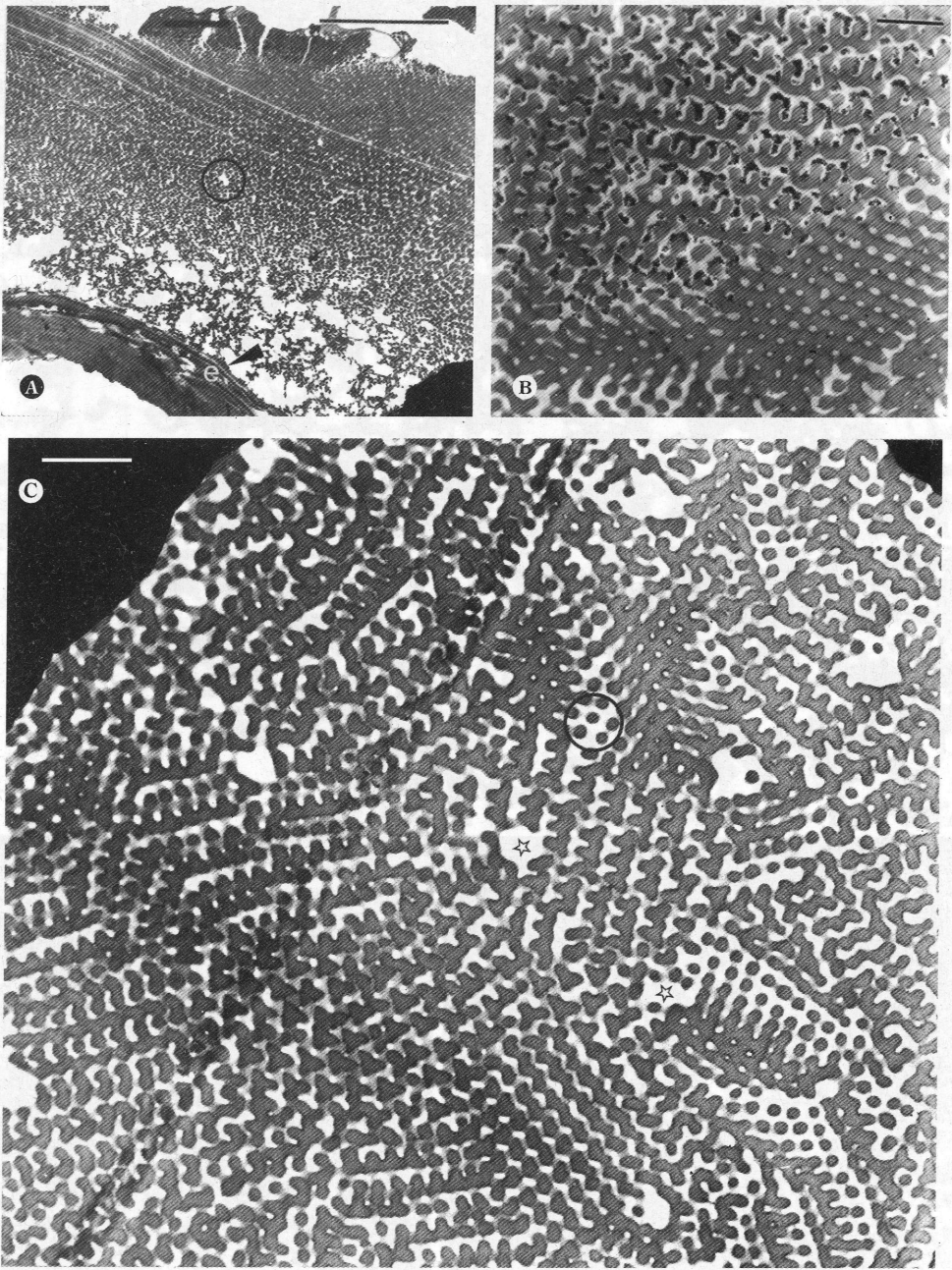
**Fig. 3. A - H.** *S. convoluta* megaspores sections at germination stage. **A:** SEM micrograph of a *S. convoluta* megaspore sectioned at a mature stage. A narrow stripe of exospore (arrowheads) is detached from the bulk of the exospore (E) except at the region (between arrowheads) of the proximal pole. At this stage there is only a narrow separation (G, gap remnant) between the two parts of the exospore. The "L" marks the great elevation of the laesura. Bar: 100  $\mu$ m. **B:** TEM section of a nearly mature megaspore near the distal pole. The protoplast has already undergone mitotic division and portions of several cells are visible (stars). A multi stratified exospore (e) has also been formed. There is a gap remnant (G) between two parts of the exospore. A narrow inner part of the exospore (arrow) is adjacent to the endospore (e). Stain: PTA - C; Bars: 10  $\mu$ m. **C:** TEM section of the exospore outer part at the distal pole. A thin black layer of silica is on the outer surface and at levels immediately below at the edge of exospore structural units. Mini gaps (asterisk) of large size are present in the middle part of the exospore. Bar: 5  $\mu$ m. **D:** TEM proximal section of an unordered exospore showing rodlets arranged in an open mesh. The presence of mini gaps (asterisk) in the middle and inner parts of the exospore are characteristic of this species. Silica darkens the outer surface and spaces between exospore rodlets in the outer levels of the exospore. "P" marks a pilum. Stain: TCH-Sp. Bar: 5  $\mu$ m. **E:** TEM section through a pilum. Silica (dark) is on the surface and between units. The base of the pilum has few structural units. Bar: 5  $\mu$ m. **F - H:** SEM micrographs of *S. convoluta* megaspores. Portions of this fractured megaspore are detailed in Fig. 2 F - I. **F:** The figure shows a megaspore seen from the proximal pole. The high laesura is on the left. It can be noticed here that the number and dimensions of the pila that constitute the ornamentation increase from the equator to the distal side. The inner part of the exospore is exposed at the fracture. Bar: 200  $\mu$ m. **G:** The figure shows one of the proximal facets in detail. The laesura is on the bottom and on the left corner. Some pila (arrowhead) are elevated on long, more or less straight units. There are few scattered low pila proximally; they increase in number and height towards the equator. Bar: 50  $\mu$ m. **H:** The micrograph is from the upper part of fracture in Fig. F (near the star): The figure shows a section of the exospore at the distal pole. In this mature megaspore the inner part of the exospore (arrow) is near the outer part of it. There is only a narrow gap remnant between the two parts of the exospore. The outer exospore (E) with many (nested) pila (arrowheads) at the surface. Bar: 20  $\mu$ m.



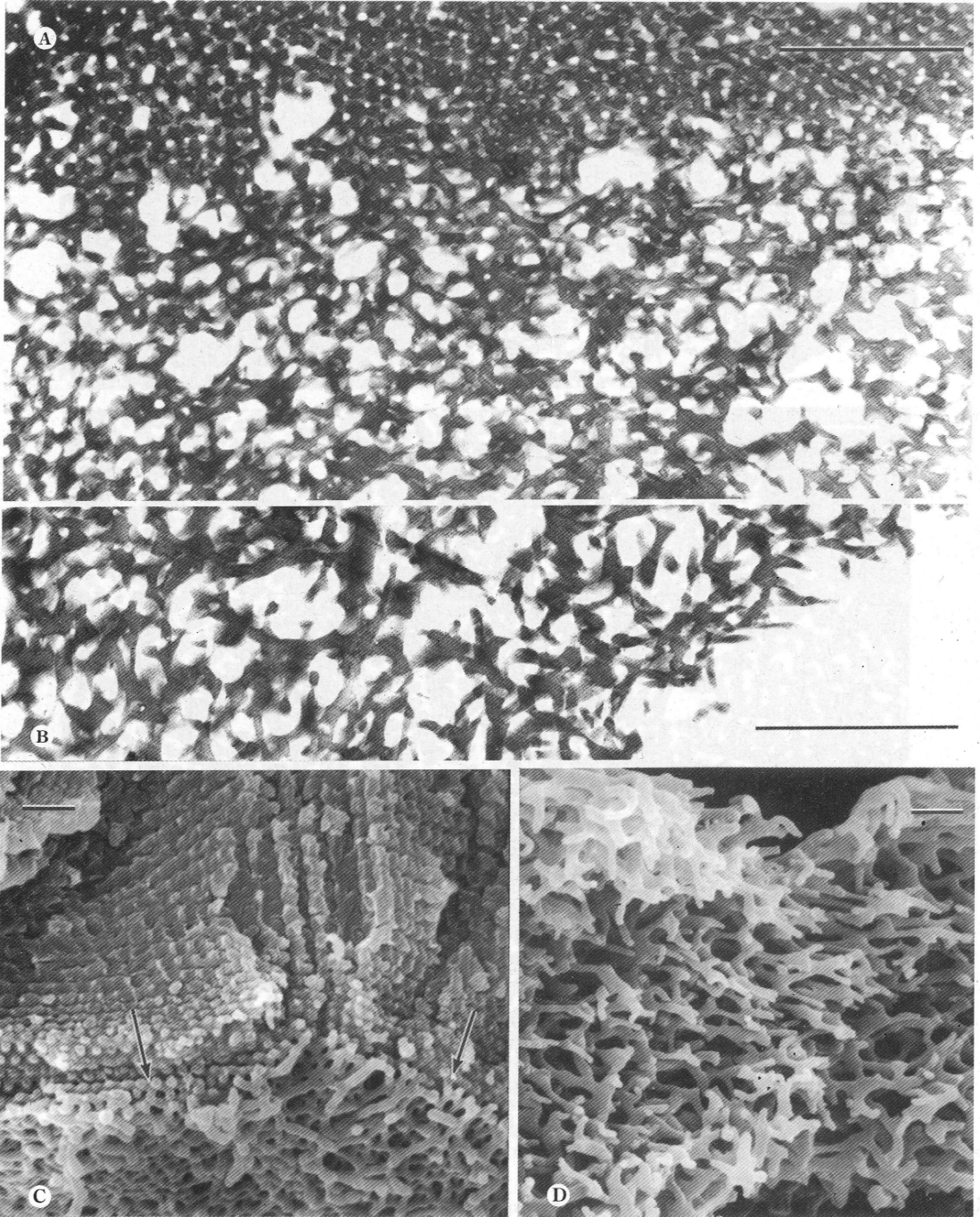
**Fig. 4.** A and B. LM photomicrographs of thick sections of a *S. marginata* megaspore near the distal pole. Two main structural parts of the exospore can be distinguished in Fig. A, an inner part that is unordered (star) and a middle region of considerable thickness that is ordered (asterisk). The inner part in Fig. A: shows the very unordered pattern of this portion of the exospore. The ordered part of the exospore (asterisk) in Fig. B: shows a moiré pattern at this magnification. The flamboyant ornaments (arrows) at the surface of Fig. A and the surface material in Fig. B are of silica on a scaffolding of exospore units. The low contrast region near the exospore surface in these figures is due to infiltration of silica (see Fig. 5 C). The inner region of the exospore in Fig. B was lost in sectioning. Stain: Toluidine blue 0 at pH 4.5. Bar: 20  $\mu\text{m}$ .



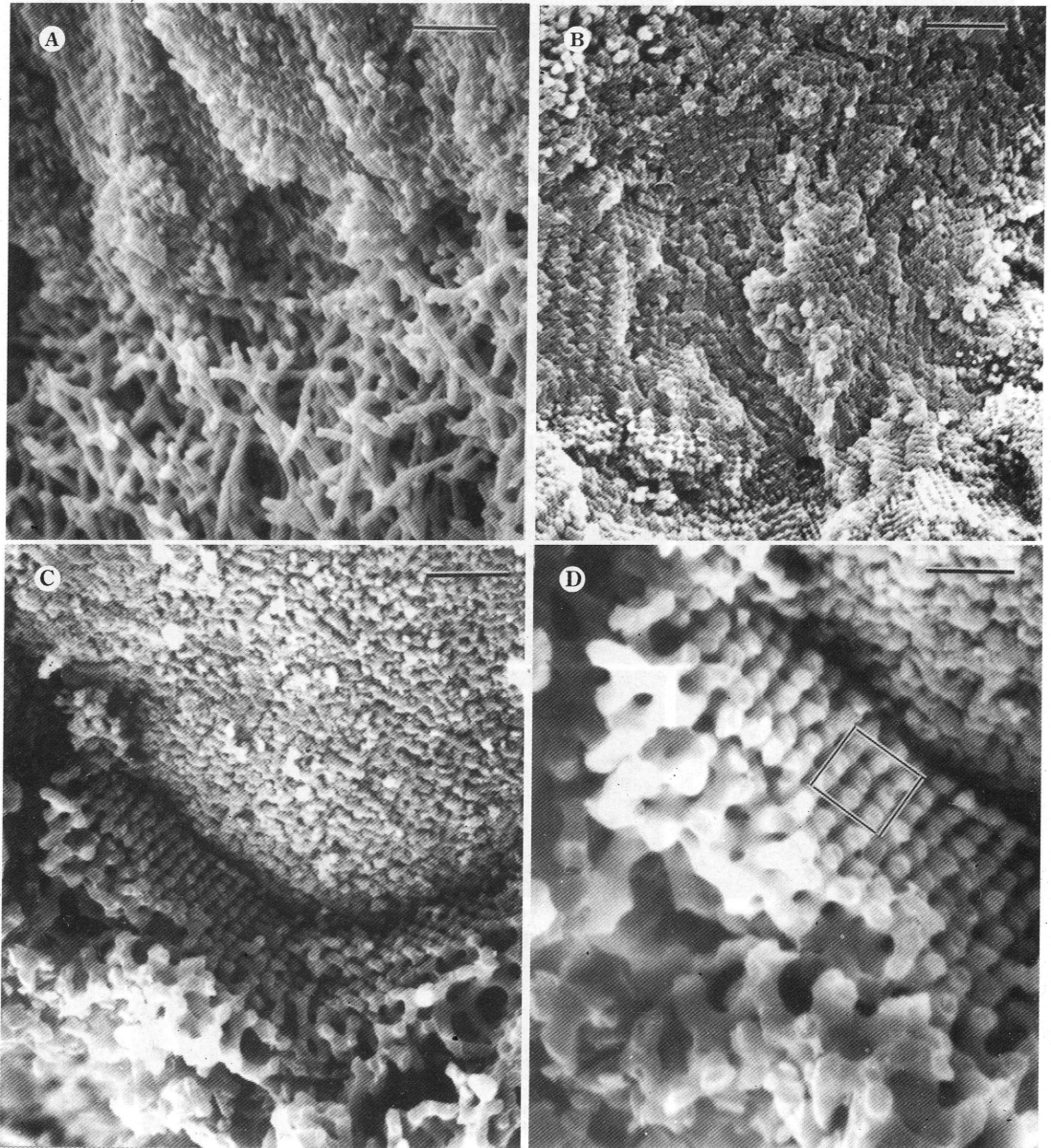
**Fig. 5.** A and B. SEM micrographs of *S. marginata* megaspores in cross section. A: megaspore sectioned in half. The proximal pole is at the bottom. The inner exospore which formed the inner surface of the major gap is folded at the inner part of the laesura region. The arrows show the outer surface of the major gap. Bar: 200  $\mu$ m. B: SEM taken of the same section as Fig. 5 A at a higher magnification. It shows the exospore with silica coating in surface and the ordered arrangement of units in the section. Bar: 20  $\mu$ m. C - D: TEM micrographs of *S. marginata* megaspore exospore. C: The high tower of silica corresponds to the flamboyant ornaments in Fig. 4 A. Silica (dark) coats the exospore units in the surface zone; these units are much less ordered than in the middle portion of the wall, as in Figs. D, E. No section stain. Bar: 5  $\mu$ m. D: The figure shows the outer portion of the exospore. The ordered part of the exospore is at the bottom of the figure. Above the ordered units (arrowhead) the organization of exospore units is unordered and in this mature stage there is silica (dark) between exospore units. Stain: PTA. Bar: 5  $\mu$ m. E: It is a section through the inner part of the exospore where there is a change from ordered (asterisk) to the unordered structural organization (star). The spaces between the unordered units are wider and less regular than in the ordered part. Bar: 5  $\mu$ m.



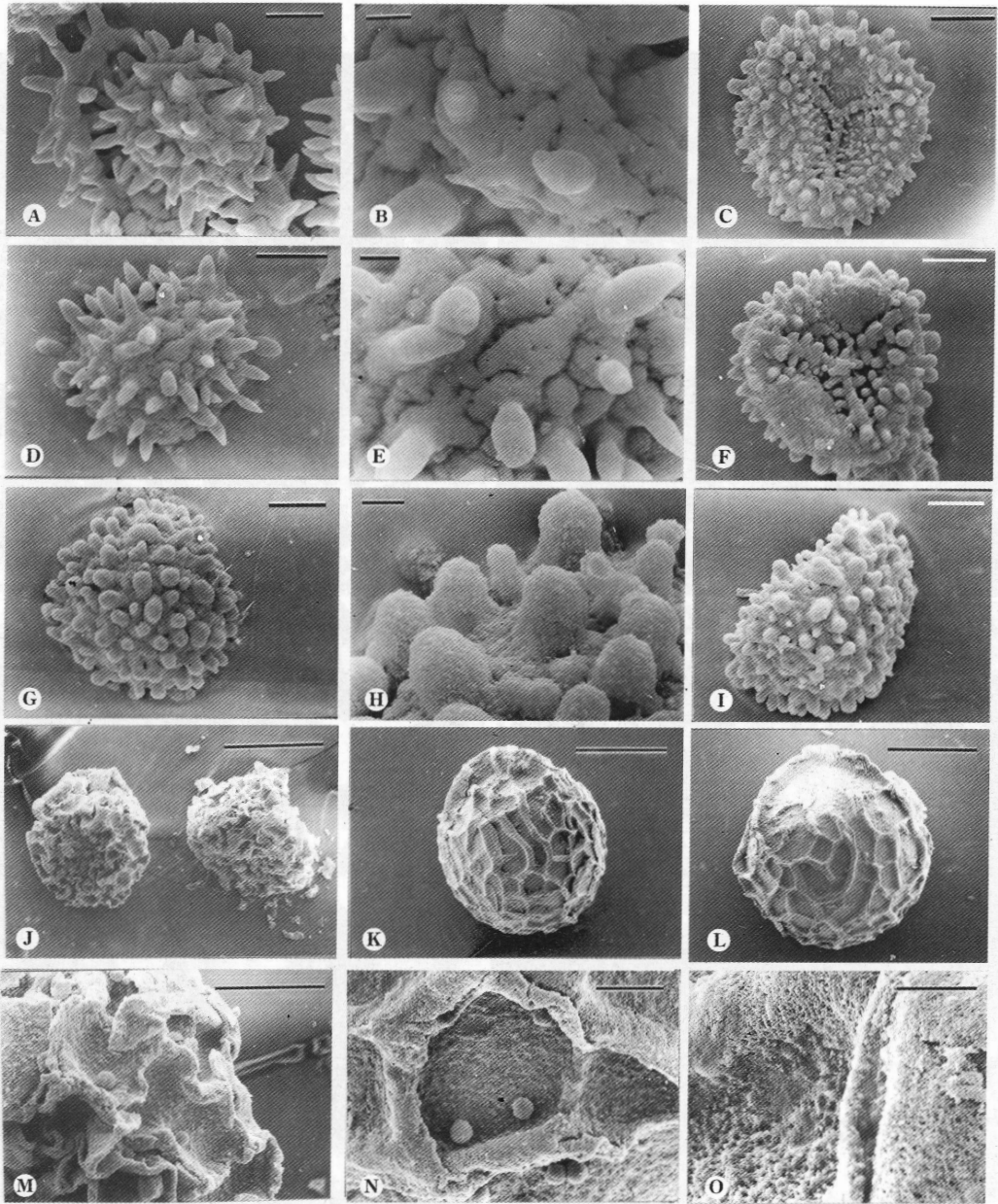
**Fig. 6.** A - C: TEM micrographs of *S. marginata* megaspore exospore. **A:** Transverse section of the exospore near the proximal pole from outside to inside there is outer surface with a thick coating of silica. Then ordered pattern that forms the main part of the exospore. There are some small mini-gaps (one is circled) in this ordered system. The inner unorderd stratum has large gaps. There is a thin layer of exospore (arrowhead) adjacent to the endospore (e). Stain: TCH-Sp. Bar: 5  $\mu$ m. **B:** The exospore units are ordered across the lower portion of the figure and less ordered above. The darkly contrasted material between units of the exospore in this unstained section is silica. Stain: none. Bar: 2  $\mu$ m. **C:** A section through the middle, ordered, portion of the exospore. The units are circular as can be seen in cross sections (six are circled). The units are helicoidally in arrangement. The units are in groups following the same orientation in planes and change directions irregularly resulting in the typical patched structure of the ordered pattern. Spaces (mini-gaps, stars) are present in sites where the groups of units change direction. The density of the silver granules of the PA-TCH-Sp reaction allows one to appreciate a 3-D aspect of the section. Bar: 2  $\mu$ m.



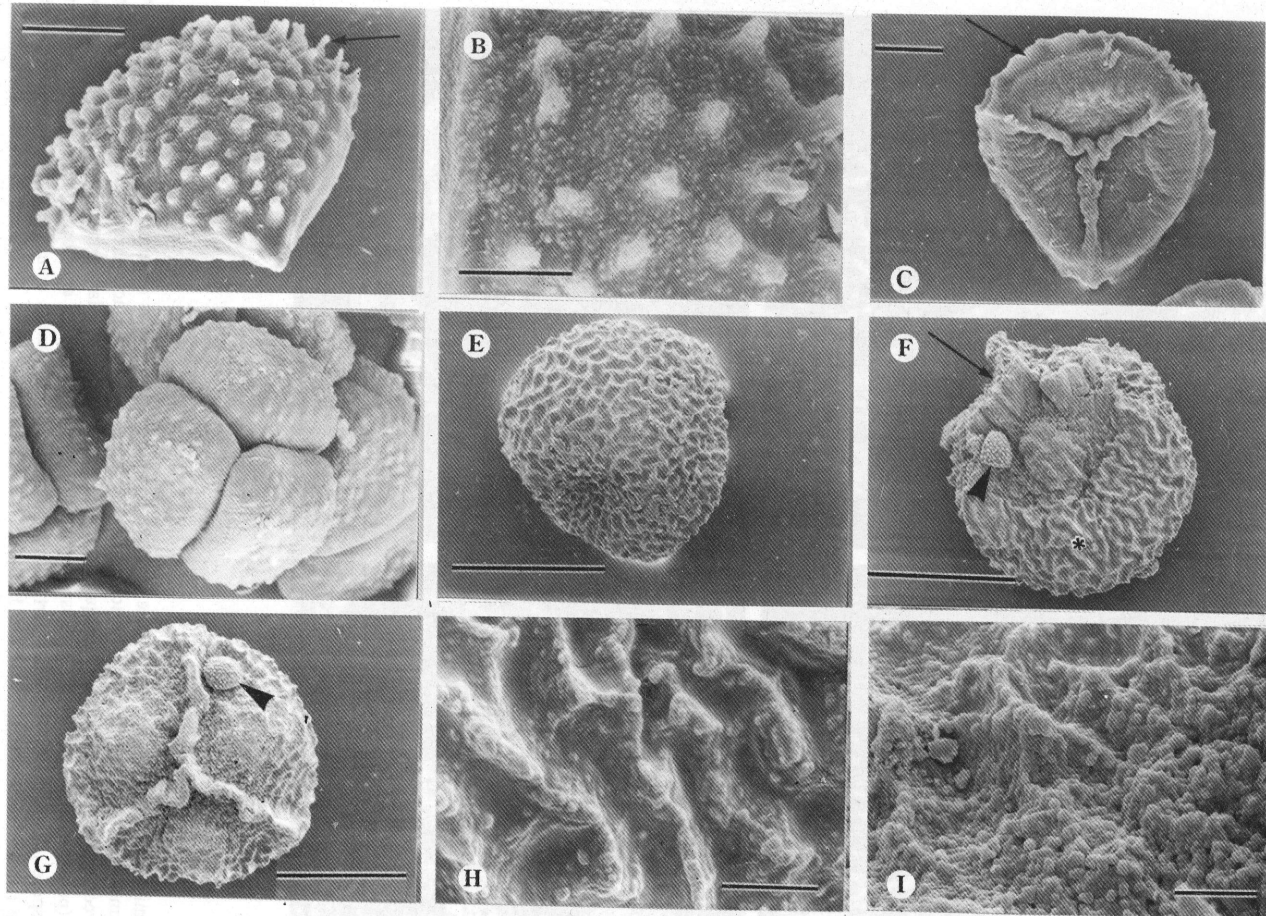
**Fig. 7. A - B:** TEM sections through the inner portion of the exospore at the distal pole. **A:** Portion of the exospore at the ordered/unordered transition. Stain: Bar: 5µm. **B:** The pattern at this level is unordered. The units are rather slender although some are flattened and thus broad in one plane. Some appear to be fused. The frequent and relatively wide spaces between units are mini gaps. The outer surface of the major gap is on the lower right corner. Stain: PA-TCH-Sp. Bar: 5 µm. **C and D.** SEM micrographs of exospore fractures of *S. marginata* megasporangia. **C:** A fracture of the exospore that shows the three-dimensional arrangement of the ordered/unordered transition. The ordered pattern is at the top and the unordered pattern is across the bottom. In several places it can be seen (arrows) that the units change from the compact arrangement to the loose unordered one. Bar: 1 µm. **D:** A fracture of the exospore at the innermost part of the unordered pattern. The structural elements at this level are thinner and some are flattened. Bar: 1 µm.



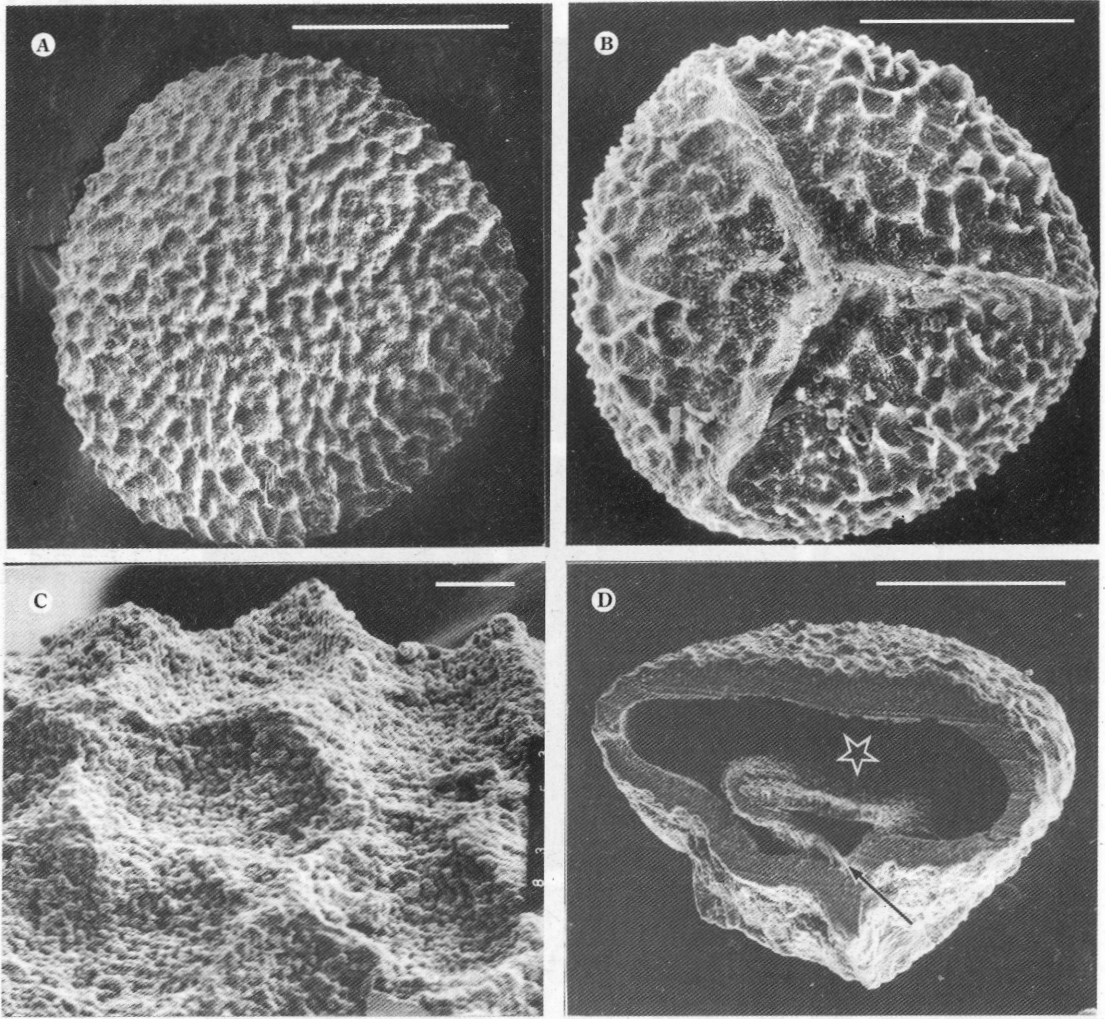
**Fig. 8. A - D:** SEM micrographs of the megaspore exospore. **A:** Fracture at the inner part of the exospore showing the ordered/unordered transition in this region. At this level there is a drastic change in the exospore organization. The structural elements do not show regularity in their distribution and the spaces between units are wide. Bar: 2  $\mu\text{m}$ . **B:** The outer surface of the sporoderm is on the top left. The fracture shows the complex and massive character of this ordered pattern. The structural elements are fractured along different orientations. The change of orientation of groups of structural units is a feature in this ordered pattern. Spaces of variable dimensions (mini-gaps) occur in places where the groups of units change direction. Bar: 2  $\mu\text{m}$ . **C:** This fracture exposes the outer portion of the exospore (at the top). One can see the transition from the ordered to an unordered pattern. The circular shape in section of the structural units is the same in both patterns. Bar: 2  $\mu\text{m}$ . **D:** This is part of the same fracture in Fig. C, but at a higher magnification. The square-frame illustrates the cubic arrangement of units in this fracture plane. Bar: 1  $\mu\text{m}$ .



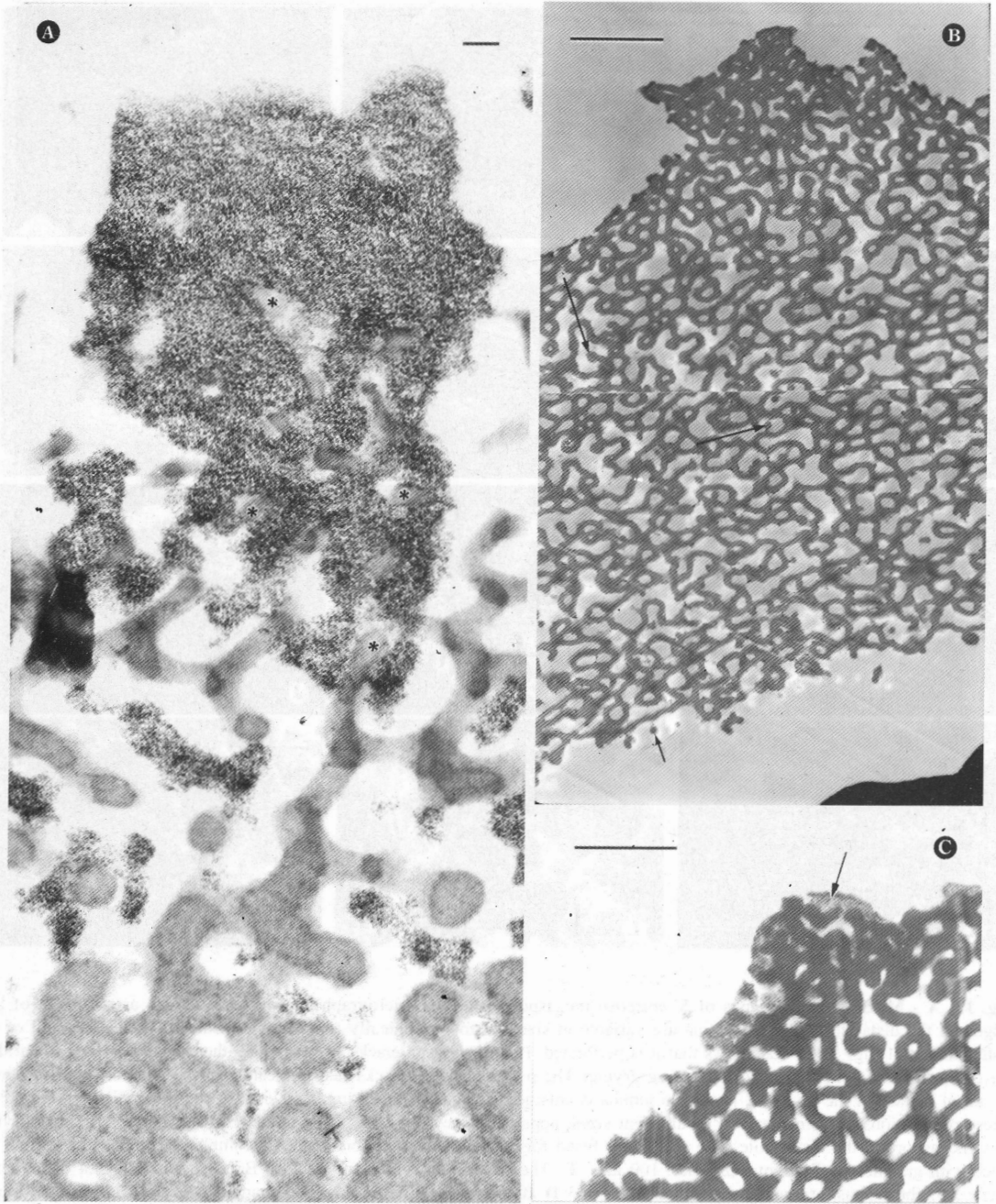
**Fig. 9.** A - I: SEM micrographs of *S. marginata* microspores. A: Several microspores having long spines. Bar: 10  $\mu$ m. B: Enlargement of a part of the central spore in Fig. 9, A: The exospore surface has small pores and is divided by deep grooves. Bar: 2  $\mu$ m. C: Proximal face showing laesura and many spines of different size and shape. Bar: 10  $\mu$ m. D: Distal surface of a microspore showing that the spines are positioned on island-like placks separated by grooves. Bar: 10  $\mu$ m. E: Enlargement of surface of Fig. 9 D. There are spheres of variable size between spines. Bar: 2  $\mu$ m. F: Proximal face with areas having verrucae and capitate processes. Bar: 10  $\mu$ m. G: Distal face with verrucae, capitate processes and cones. Bar: 10  $\mu$ m. H: Detail of the distal surface. The surface of the processes is scabrate. Perforations are located between processes and also at their bases. Bar: 2  $\mu$ m. I: Equatorial view showing processes smaller in diameter on the proximal face (left side) than distally. Bar: 10  $\mu$ m. J - O: SEM micrographs of *S. marginata* megaspores. J - L: Are the four megaspores from a same megasporangium. They are in equatorial view. J: These two megaspores are not fully developed. Note the difference in ornamentation features between those two megaspores and the more fully developed ones in K and L. Bars: 200  $\mu$ m. M: A detail of the exospore surface in one of the incompletely developed megaspores. Bar: 50  $\mu$ m. N: A detail of the distal reticulated surface of the megaspore in K. There are cracks on the surfaces of some muri. Bar: 20  $\mu$ m. O: A detail of the distal surface of the megaspore in L. The surface of a murus can be seen at the right. There is less silica on the surface of the lumina than on the murus. The surface is perforated. Bar: 10  $\mu$ m.



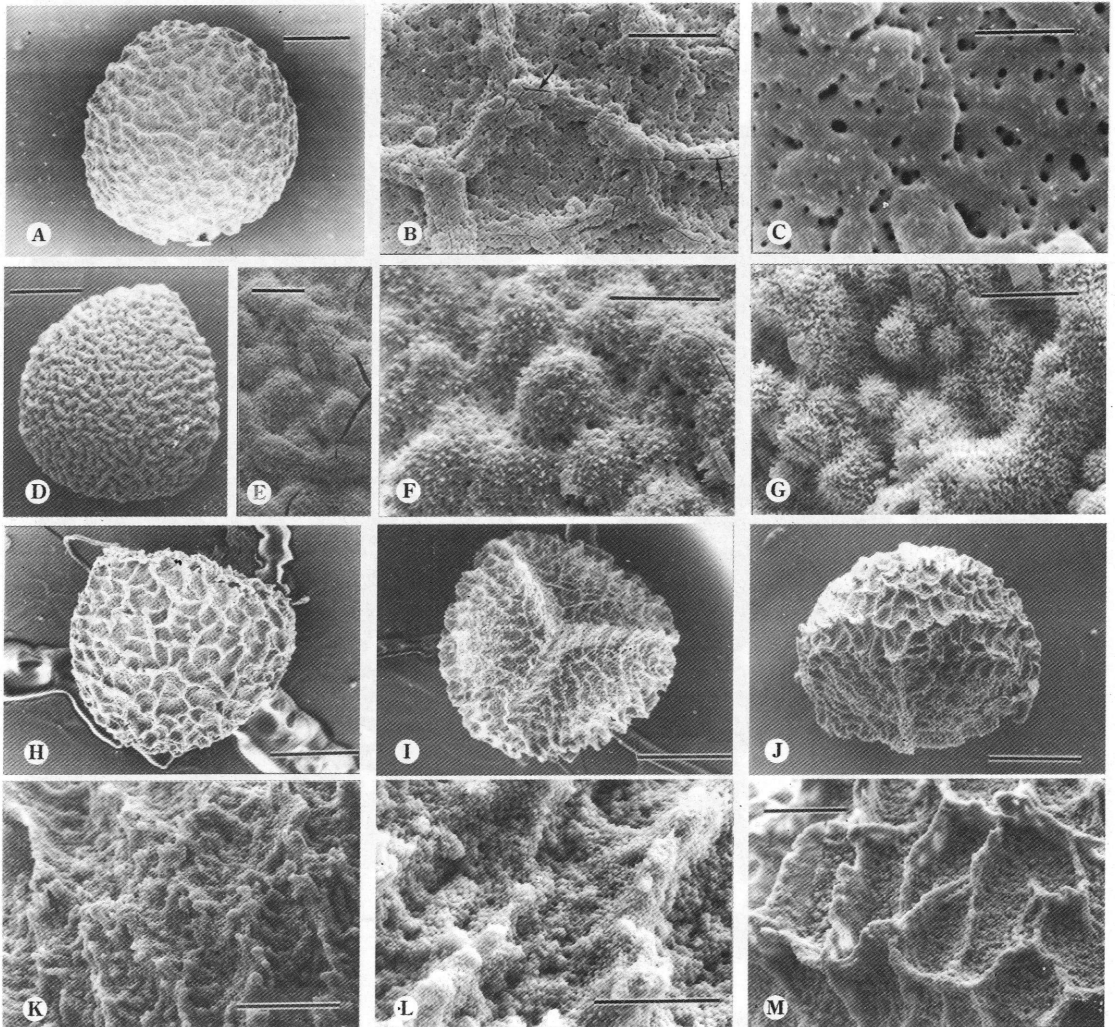
**Fig. 10.** A - D: SEM micrographs of *S. microphylla* microspores. **A:** Tilted distal surface shows truncated spines. Some processes are capitated (arrow), Bar: 10  $\mu\text{m}$ . **B:** The distal surface has small gemmae as well as spines. Bar 5  $\mu\text{m}$ . **C:** The proximal surface of a microspore showing the laesura and pronounced equatorial ridge (arrow) Bar 10  $\mu\text{m}$ . **D:** Tetrad of microspores that are expanded. In the microspores that are not collapsed it is evident that the equatorial surface is like the proximal surface, and is without the ridge seen in Fig. C. Bar: 10  $\mu\text{m}$ . **E** - **I:** SEM micrographs of *S. microphylla* megaspores. **E:** The reticulate ornamentation of the distal surface has high ridges. Bar 100  $\mu\text{m}$ . **F:** A tilted megaspore showing portions of both the proximal surface, with a high laesura (arrow) and the equatorial region of the distal surface. The distal surface is reticulate but near the proximal side the ridges tend to be parallel (asterisk). A microspore (arrowhead) is on the proximal region near the laesura. Bar 100  $\mu\text{m}$ . **G:** Proximal face showing the relatively low ridges of the irregular reticulated surface. At the right there is a microspore (arrowhead) near the laesura. Bar 100  $\mu\text{m}$ . **H** and **I:** Details respectively of the distal and proximal surfaces. Bar: 10  $\mu\text{m}$ .



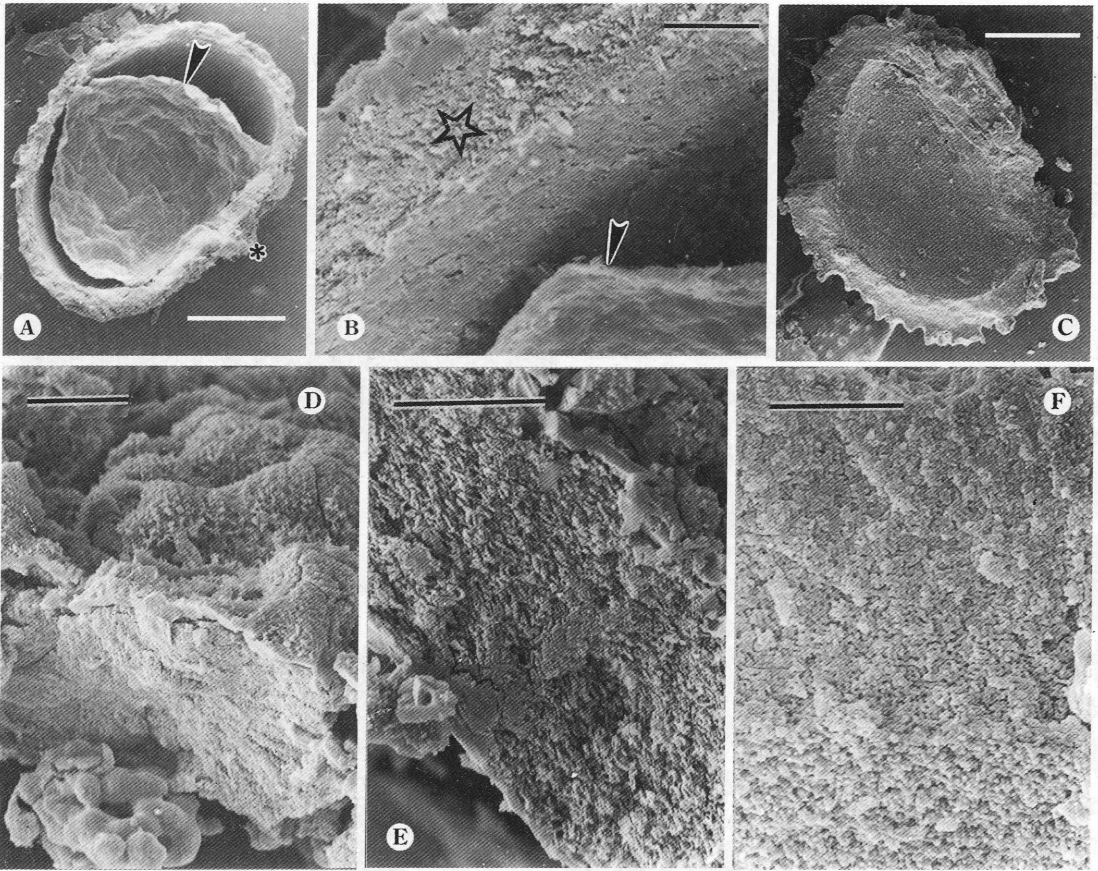
**Fig. 11. A - D:** SEM micrographs of *S. microphylla* megaspores. **A** and **C.** Distal views showing a reticulate sculpture. **A.** Bar: 100 µm. **B.** Proximal view showing the reticulate sculpture. The ridges on this surface surround wide lumina. In some cases connections between ridges are incomplete. The equator is marked by arcs of irregular surface. Bar: 100 µm. **C:** shows that there are spaces, (perforations, holes) between exospore units. Bar: 5 µm. **D:** Is an equatorial section of an immature megaspore. The mesospore is covered by the inner part of the exospore (arrow) that is connected to the exospore of the proximal pole. The gap (star) is wide at this stage. Bar: 100 µm.



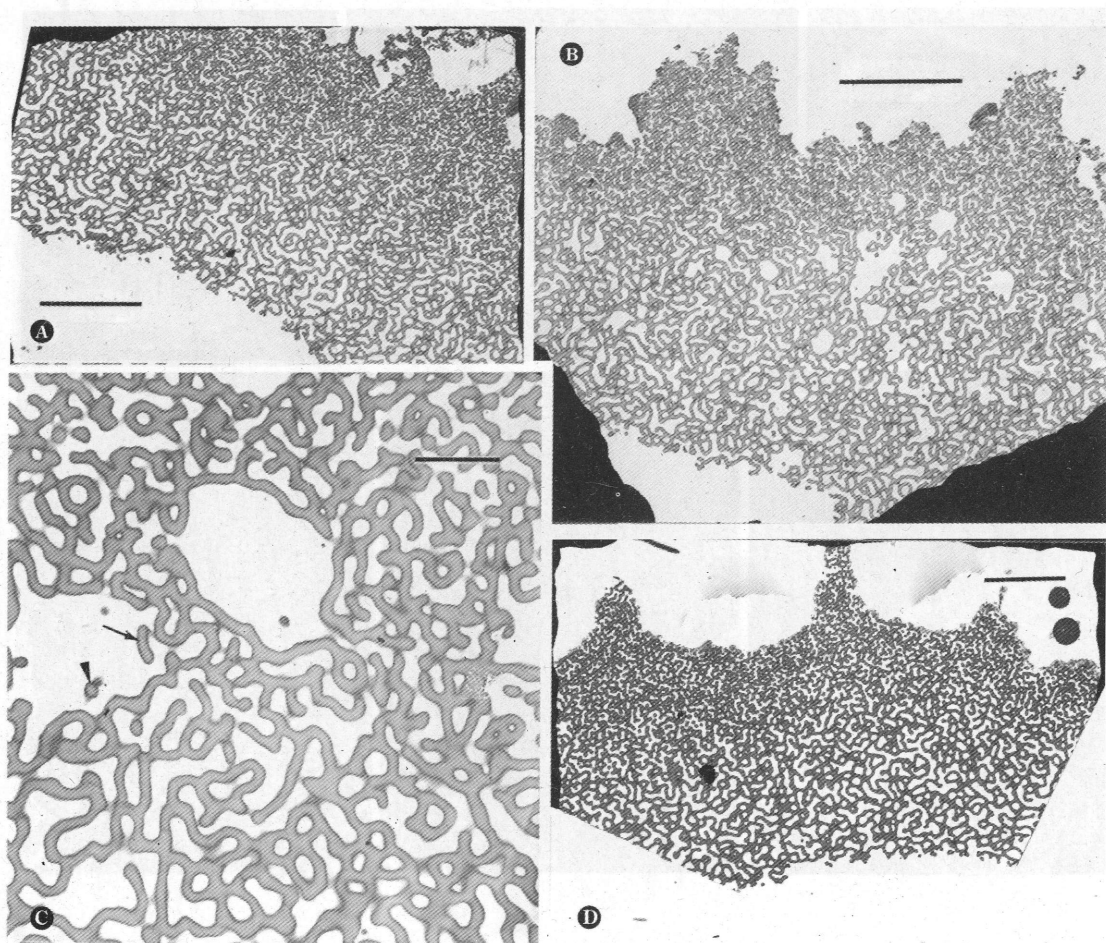
**Fig. 12.** A – C: TEM micrographs of *S. microphylla* megaspore exospore in cross section. **A:** Is a detail of the exospore surface. The silica (dark) containing rods are located between exospore units (asterisks). The rods are considered to be remnants of wicks. Bar: 0.1  $\mu\text{m}$ . **B:** Is a section near the distal pole. It is a typical unordered exospore. The spaces between units are interconnected. Exospore units are bars and circular in section (arrows). The elements of the ornamentation (ridges) have the same structure as the rest of the exospore. Stain: UA - Pb. Bar: 10  $\mu\text{m}$ . **C:** The figure includes a section through one ridge which shows accumulation of silica at the surface (arrow). Silica also fills some spaces between units within the ridge. The structure of the ridge is the same than the rest of the exospore. Stain: UA - Pb. Bar: 5  $\mu\text{m}$ .



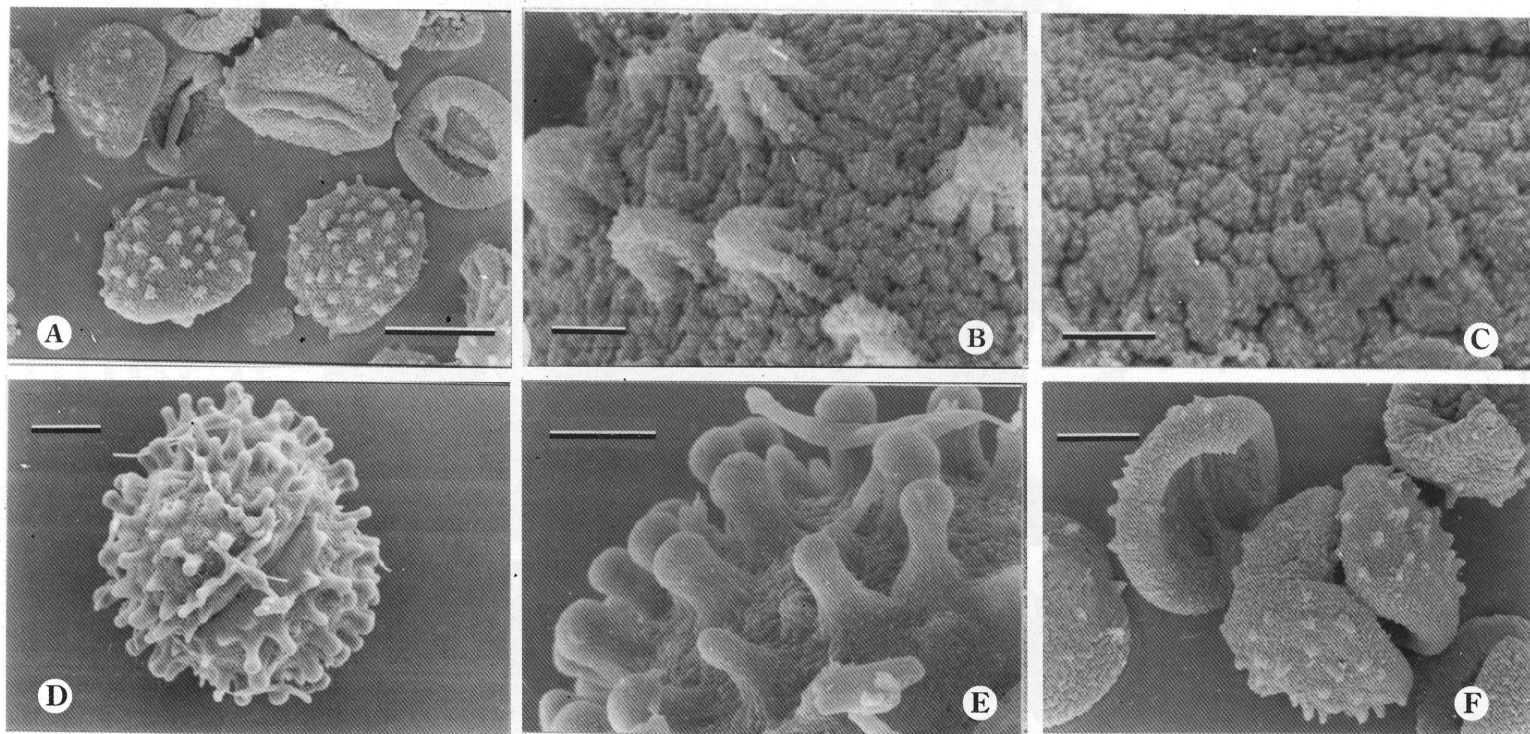
**Fig. 13.** A - M. SEM micrographs of *S. muscosa* megaspores. **A:** The micrograph shows the reticulate arrangement of low ridges on the distal surface. The lumina are variable in size and topographically and irregular. Bar: 100  $\mu$ m. **B:** Detail of the distal surface of Fig. 13: A that shows that it is perforated. Ridges show a crack (arrows) along their axis. We suggest that they are compression-cracks that occurred during drying. The position of the cracks may indicate that the silica coating is thin at the distal surface of the ridges. A part of a lumina is enlarged in Fig. C. Bar 20  $\mu$ m. **C:** Detail of the perforations in Fig. 13 B. These perforations are irregular and of different sizes; apparently they are both single or joined. Bar: 5  $\mu$ m. **D:** General view of a megaspore at the distal pole. The ridges are fused forming an irregular, some times incomplete reticule. The lumina are filled with granules of different sizes. Bar: 100  $\mu$ m. **E:** The surfaces of ridges are cracked. Bar: 10  $\mu$ m. **F:** Detail of the distal surface of the megaspore coated by silica in Fig. 13 D at higher magnification. The ornamentation is composed by single verrucae and ridges formed by coalescence of verrucae. The whole surface is echinulate/perforated. The perforations are numerous over the entire surface but largest in valleys between ridges. The spiky surface is due to silica (see Fig. 13 G). Bar: 10  $\mu$ m. **G:** The extremely echinate surface on this megaspore is due to silica (see Fig. 19 B). Bar: 10  $\mu$ m. **H - M.** These figures show mature megaspores in a different specimen from A - G. **H:** The ornamentation is reticulate distally. Bar: 100  $\mu$ m. **I:** Although the ornamentation is reticulated proximally the muri are radially oriented in each proximal facet. Bar: 100  $\mu$ m. **J:** The figure shows a megaspore in equatorial view. The curvature is evident for the lateral fusion of the muri at the equator. Bar: 100  $\mu$ m. **K:** A detail of the surface of the megaspore in Fig. 13 H. Bar: 20  $\mu$ m. **L:** A detail of the surface of the megaspore in Fig. 13 I. Bar: 10  $\mu$ m. **M:** A detail of the curvature of the megaspore in Fig. 13 J. Bar: 20  $\mu$ m.



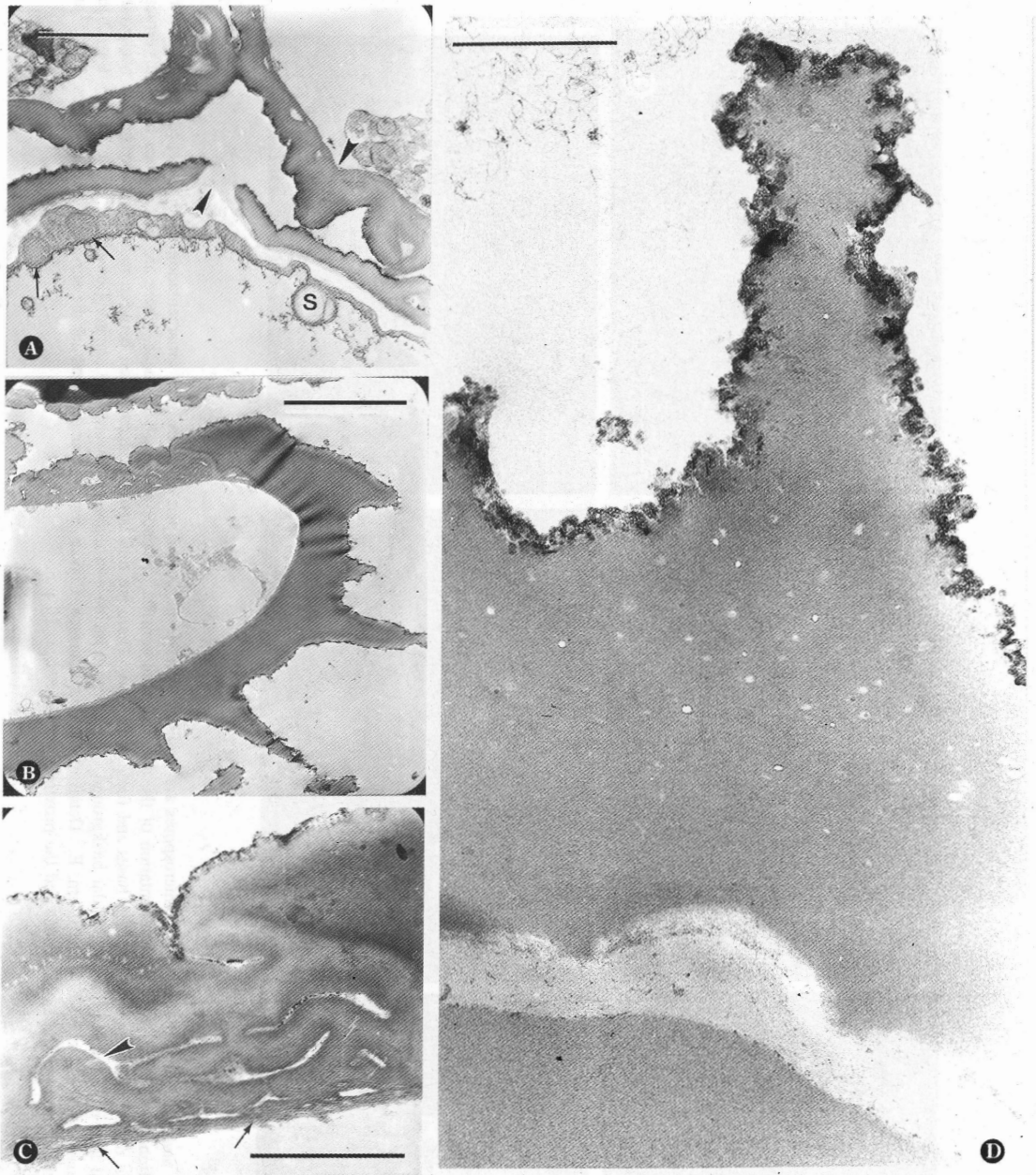
**Fig. 14.** A - F: SEM micrographs of sections or fractures of *S. muscosa* megaspore exospore. **A:** A megaspore sectioned in half at a mature stage. The inner exospore (arrowhead) is thin and relatively near to the outer exospore. The inner exospore is only attached to the outer exospore in the proximal pole region (asterisk). Bar: 100  $\mu\text{m}$ . **B:** A portion of the fracture in Fig. A showing the inner (arrowhead) and outer parts of the exospore (star). Bar: 10  $\mu\text{m}$ . **C:** In fracturing this megaspore the distal parts of the exospore broke into several pieces. Portions of these are seen in Fig. D. Bar 100  $\mu\text{m}$ . **D:** This is a fracture that offers a detail of the exospore. A compact layer of a few  $\mu\text{m}$  on the outer part of the exospore that forms the sculpture. It is perforated (cf Fig. 13, C and F) and often cracked (cf Fig. 13, B and E). Bar : 10 $\mu\text{m}$ . **E:** A section at the proximal pole where there is no separation between parts of the exospore. The inner surface is at the lower left and the outer surface at the upper right corner. Bar: 10 $\mu\text{m}$ . **F:** The outer surface of the exospore is at the top and the inner surface (outer surface of the former gap) at the bottom. There are many spheres sitting along the rods structural elements on the inner surface. The labyrinthine unordered wall has broader spaces between units inwards (towards the bottom). Bar: 10  $\mu\text{m}$ .



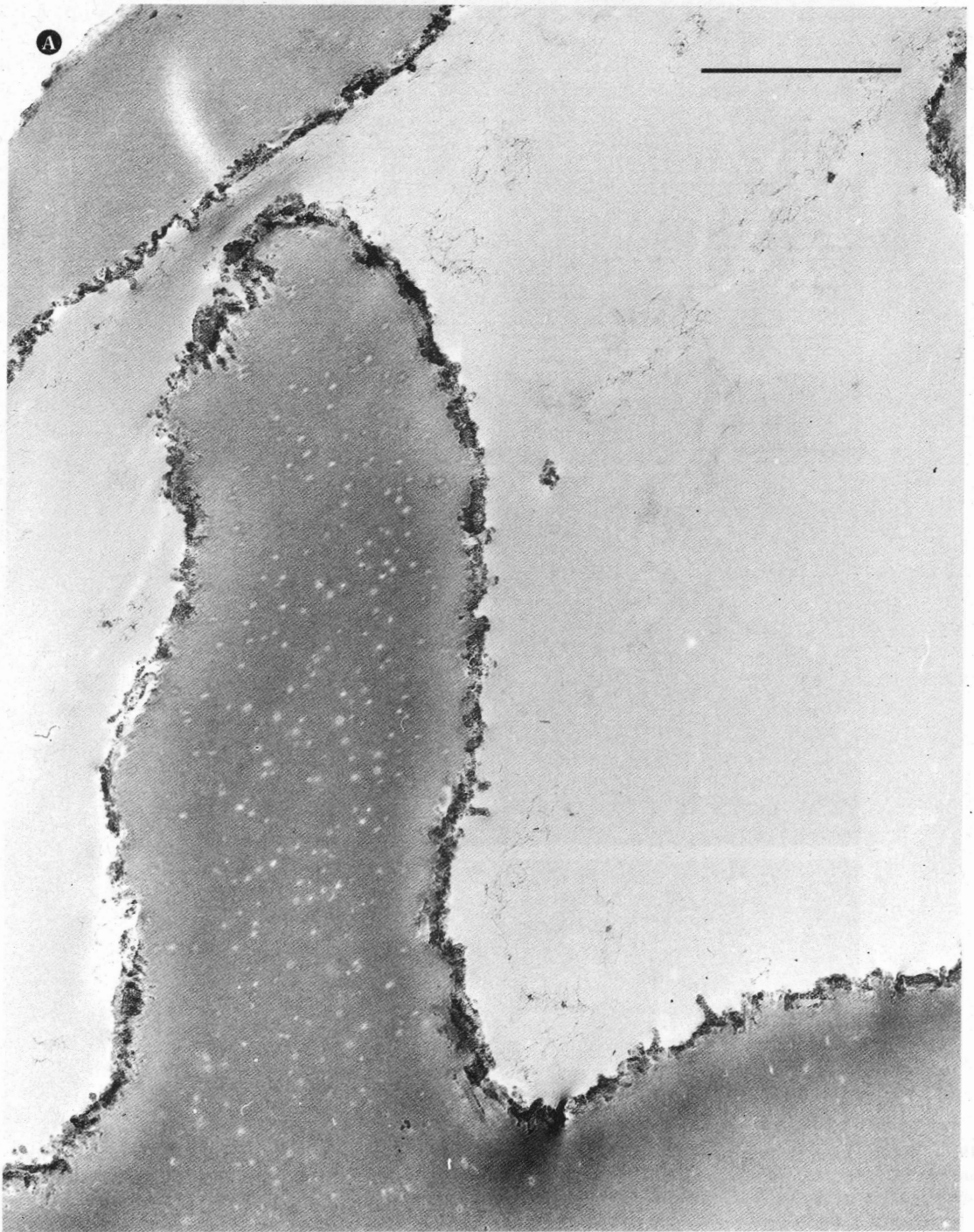
**Fig. 15. A - D:** TEM micrographs of *S. muscosa* megaspore exospore. **A:** Section through the exospore at the distal pole with distal muri at the top. The muri have the same structure as the rest of the exospore. The structure is basically the same throughout the exospore. Stain: UA-Pb. Bar: 10  $\mu$ m. **B:** Section through the exospore at the distal pole. The outer surface is at the top. This section is serial with fig. A. It shows many mini gaps of different sizes, most of them concentrated in the middle portion of the exospore. The fact that figures A and B correspond to different planes of sectioning through the same part of the exospore of the same megaspore shows that the gaps are not extensive in width, i.e., they do not form continuous tunnels running with tangential orientation. Note that both surfaces (inner and outer) of the exospore are discontinuous. Stain: UA - Pb. Bar 10  $\mu$ m. **C:** A section similar to the one in Fig. 15 B although at increased magnification. Mini gaps have exospore units extending through them. The arrowhead marks the circular shape of a unit cut in cross section and the arrow one cut obliquely. Bar: 2  $\mu$ m. **D:** A section of the whole exospore that shows tall ridges (muri) at the distal pole. They have the same structure as the rest of the exospore. The diameter of the structural processes and spaces between them increase towards the inner surface. The outer surface is discontinuous. Spaces (conduits) are continuous throughout the wall. Stain: UA-Pb. Bar: 10  $\mu$ m.



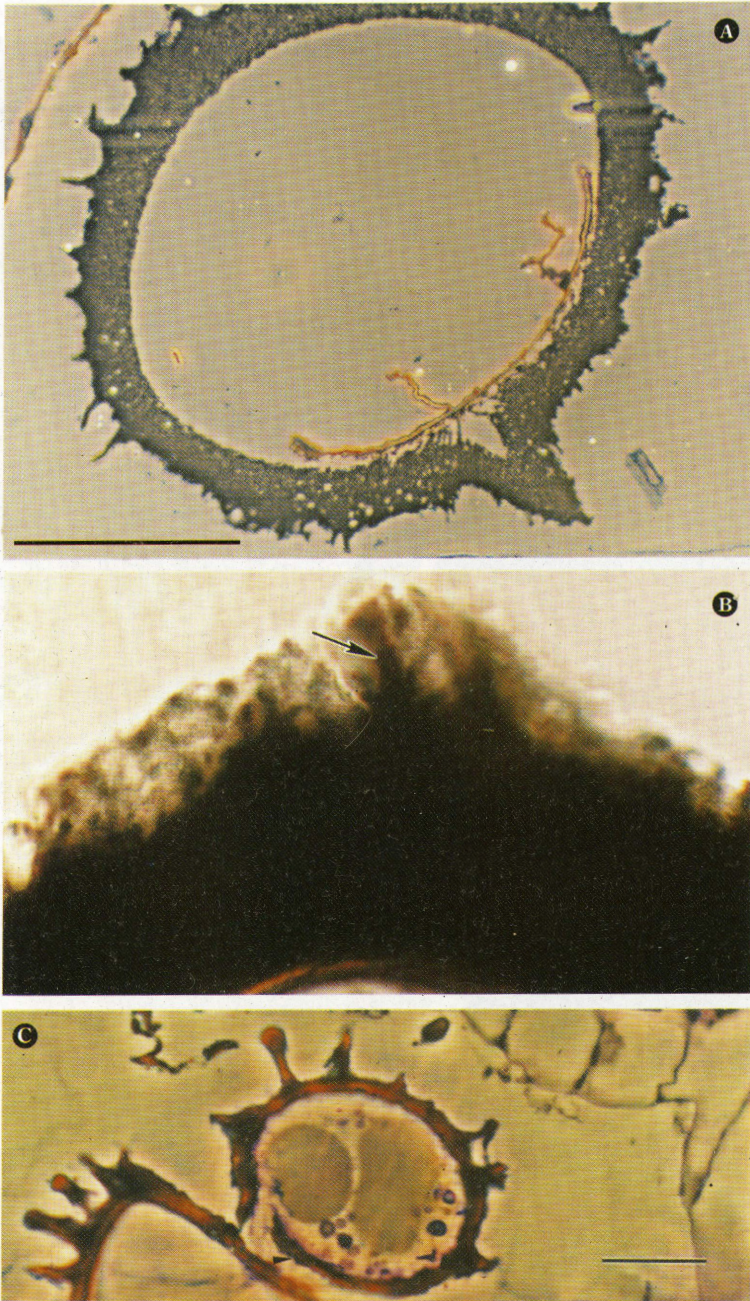
**Fig. 16. A - F:** SEM micrographs of *S. muscosa* microspores. **A:** Two microspores are in distal views in the lower part of figure. The ornamentation is cone-shaped. There are three monolete microspores in the upper portion. Bar 20  $\mu\text{m}$ . **B:** The ornamentation of the distal surface consists of cones and small verrucae. There are perforations between verrucae. Each cone is built by several concrescent rods (rods independent at their bases and fused at the top). There are tiny lateral bridges (links) between rods in each cone. The background is granular. Bar: 2  $\mu\text{m}$ . **C:** The proximal face is verrucated with a granular background. The verrucae increase in diameter towards the equator (lower part of the figure). Bar: 2  $\mu\text{m}$ . **D:** Two microspores in a tetrad. The ornamentation is clavate. Bar: 10  $\mu\text{m}$ . **E:** Detail of the distal surface. Clavae. Small strands constitute the surface of the processes. Bar: 5  $\mu\text{m}$ . **F:** Microspores in tetrads. The distal surface in these spores has cones and the proximal verrucae. Bar: 10  $\mu\text{m}$ .



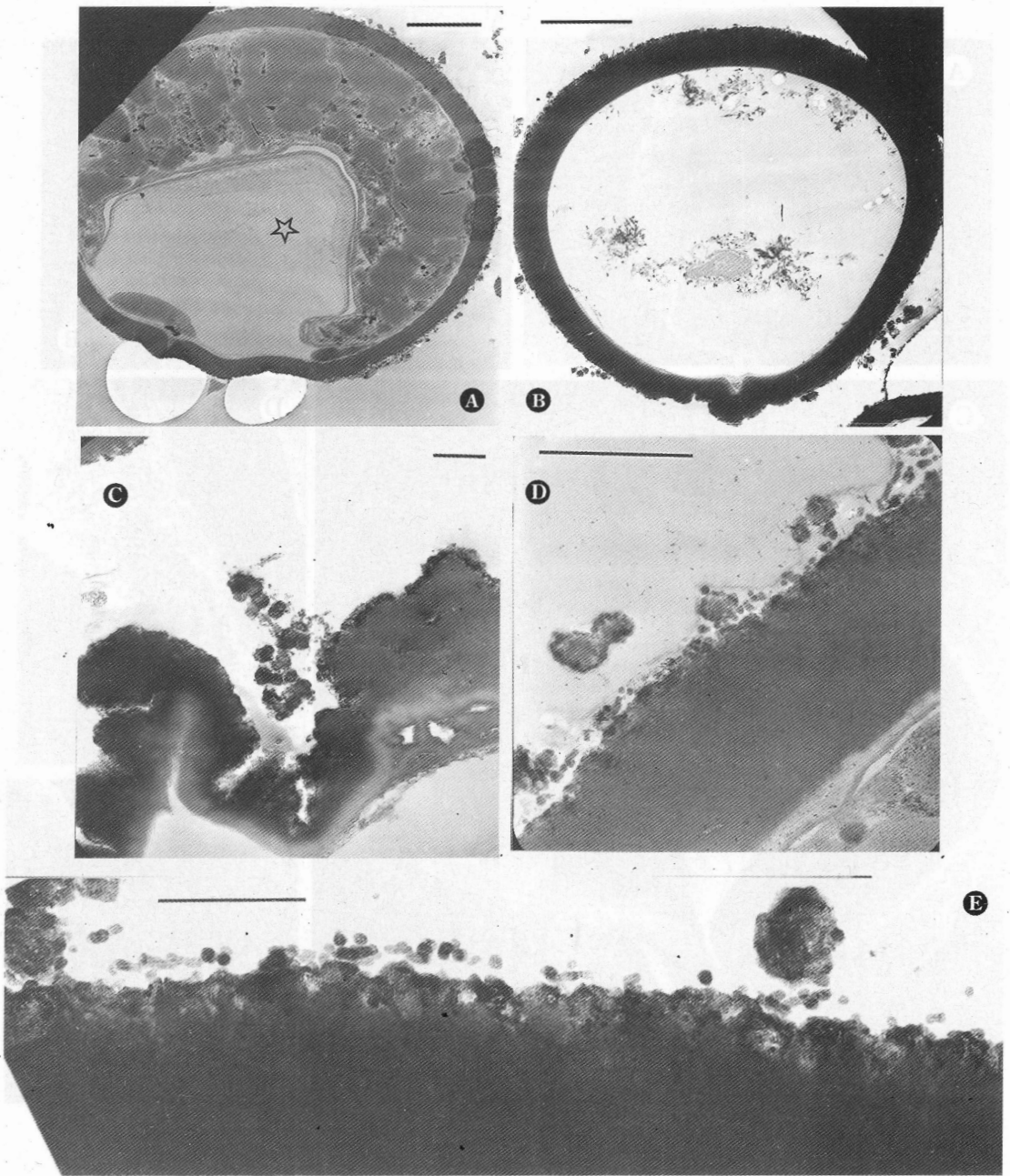
**Fig. 17. A - D:** TEM micrographs of *S. muscosa* microspores. Stain: UA-Pb. **A:** The proximal region of three microspores. Laesurae (arrowheads) are apparent in two of them; in the lower one the laesura is open. The thin cytoplasm around a large vacuole shows mitochondria (arrows) and a plastid with starch (s). At the inner surface of these exospores there is a thin lamellated zone. (see Fig.17 B and C ). There is also a rather wide zone of low contrast. (see Fig.17 D). Bar: 2  $\mu$ m. **B:** The section shows the exospore structure in proximal and distal regions. Proximally there are cavea and the innermost part is lamellated. The proximal surface is verrucated. The structure of the distal exospore is compact and the innermost part of it is lamellated. The sculpture is echnated. Bar: 5  $\mu$ m. **C:** The exospore structure at the proximal pole has aligned gaps (arrowhead). Then gaps and a zone of cavea. The innermost region is lamellated (arrows). The dark surface coat is shown in greater detail in Fig. 17 D and Fig. 18 A. Bar: 1  $\mu$ m. **D:** The surface coat can be seen to consist of tubules that are ca 10 - 15 nm in diameter with a low dense core. The zone of low contrast below the exospore is seen to include strands and lamellations. Low contrast sites (holes) are apparent here and in Fig. 18 A. Bar: 0.5  $\mu$ m.



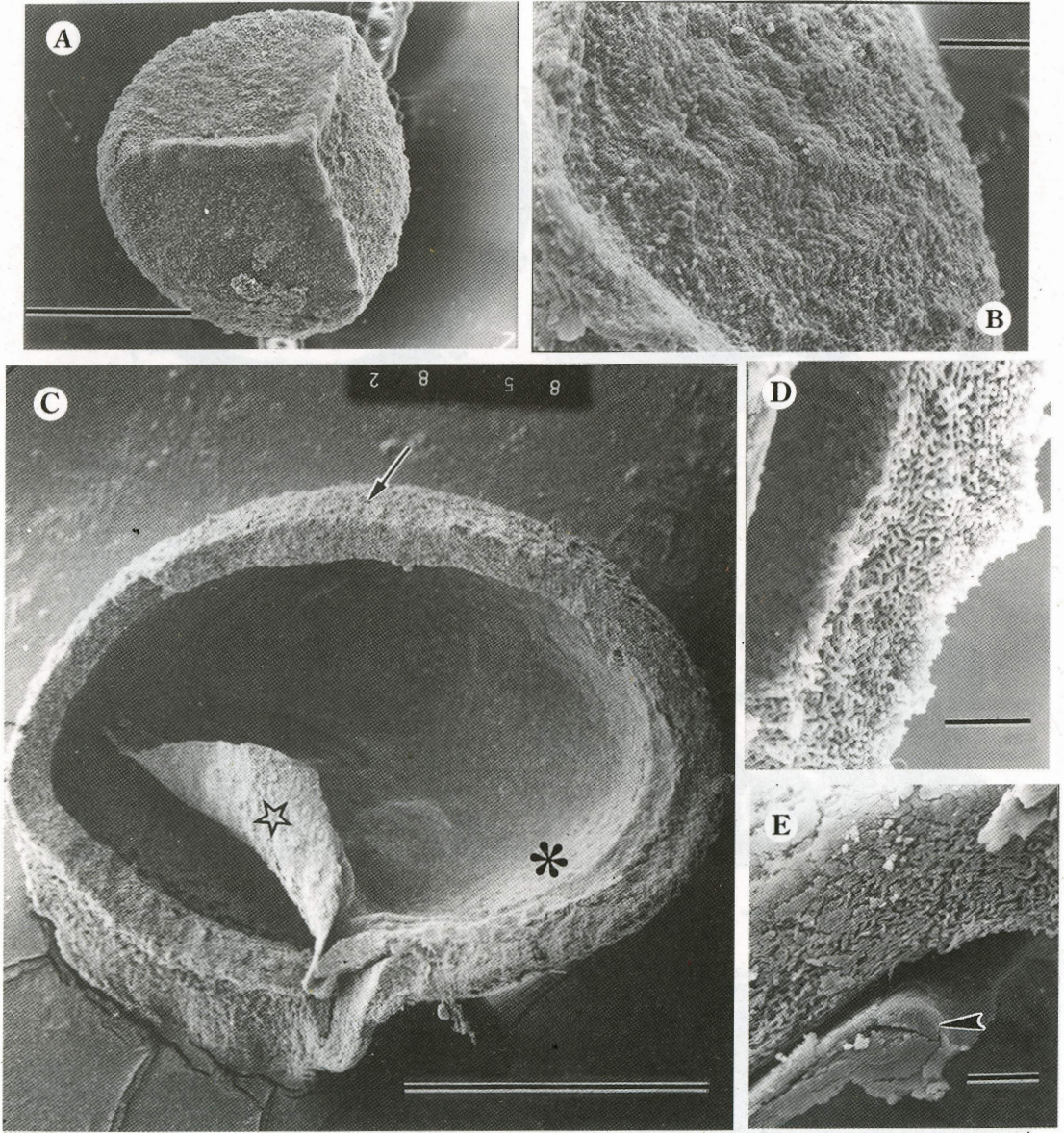
**Fig.18. A:** Spine with a coating of small tubules. Some of them are seen in section. Low contrast sites (holes) are apparent within the spine and also within the bulk of the exospore. Bar: 1  $\mu$ m.



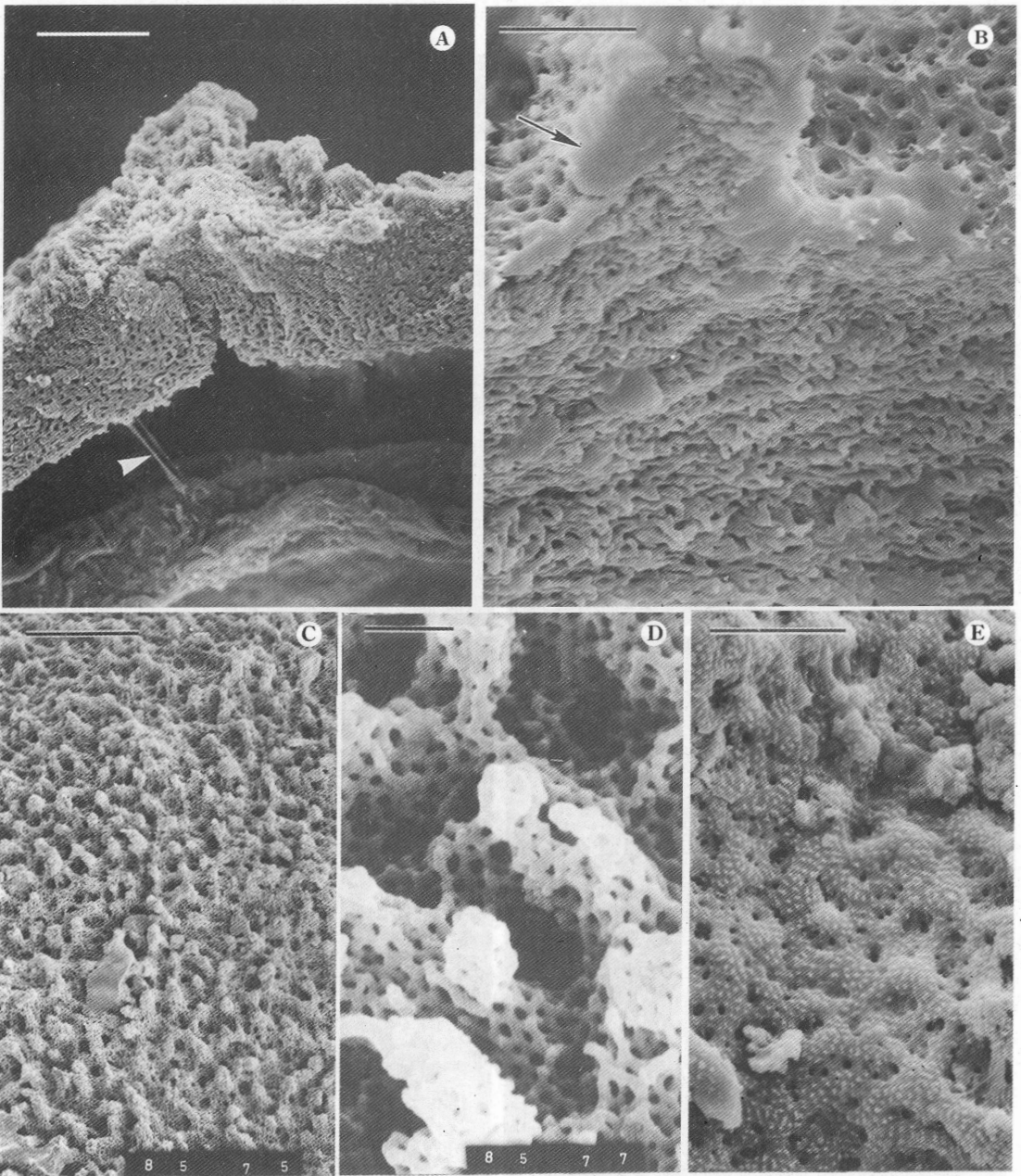
**Fig. 19.** **A and B:** LM photomicrographs of thick sections of *S. muscosa* megaspores. The sections were stained with toluidine blue. **C:** LM photomicrograph of *S. novae-hollandiae* microspores within a microsporangium. **A:** The section is of the same megaspore cut for the thin sections in Fig 15 B, C and D. The narrow stripe (pink/red in color) of inner exospore is partly folded against the inner part of the proximal exospore. The inner exospore is attached to the outer exospore only at either side of the laesura near the proximal pole. A section of the laesura is at the lower right. Mini gaps of varying size occur throughout the exospore although their frequency is greatest in the proximal region. There are tall and slender spines that are part of the exospore surface. Bar: 100  $\mu$ m. **B:** A section from the above megaspore but cut much thicker. This thick section shows an exospore spine (arrow) bordered by silica. Bar: 25  $\mu$ m. **C:** Sections of *S. novae-hollandiae* microspores within a microsporangium. One microspore has been sectioned in an equatorial plane. The proximal surface is granular (arrowhead) and the distal surface has tall processes of variable shape. The protoplast is preserved. There is part of another microspore to the left. At the top right corner is the sporangium wall. Stain: toluidine blue. Bar: 20  $\mu$ m.



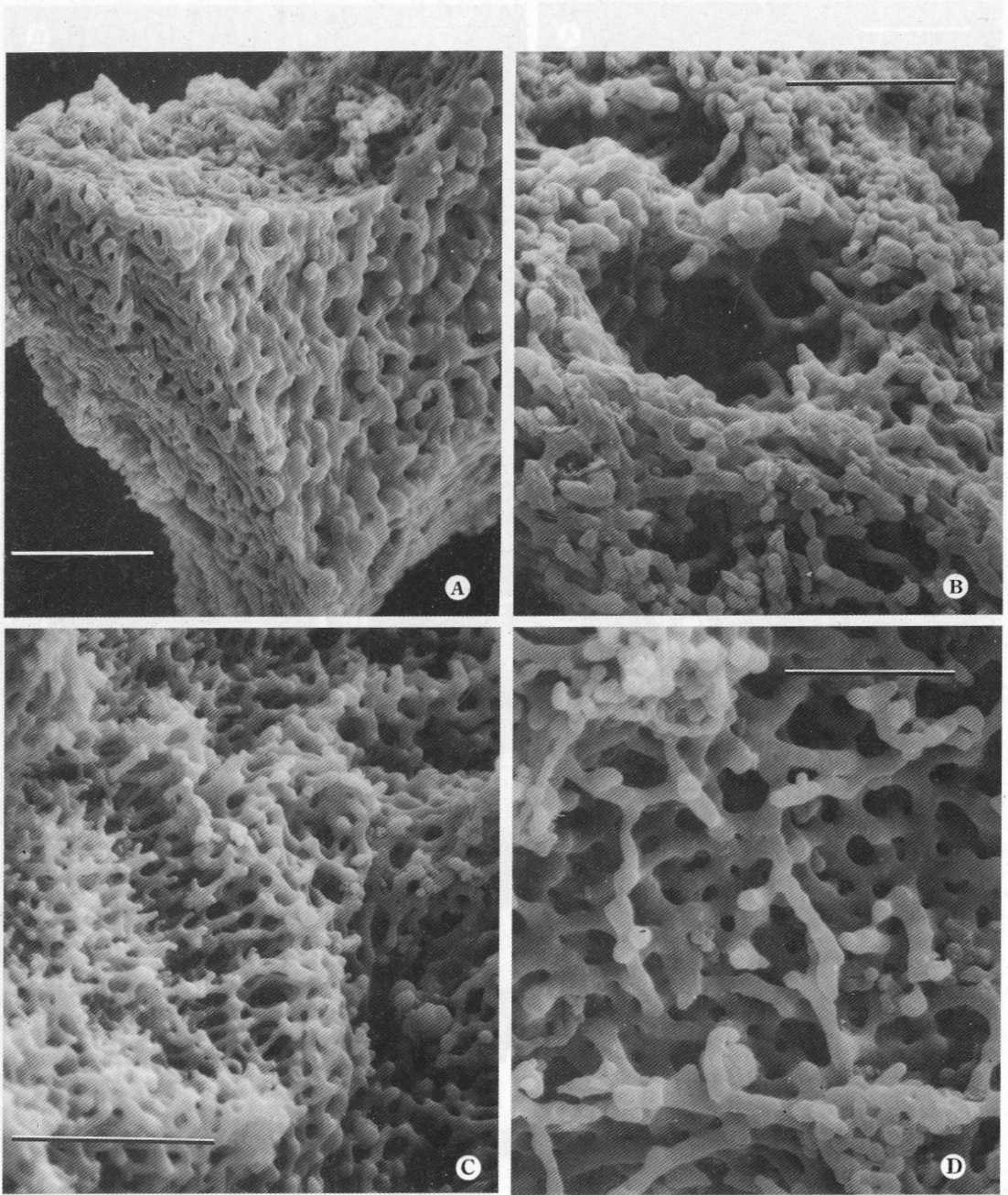
**Fig. 20.** A - E: TEM micrographs of *S. novae-hollandiae* microspores. Stain: UA-Pb. A - B: Show sections of whole microspores. A: shows the cellular content which appears to be largely lipoidal. Vacuole (star). The exospore is thicker in the distal region and at the equator than in the proximal region. Sections of verruca are seen in the proximal region. Bars: 5  $\mu\text{m}$ . C: The exospore is irregular at the proximal surface. There are many small rods and granules. (ca. 0.3  $\mu\text{m}$  in diameter) on and near the surface. Cavea are present in the inner part of the exospore at both sides of the laesura. Bar: 1  $\mu\text{m}$ . D: The distal surface is relatively smooth but has many small rods like the proximal surface. The endospore is evident. Bar: 1  $\mu\text{m}$ . E: The rods on the surface of microspores are ca 50 nm in diameter and have a central low dense core that is ca 10 nm in diameter. The relatively large granules (many are ca 0.3  $\mu\text{m}$  in diameter) show low dense sites (holes) that are ca 10 nm in diameter. Apparently these "large granules" are collections of the ca 50 nm rods. Bar: 0.5  $\mu\text{m}$ .



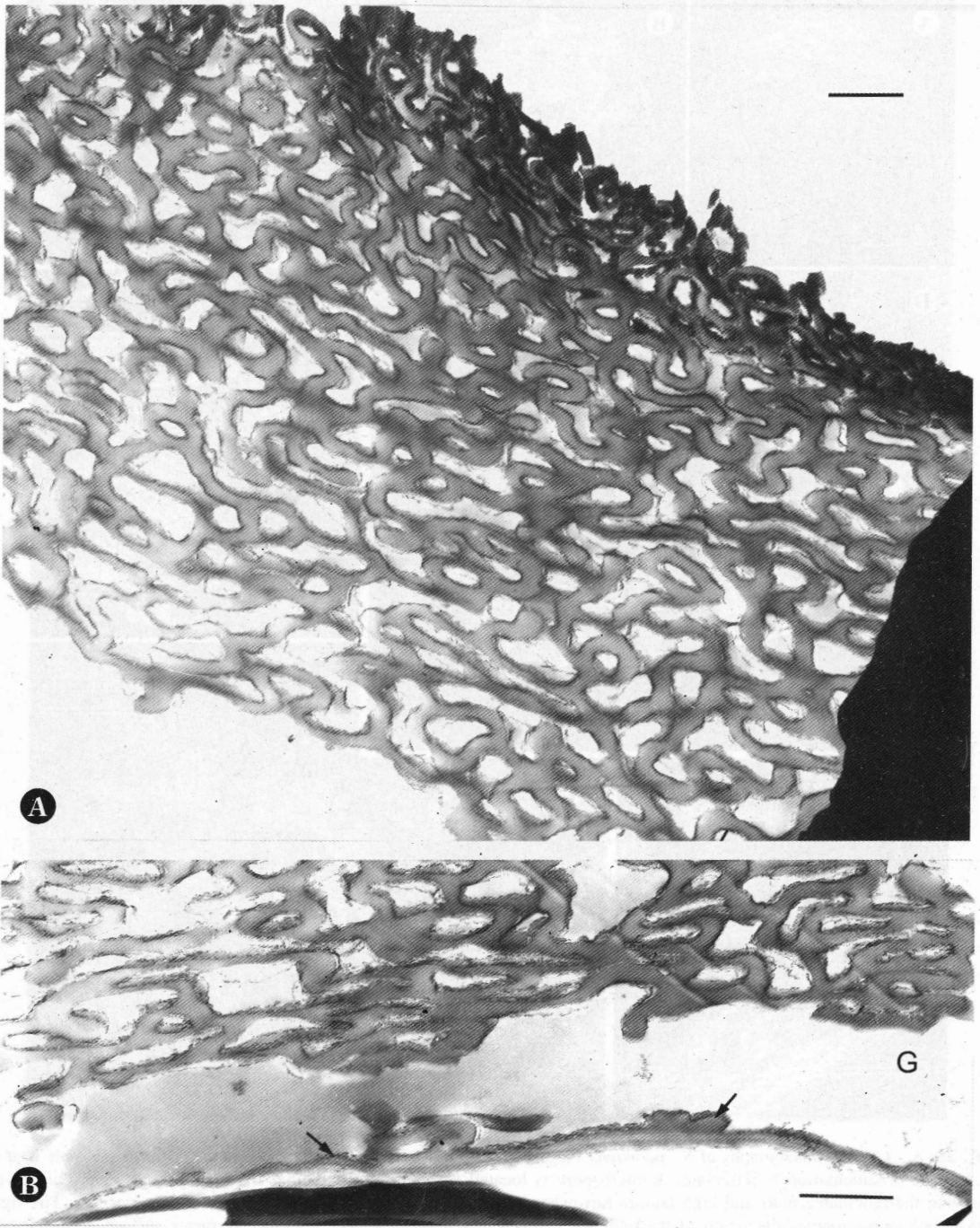
**Fig. 21. A - E:** SEM micrographs of *S. novae-hollandiae* megaspores. **A:** Proximal surface showing the high elevation of the triradiate laesura. The low profile of the crenated arc is evident in the upper left sector. Bar: 100  $\mu$ m. **B:** Detail of the proximal surface. It is slightly rugulated. Bar: 20  $\mu$ m. **C:** A megaspore sectioned in half showing the surface of the outer exospore (arrow) and both surfaces of the inner exospore (inside= asterisk and outside=star). The distal surface is at the top of the figure. Bar: 100  $\mu$ m. **D:** A section through the distal exospore. It shows the outer and inner parts of the exospore at both sides of the former gap. The structure of the exospore in this species is labyrinthine. Bar: 5  $\mu$ m. **E:** Section of the exospore in the equatorial region showing the outer exospore above the inner exospore (arrowhead). The inner exospore is folded back at the arrowhead. Bar: 5  $\mu$ m.



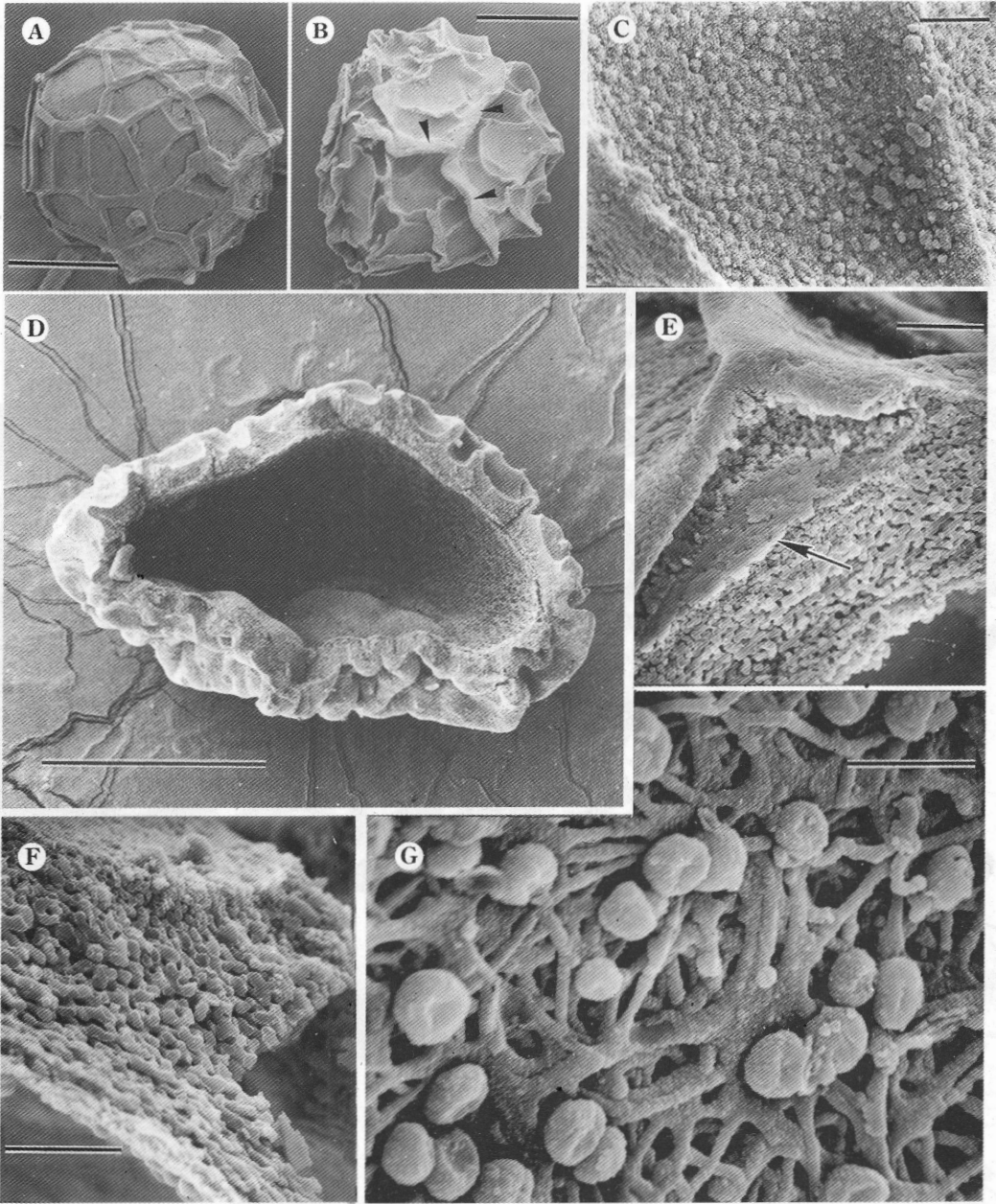
**Fig. 22.** A - E: SEM micrographs of the proximal surface of the *S. novae-hollandiae* megaspores. A: A fracture across an arm of the laesura perhaps near the equatorial region. The spaces between structural units are widest inwards; there are two unit-structures (arrowhead) extending from the outer part of the exospore across the gap to the inner part of the exospore. Bar: 10 µm. B: A fracture across the exospore. The surface differs from the underlying unordered structure. Holes are apparent in the surface coating of silica. The thickness of the outer layer (coating) of silica can be appreciated on the left side of the sculptural process (arrow). Bar: 5 µm. C and D: The proximal surface has complicated processes which may appear to be capitate. These consist of a three-dimensional reticulum. Bars: C: 20 µm, D: 2 µm. E: The ornamentation is due to silica on the surface. There are also perforations in the silica coating. Bar: 5µm.



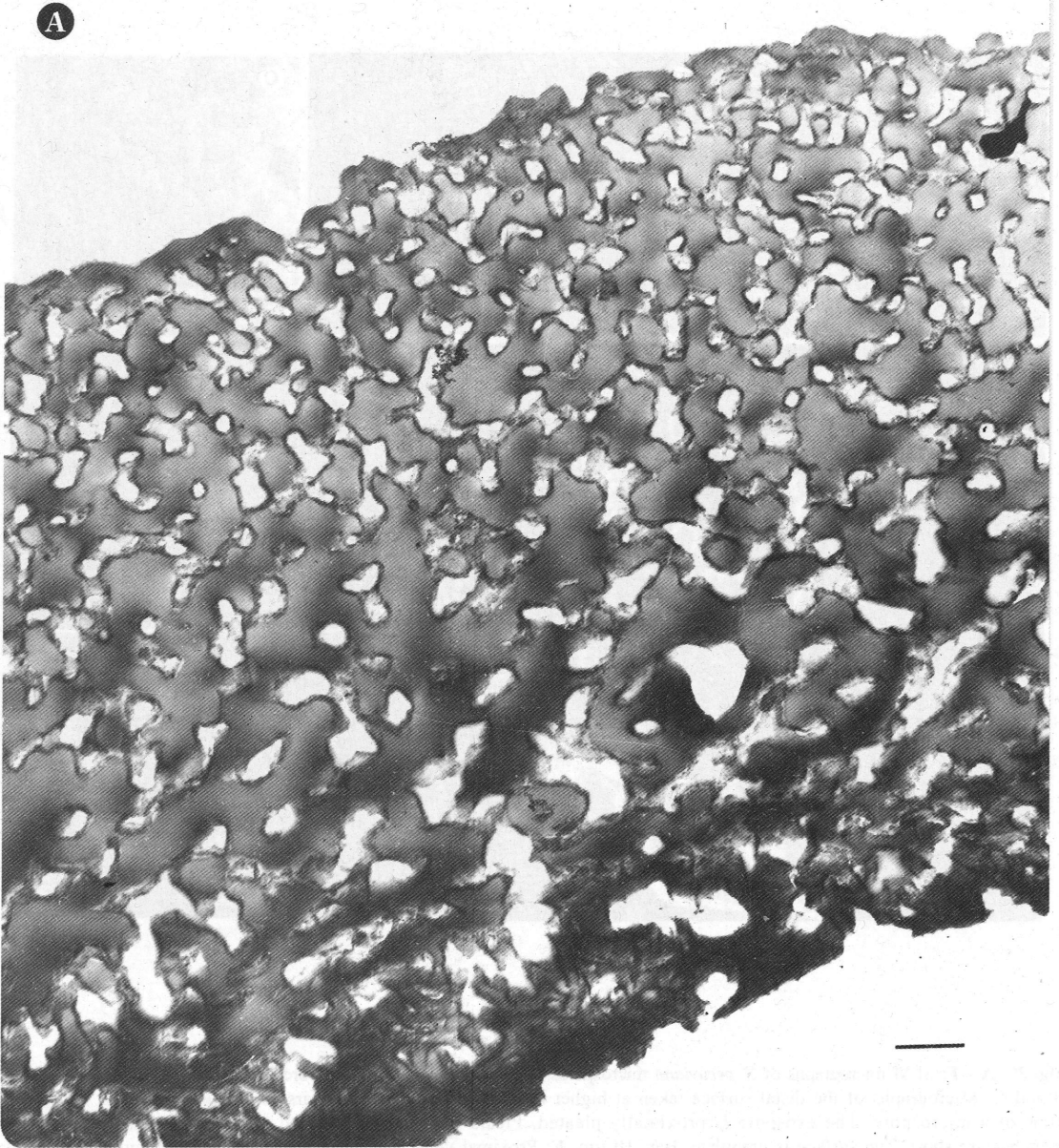
**Fig. 23. A - D:** SEM micrographs of *S. novae-hollandiae* megaspore exospore. **A:** A three dimensional section of the exospore. The inner surface of the outer part of the exospore (outer surface of the former gap) faces right. Bar: 5  $\mu$ m. **B:** Detail of the exospore surface. The proximal surface (apparently without silica coating) is very irregular. Many structural units of different diameter are seen on end. Bar: 3  $\mu$ m. **C:** The outer reticulated surface. Many units of different diameter are seen on end. Bar: 5  $\mu$ m. **D:** The reticulated outer surface of the exospore. The ends of the units are free at the surface of the SEM fracture. Bar: 3  $\mu$ m.



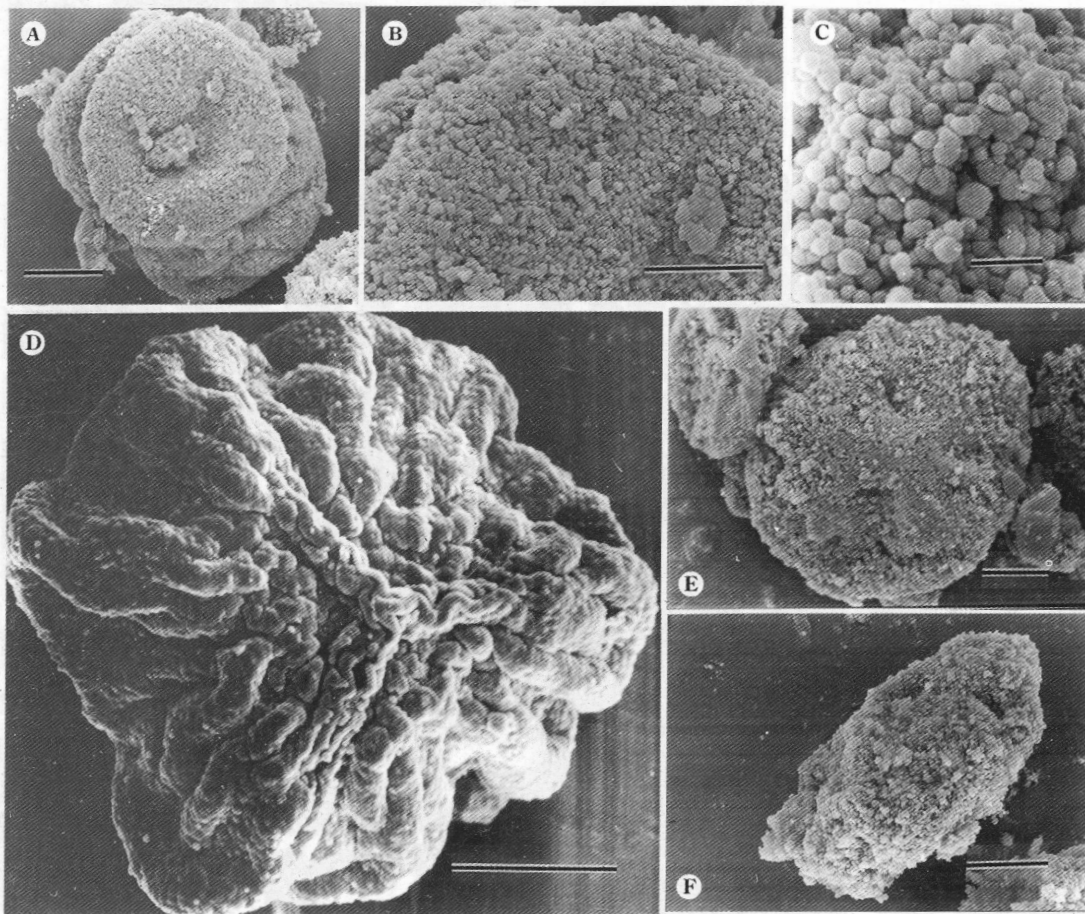
**Fig. 24. A - B:** TEM micrographs of *S. novae-hollandiae* megaspore exospore. **A:** The outer part of the exospore. Silica (darkly contrasted) is on the surface and between exospore units in levels below of the surface. Bar: 1  $\mu\text{m}$ . **B:** The inner part of the exospore below the gap (G) is only a narrow stripe (arrows) in this section. Bar: 1  $\mu\text{m}$ .



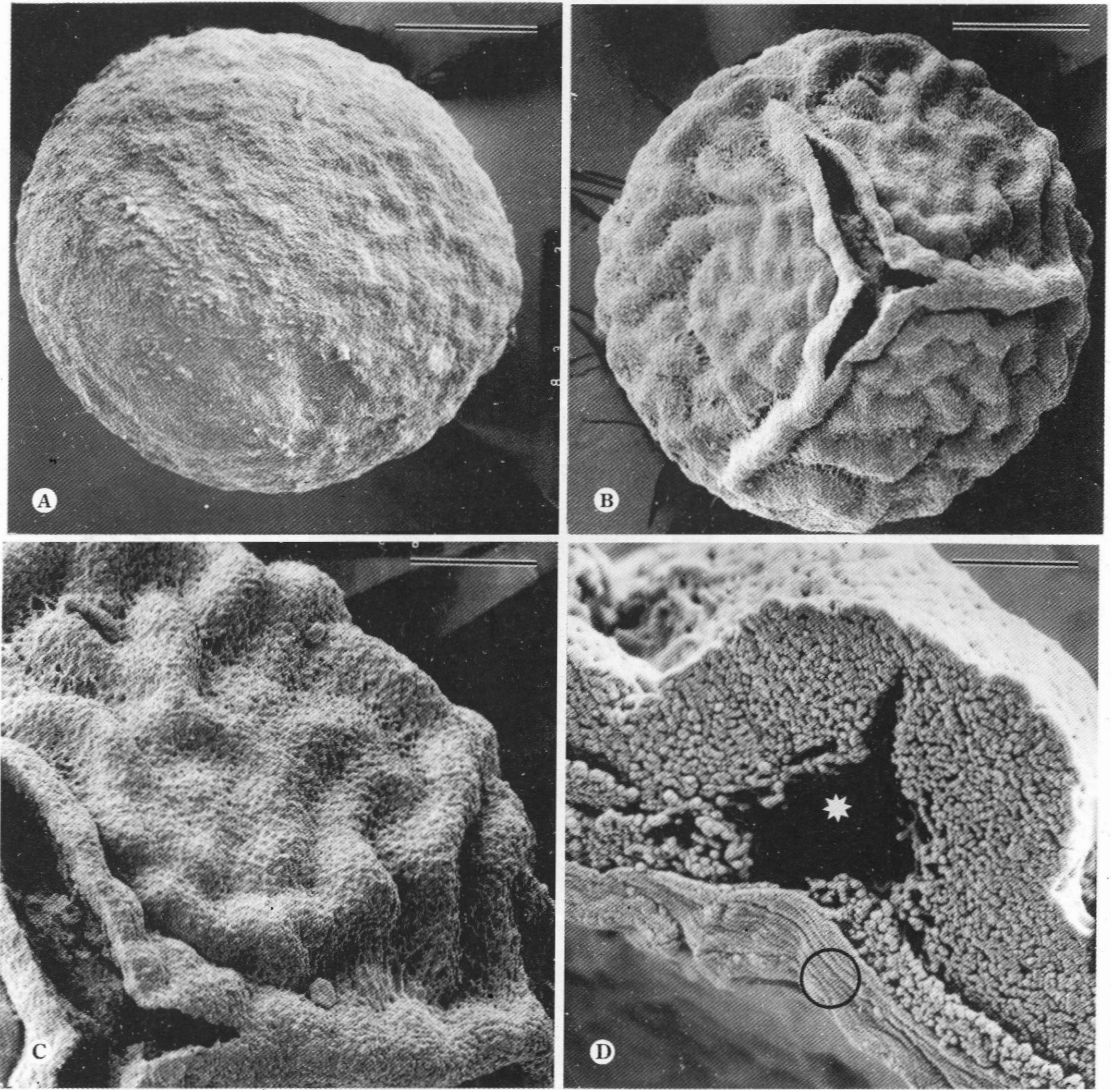
**Fig. 25.** A - G: SEM micrographs of *S. peruviana* megaspores. **A:** Equatorial view of a megaspore. The proximal pole is at the right. The ornamentation is reticulate. A microspore is located within a distal lumina. Bar: 200  $\mu$ m. **B:** A proximal view showing the reticulated muri and high laesura (arrowheads). Bar: 100  $\mu$ m. **C:** The distal surface of a megaspore. The figure shows one murus running diagonally at the right and many scattered verrucae. The granulate-verrucate surface is considered to be due to silica. Bar: 5  $\mu$ m. **D:** A megaspore sectioned in half in an equatorial plane. This mature megaspore has the inner exospore adjacent to the outer exospore all around. The high ridges of reticulations and laesurae are apparent on the proximal surface (cf. Fig. 25 B). The ridges on the distal surface differ in shape (cf. Fig. 25 A) from those proximally (cf. Fig. 25 B). Bar: 200  $\mu$ m. **E:** The outer surface at the top shows part of the triradiate branch of the aperture. Under this there are protruding exospore units. There is a fracture of the exospore across the lower portion of the figure. The arrow points the cover of silica. Bar: 5  $\mu$ m. **F:** A fracture through the whole exospore at the distal pole. The outer surface is at the top. Units are circular in section. Several loops had been sectioned. The diameter of units and spaces between them increase inwards. Bar: 1  $\mu$ m. **G:** The inner surface of the outer part of the exospore (i.e., outer surface of the former gap). It shows spheres on rod structural-units. Bar: 1  $\mu$ m.



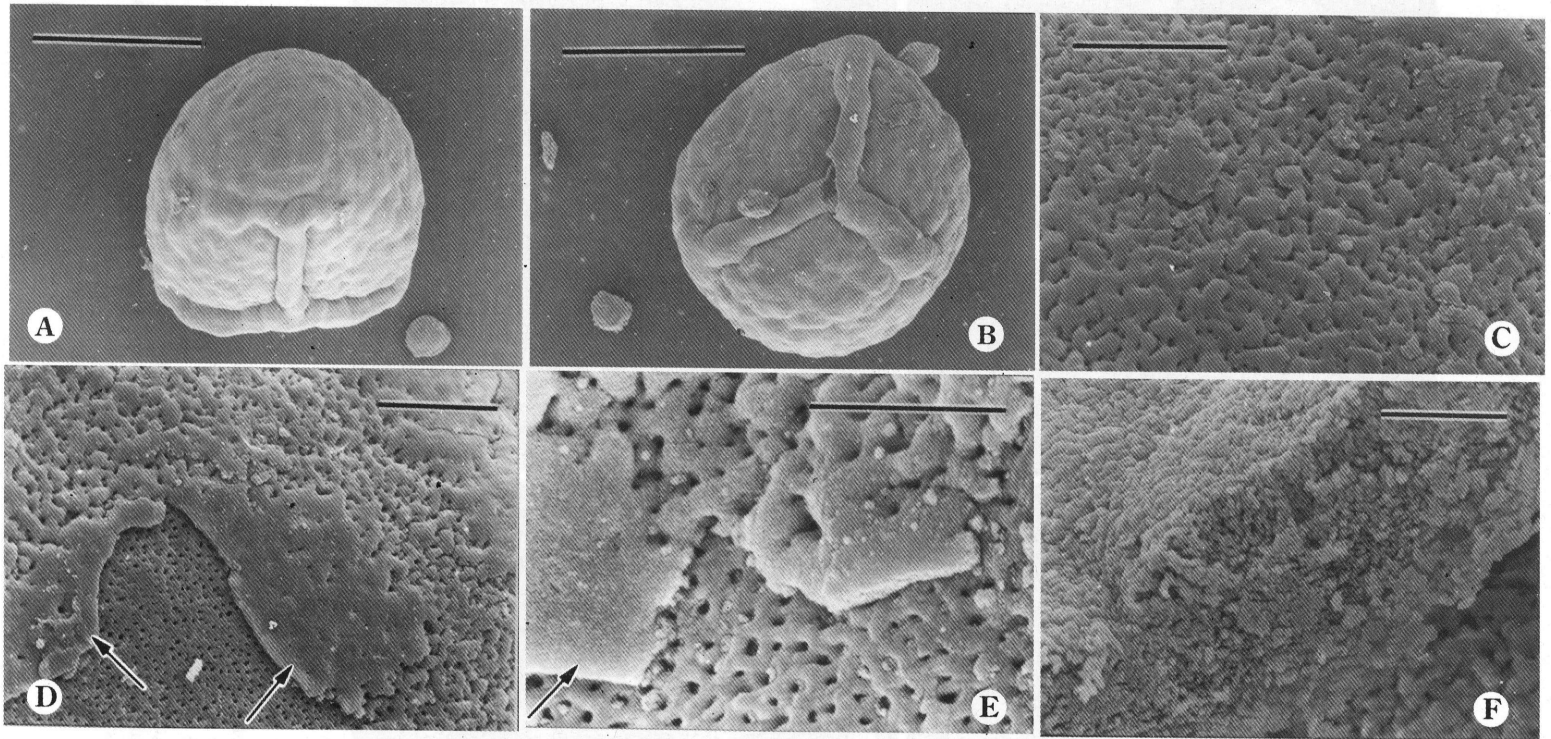
**Fig. 26. A:** TEM micrograph through the *S. peruviana* megaspore exospore near the distal pole. In the middle portion of the wall the units are thick. In some planes of section the units seem to be flattened. The spaces between units are smaller towards both outer and inner surfaces. Bar: 1  $\mu\text{m}$ .



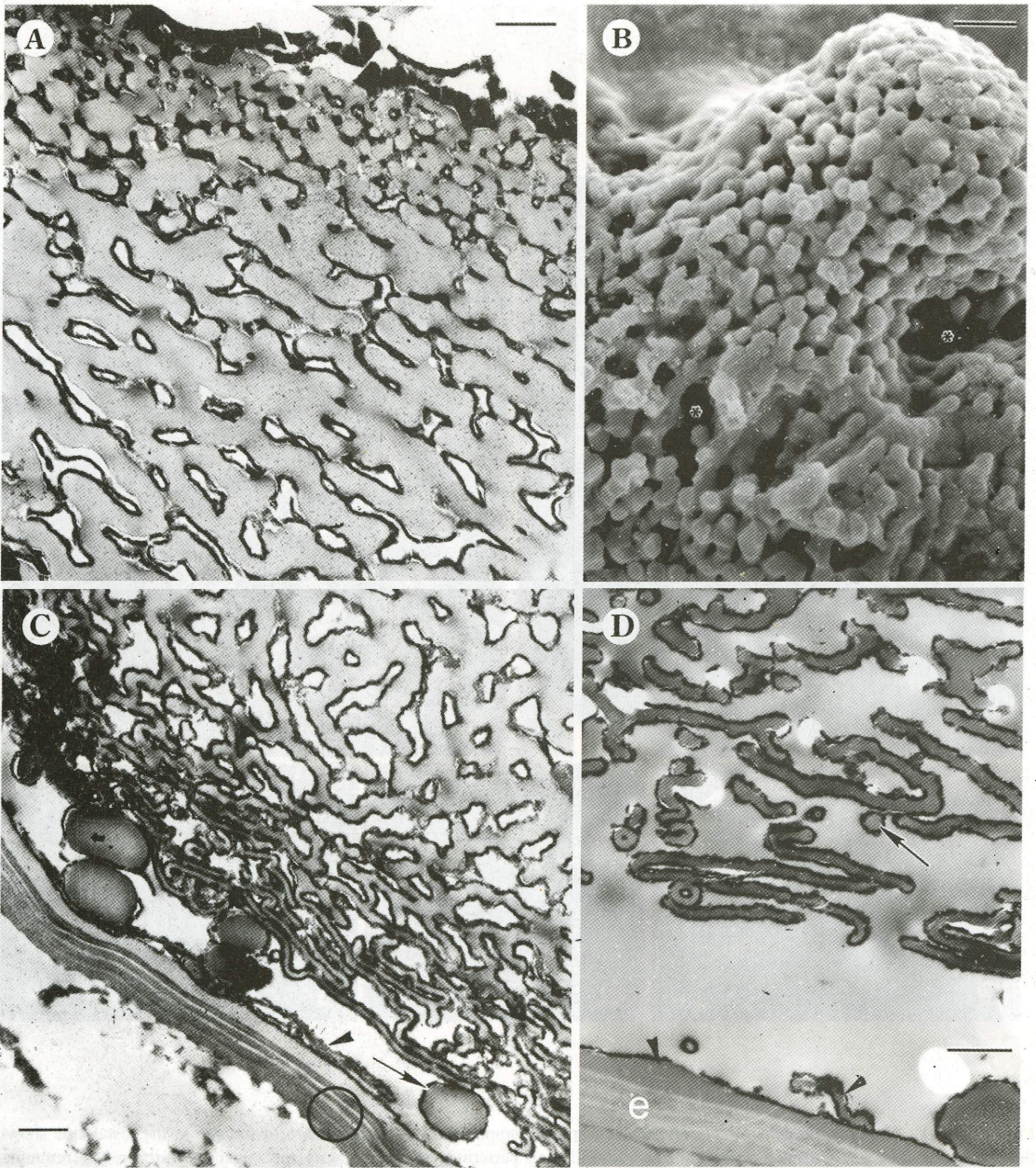
**Fig. 27.** A - F: SEM micrographs of *S. peruviana* microspores. **A:** Distal view of a microspore that is part of a tetrad. Bar: 20 µm. **B** and **C:** Micrographs of the distal surface taken at higher magnification than in **A**. Bars, **B:** 10 µm, **C:** 2 µm. **D:** Proximal view of a microspore. The exospore is proximally pleated. Pleats are radially oriented in each proximal facet. Laesura branches are short. The surface is granulate. Bar: 10 µm. **E:** Proximal view. The surface is covered by granules. Bar: 10 µm. **F:** Equatorial view. The proximal facet is pleated. The surface is coarsely granulated. Bar: 10 µm.



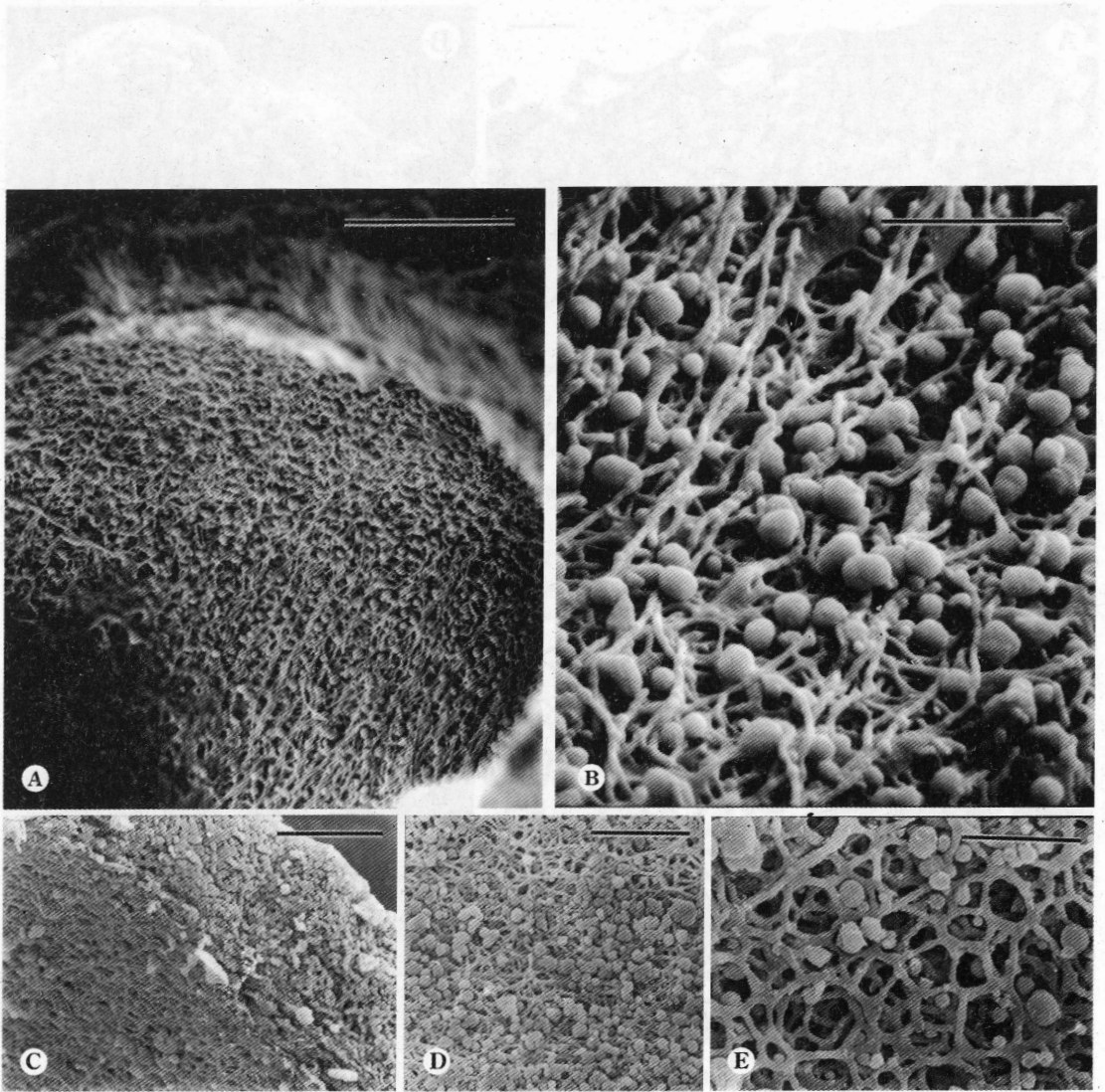
**Fig. 28. A - D:** SEM micrographs of *S. sellowii* megaspores. **A:** The distal surface is weakly rugulate. Bar: 100  $\mu$ m. **B and C:** On the proximal surface the exospore is strongly rugulate. There are perforations of small diameter over the whole surface and relatively large perforations in the valleys between the ridges (see Fig. 28 C). The crassimarginate laesura is partially open. Bars: 100  $\mu$ m and 50  $\mu$ m. **D:** Megaspore sectioned through the exospore and endospore in the laesura region. The exospore is thickened at margins of the laesura (crassimarginate). The laesura chamber between the margins is marked by a star. The multilayered endospore (circled) is across the bottom of the figure. Bar: 50  $\mu$ m.



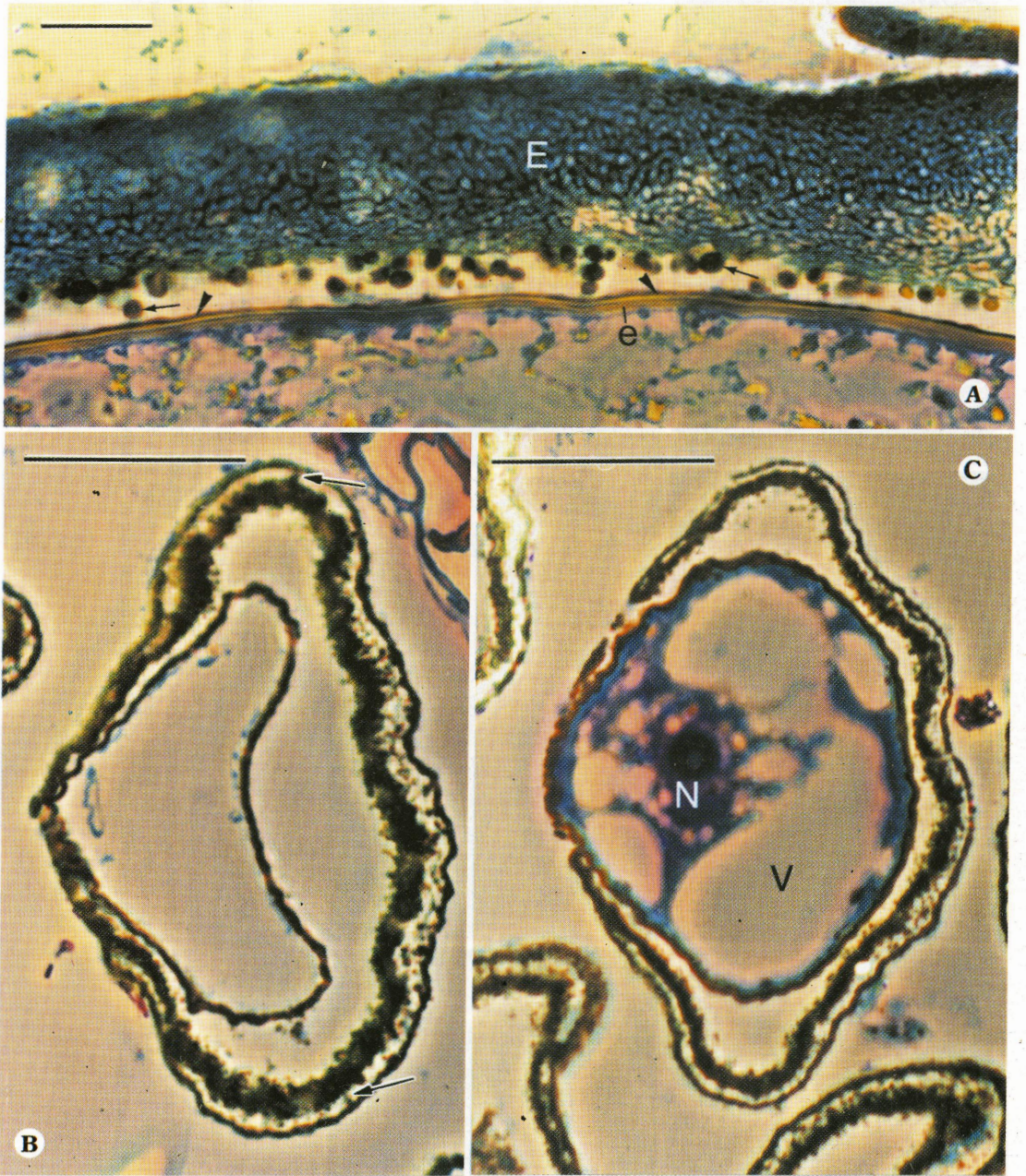
**Fig. 29. A - F:** SEM micrographs of *S. sellowii* megaspores. **A:** A general view of a mega and a microspore in equatorial and distal views respectively. The megaspore surface is rugulated and the laesurae are crassimarginated. Bar: 200  $\mu\text{m}$ . **B:** A megaspore in proximal view and several microspores. The megaspore ornamentation on proximal surface is rugulate. Bar: 200  $\mu\text{m}$ . **C:** The megaspore ornamentation consists of low and broad ridges covered by rough-textured material that is perforated. Bar: 10  $\mu\text{m}$ . **D and E:** Under the SEM beam the textured and perforated surface material was occasionally folded back (arrows) revealing the relatively smooth but much perforated surface of the exospore. The textured surface layer that can be separated is at least, in part, of silica. Bar: 10  $\mu\text{m}$  and 5  $\mu\text{m}$ . **F:** A fracture across the exospore. The outer surface is at the top (compare with the surface in Fig. 29 C). Bar: 10  $\mu\text{m}$ .



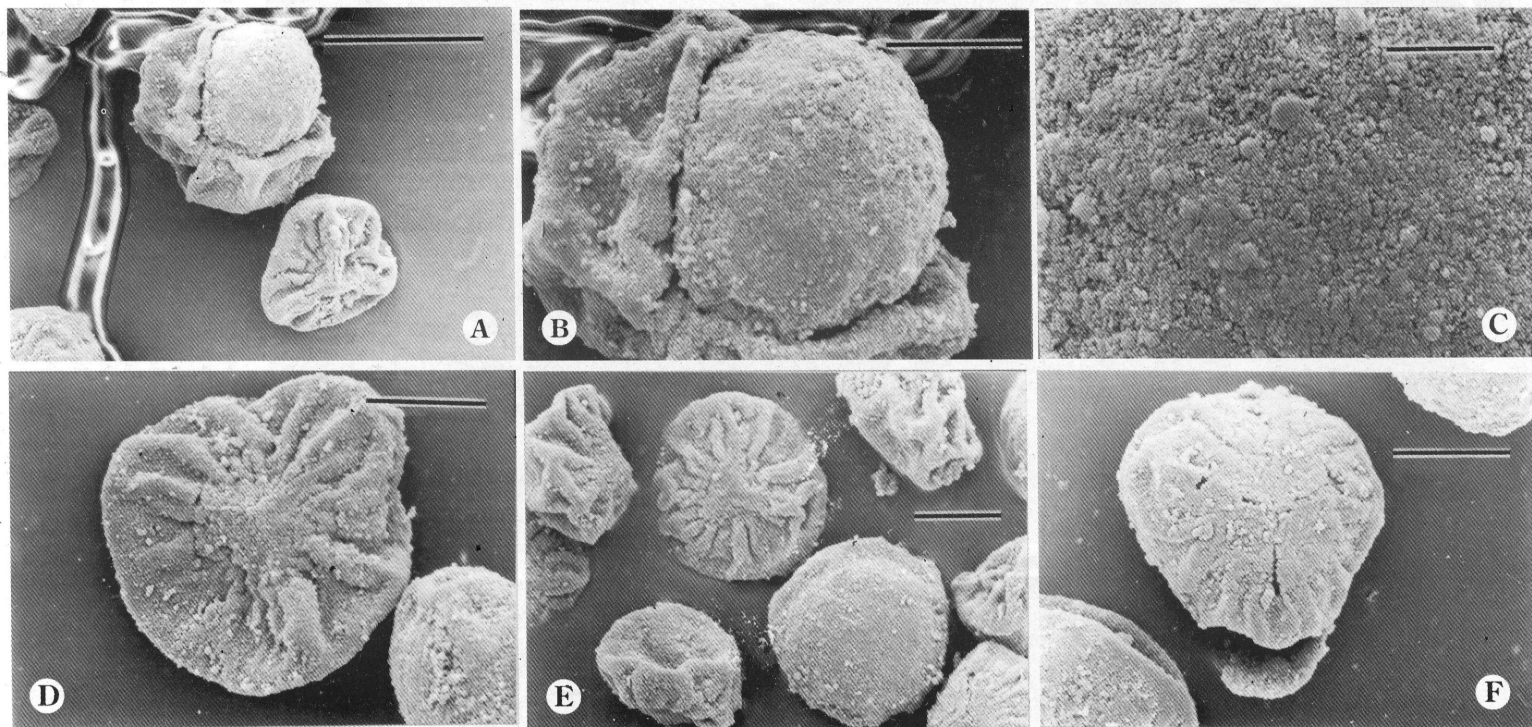
**Fig. 30.** A - D: TEM micrographs of *S. sellowii* megaspores. **A:** TEM micrograph of a section showing an outer portion of the exospore with a layer of silica. The diameter of the exospore units decreases inward from the surface. The distance between units increases in the same direction. There is silica, seen as dark in TEM micrographs, on the outer surface and on the edges of units. Silica is also deposited on the units. Stain: UA-Pb. Bar: 1  $\mu$ m. **B:** SEM micrograph of rugulate ridge on a *S. sellowii* megaspore. Many exospore units can be seen on end at the top and side of the ridge. There are large openings (asterisks) at the base of the ridge (please see Fig. 28 C). Bar: 2  $\mu$ m. **C:** TEM micrograph of a transverse section of a megaspore wall. The diameter of the exospore units in this unordered wall is greatly diminished toward the inner surface. This inner surface is, however, the outer surface of the gap remnant. This remnant of the gap holds spheres (arrow) which are attached to either outer and inner surface of the gap remnant. The inner surface (arrowhead) of the gap remnant is adjacent to the endospore (circled). The dark material bordering exospore units and the spheres is silica. Bar: 1  $\mu$ m. **D:** A detail of the inner part of the megaspore wall. The very slender rods next to the outer wall of the gap can be seen laterally and in section (arrow). The inner surface of the gap (arrowheads) remnant is very thin and discontinuous. The endospore is marked "e". Stain: Sp only. Bar: 1  $\mu$ m.



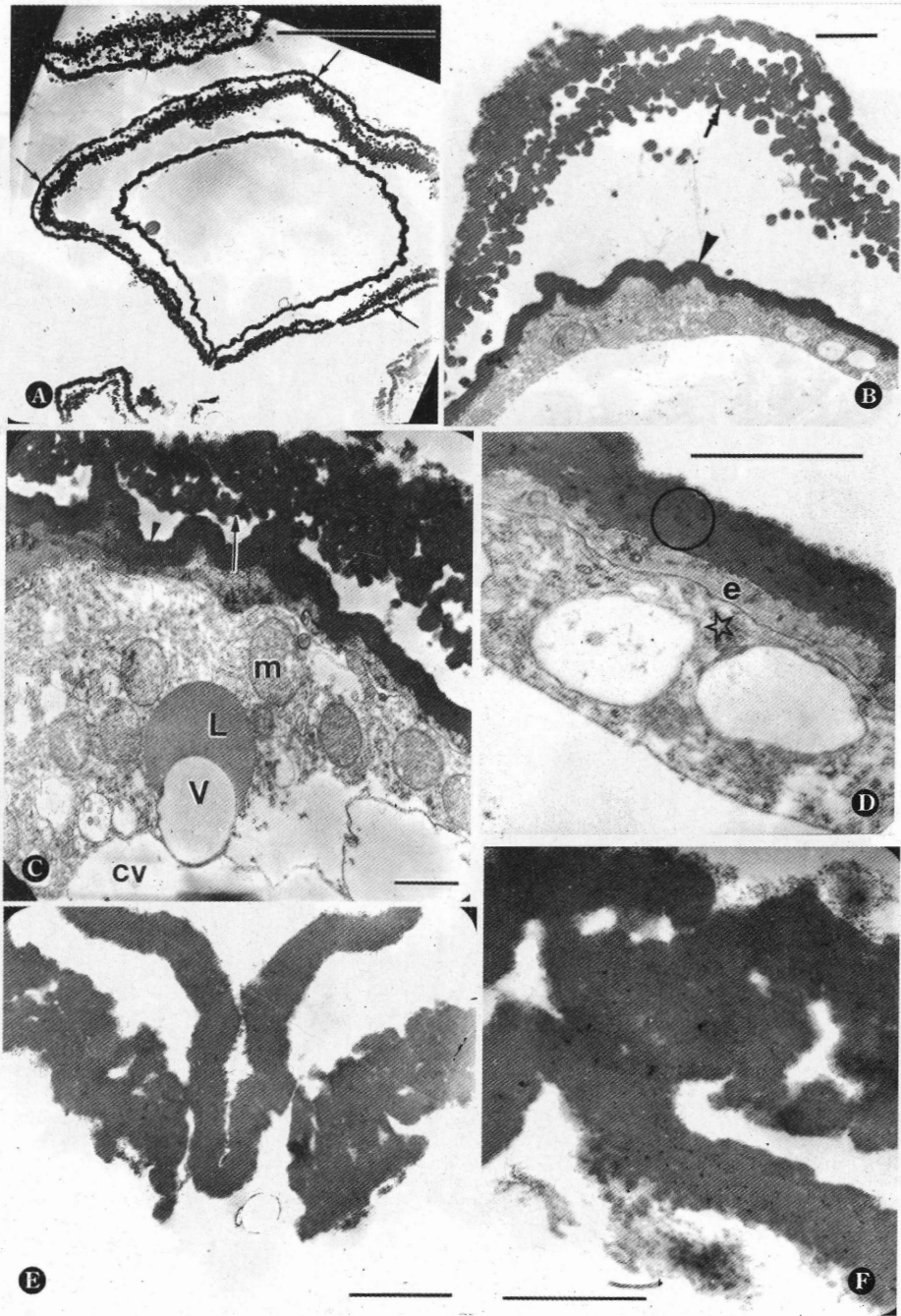
**Fig. 31.** A - E: SEM micrographs of *S. sellowii* megaspore components. A: Outer surface of the gap at the distal side showing the large area that was within the gap and the complicated pattern involving spheres and exospore units in this remnant of the gap. The outer exospore and megaspore surface is out of focus at the top. Bar: 20  $\mu$ m. B: The surface of part of Fig. 31 A at higher magnification. Many exospore units near the surface of the image run more - or - less parallel; these may have been stretched across the gap (please see Figs. 47 and 48 in Rowley & Morbelli, 1995). Bar: 5  $\mu$ m. C: Section through the outer part of the exospore at the distal pole. The outer surface of the former gap is at the bottom. Bar: 10  $\mu$ m. D and E: The outer surface of the former gap that illustrates the 3-D character of the spheres and units of the exospore. Bars, D: 10  $\mu$ m and E: 5  $\mu$ m.



**Fig. 32. A - C:** LM photomicrograph of transverse sections of mega and microspores of *S. sellowii*. **A:** Thick (ca 5-10  $\mu\text{m}$ ) transverse section of a megaspore wall near the distal pole. The section shows the unordered exospore (E) and spheres (arrows) within the gap remnant. A thin and disjoint exospore stripe (arrowheads) is the remnant of the inner exospore. It is attached to the multilayered endospore (e). The blue/green color after toluidine blue staining indicates the presence of polyphenolic compounds. Bar: 10  $\mu\text{m}$ . **B and C:** Almost mature microspores. Sections stained with toluidine blue. Section thickness ca 3  $\mu\text{m}$ . Bar: 20  $\mu\text{m}$ . **B:** The section shows the microspore in equatorial view. In the outer exospore there are columellar-like rods (arrows). The inner exospore is near the outer exospore at the proximal pole. See TEM of a microspore section on a similar plane in Fig. 34 A. Bar: 20  $\mu\text{m}$ . **C:** The plane of sectioning is similar to that in Fig. 32 B but the cytoplasm is preserved within the inner exospore. Nucleus (N), vacuole (V). Bar: 20  $\mu\text{m}$ .



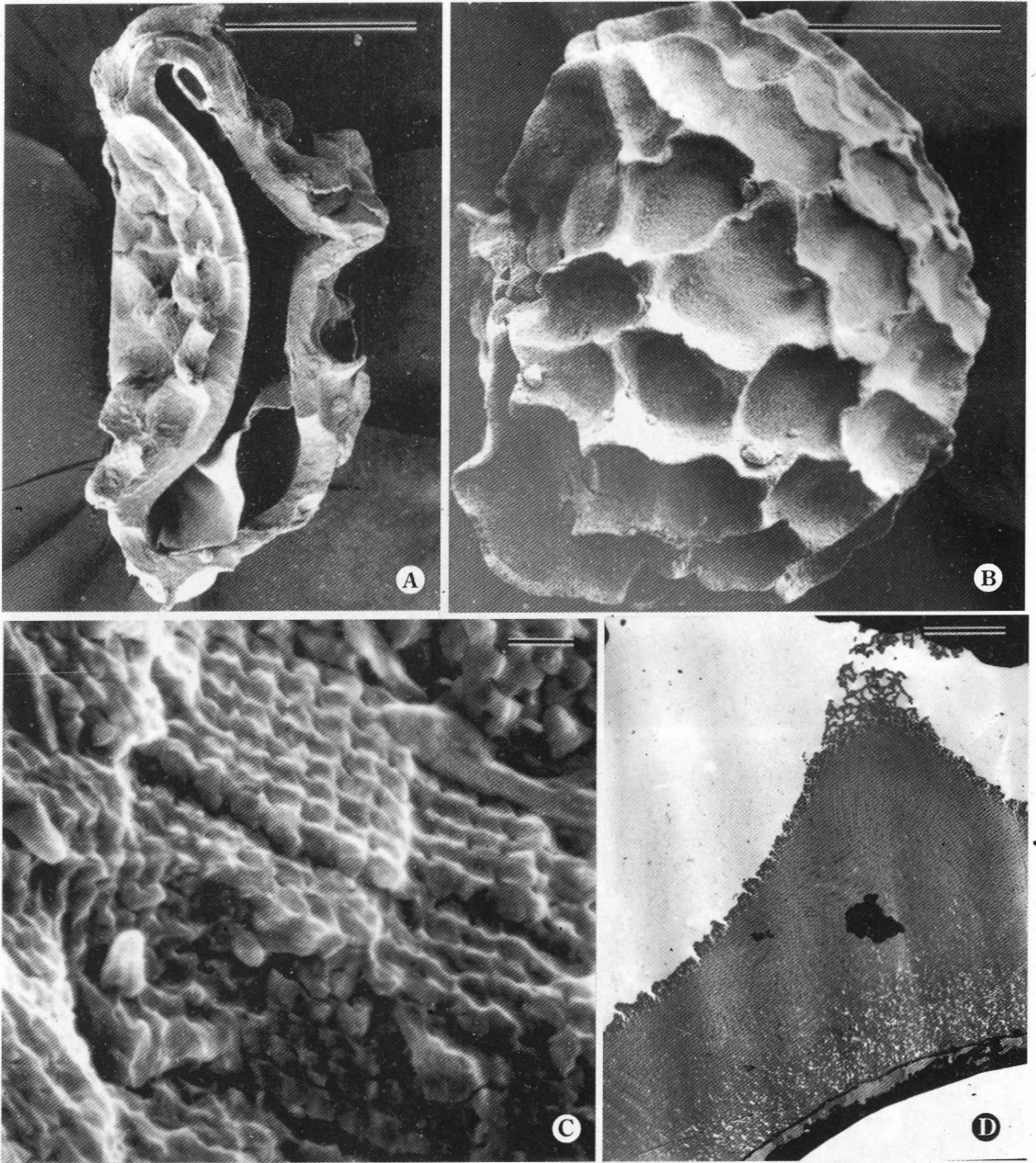
**Fig. 33. A - F:** SEM micrographs of *S. sellowii* microspores. **A:** A microspore tetrad and an isolated microspore in proximal view. The microspores in this species have short laesurae and the proximal face is wrinkled ("pleated") when dry. The distal pole has a granulated surface. Bar: 50  $\mu\text{m}$ . **B:** A detail of tetrad in Fig. 33 A, showing the distal heterogranulated surfaces of three microspores. Bar: 20  $\mu\text{m}$ . **C:** This heterogranulated ornamentation is the same over the entire microspore surface. Bar: 5  $\mu\text{m}$ . **D:** A proximal view of a microspore showing the radial distribution of the pleats. Bar: 20  $\mu\text{m}$ . **E:** Microspores in proximal, distal and equatorial orientations. The distal surface is granular and the equatorial margin is broad. The proximal view shows the pleated nature of the proximal pole. The pleats have a radial distribution and as a result the equatorial outline appears undulated. Both the proximal pleating and equatorial margin are pronounced in this dry condition. Bar: 20  $\mu\text{m}$ . **F:** The proximal side of this spore is less collapsed than those in, e.g., Fig. 33 D, E, and the pleating is much less. Bar: 20  $\mu\text{m}$ .



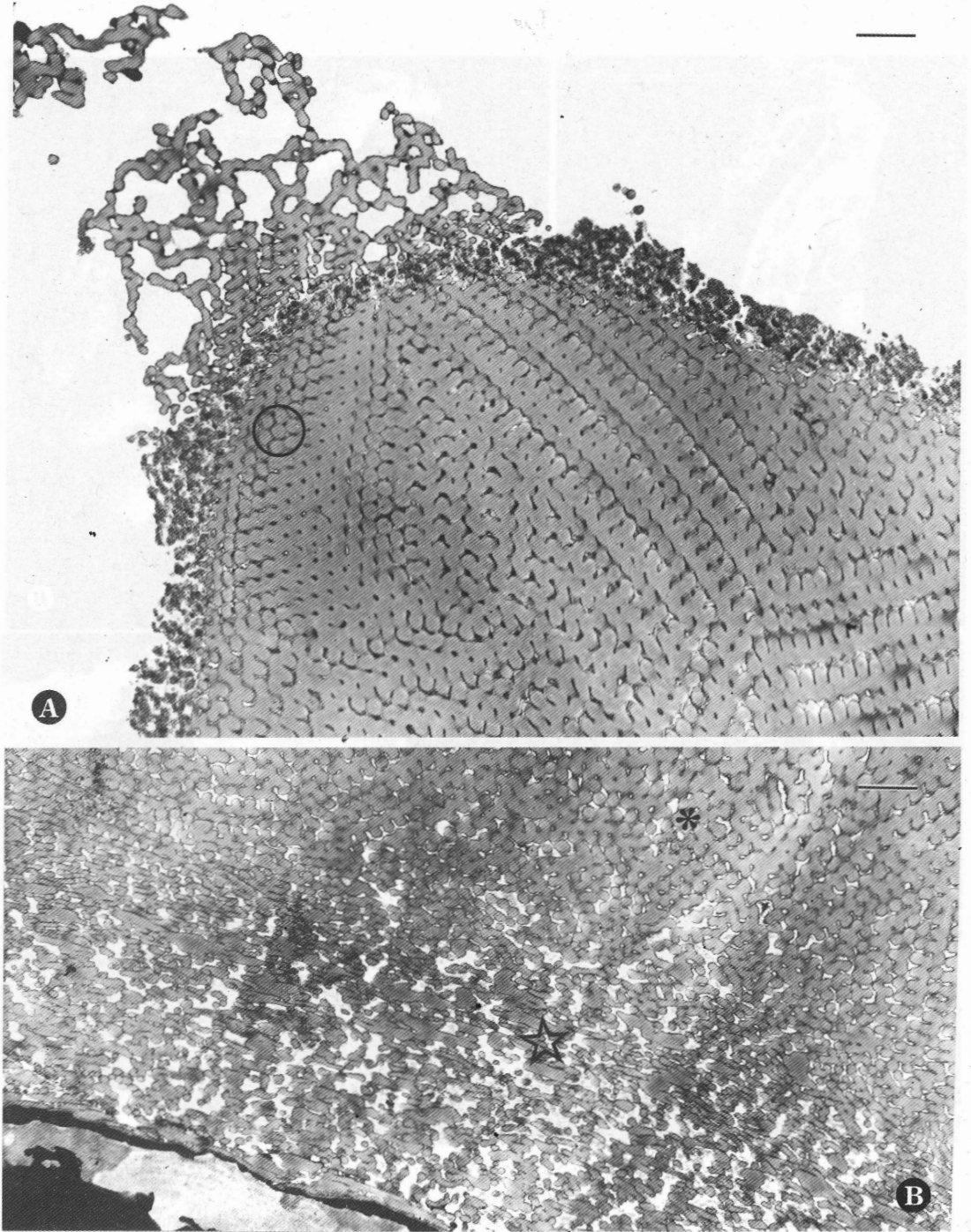
**Fig. 34.** A - F: TEM sections of *S. sellowii* microspores at a late stage of development. Stain: UA-Pb. **A:** Section showing distal, equatorial and proximal regions. In the outer exospore there are columellar-like rods (arrows). The inner exospore is near outer exospore at the proximal pole (see Fig. 34, E). See microspore section on a similar plane in Fig. 32 B. Bar: 20  $\mu$ m. **B:** In the equatorial sector the outer (arrow) and inner (arrowhead) exospore are separated. The units of the outer exospore are circular in cross-section. Bar: 2  $\mu$ m. **C:** Section near the proximal pole. The outer (arrow) and inner (arrowhead) exospore are in contact or close. The cytoplasm shows many mitochondria (m), lipoidal globules (L) and vesicles (V). The central vacuole (CV) is large (see Fig. 32 C). Bar: 1  $\mu$ m. **D:** The inner exospore is adjacent to the cytoplasm (star) and the endospore (e). The inner exospore has many contrasted sites (circled) like those in the outer exospore (see Figs. 34 E, F). Bar: 1  $\mu$ m. **E:** Inner exospore protrudes in this section between the proximal opening in the outer exospore. Bar: 1  $\mu$ m. **F:** Contact between the inner and outer exospore in the proximal region. Bar: 0.5  $\mu$ m.



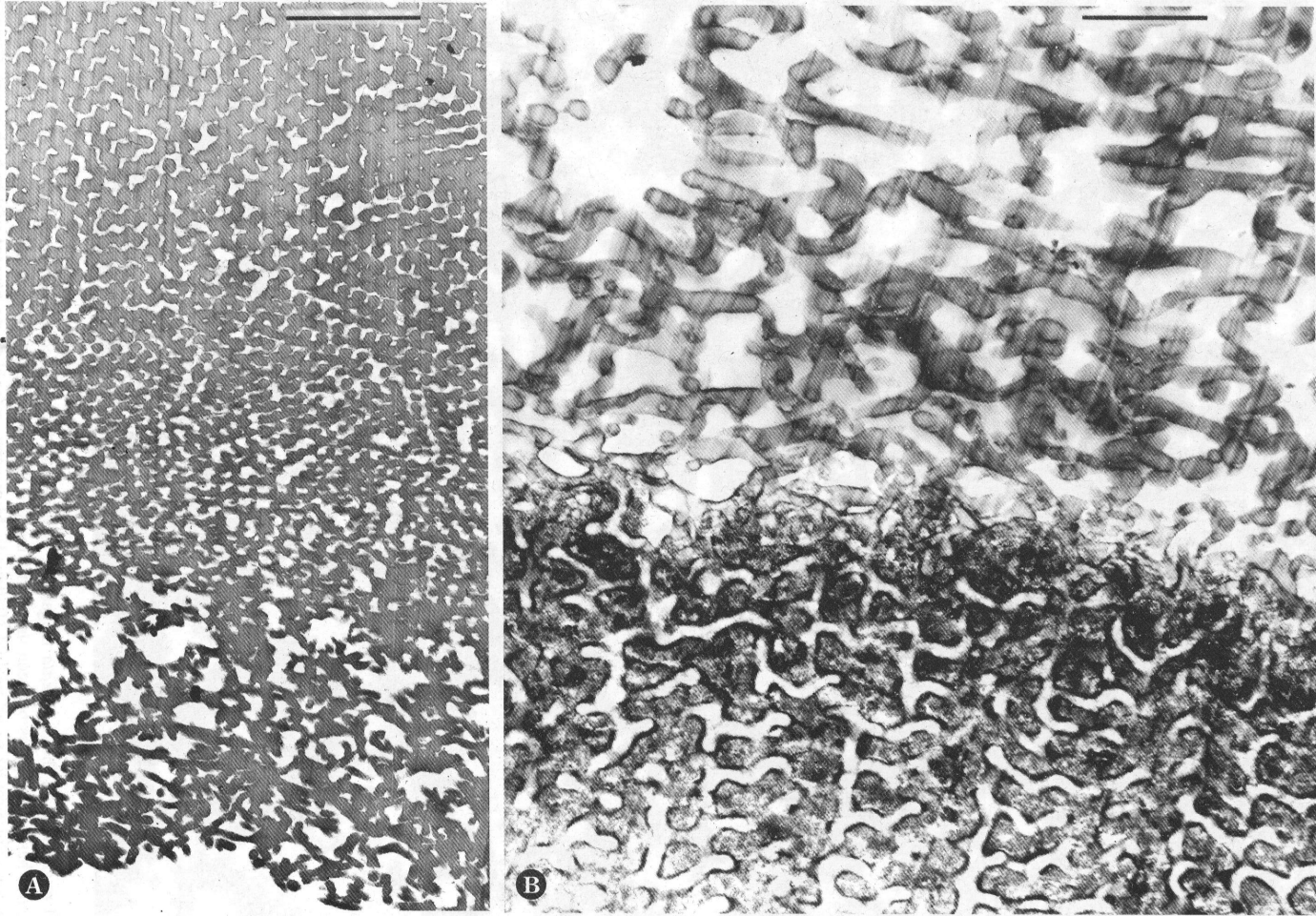
**Fig. 35. A - C:** TEM micrographs of *S. sellowii* microspores. **A and B:** A surface coating is apparent on the outer part of the exospore. This is the surface that is positive for polyphenols (green in toluidine blue) in Fig. 32, **B** and **C**. There are many dark sites ("microchannels") within the exospore. Bars, 5  $\mu\text{m}$  and 1  $\mu\text{m}$ . **C:** Inner part of the exospore. Like the outer part of the exospore, this has many darkly contrasted sites which are equivalent to microchannels. Bar: 0.1  $\mu\text{m}$ .



**Fig. 36. A - D:** SEM micrographs of *S. sulcata* megaspores. **A:** A megaspore sectioned in half. The surface is reticulate. The inner part of the exospore is thin and attached to the outer part of the exospore at the laesura but detached elsewhere. Bar: 200  $\mu\text{m}$ . **B:** An equatorial view of a whole megaspore. The ornamentation is reticulate with broad lumina and thin, sinuous muri. Bar: 200  $\mu\text{m}$ . **C:** A fracture of the exospore at the distal pole. The outer surface is at the top. The exospore units are orientated in different planes. Bar: 1  $\mu\text{m}$ . **D:** A section of the exospore, including a murus near the proximal pole. The tip of the murus has unordered units but most of the exospore consists of highly ordered units. There is a change again to the unordered condition toward the inner surface. Bar: 10  $\mu\text{m}$ .



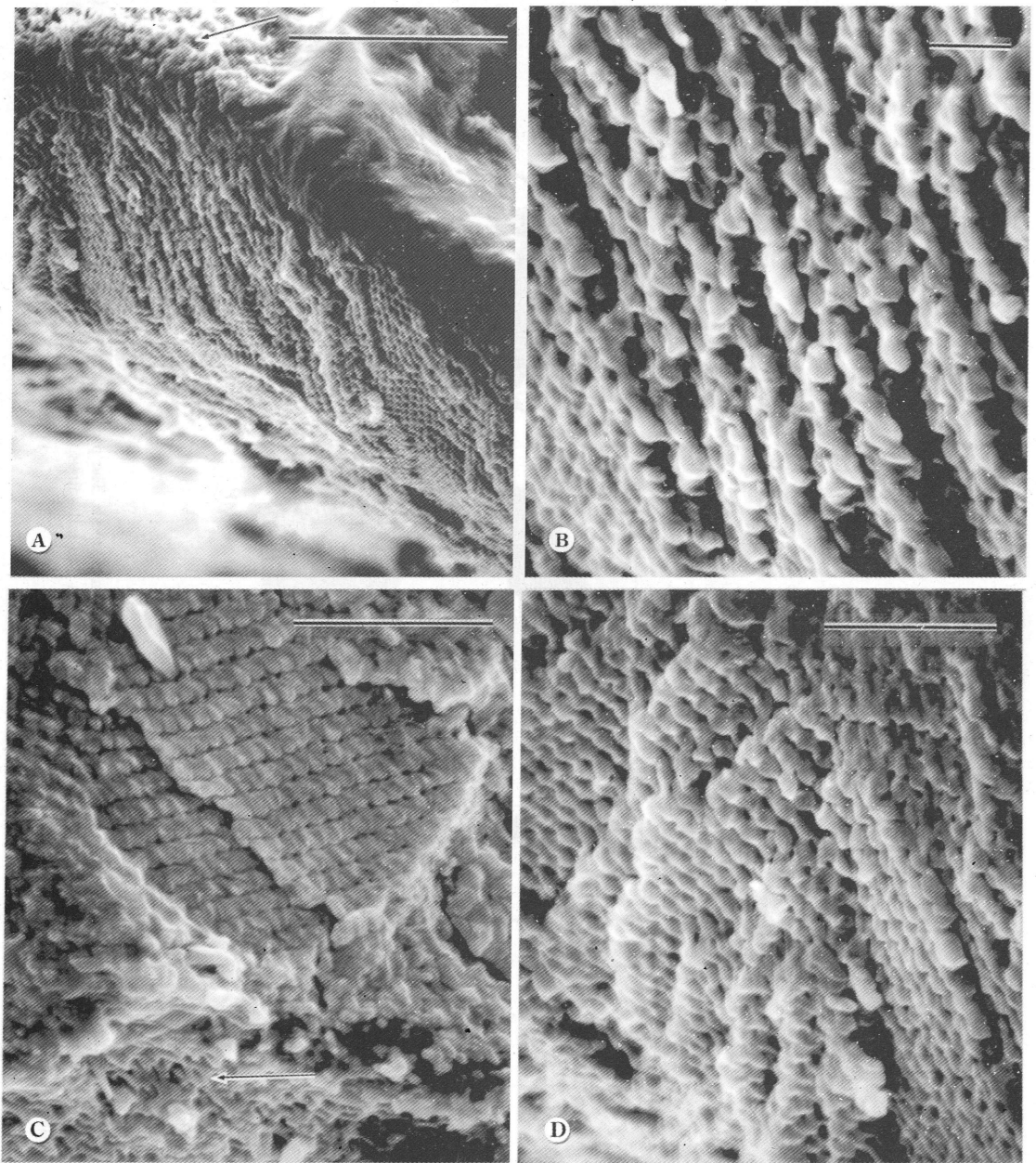
**Fig. 37. A - B:** TEM micrographs through the megaspore exospore of *S. sulcata*. **A:** The structural elements are highly ordered and close packed below the outer surface of this murus. Silica covers the units of the outer surface. There is silica (dark) on the surface of the units. Where the section passes through units arranged perpendicular to the plane of the section it can be seen that their units are hexagonally close packed (see circled region). Bar: 2  $\mu\text{m}$ . **B:** The inner portion of the exospore at the proximal pole. Different structural zones can be seen from the outer (see Fig. 36 D and upper part of this figure) ordered zone (asterisk) to the unordered (star) inner part of the exospore. The diameters of the structural elements in section change from the outer (where they are larger) to the inner parts of the wall (where they are smaller). The spaces between units are wider in the inner part of the wall. Bar: 2  $\mu\text{m}$ .



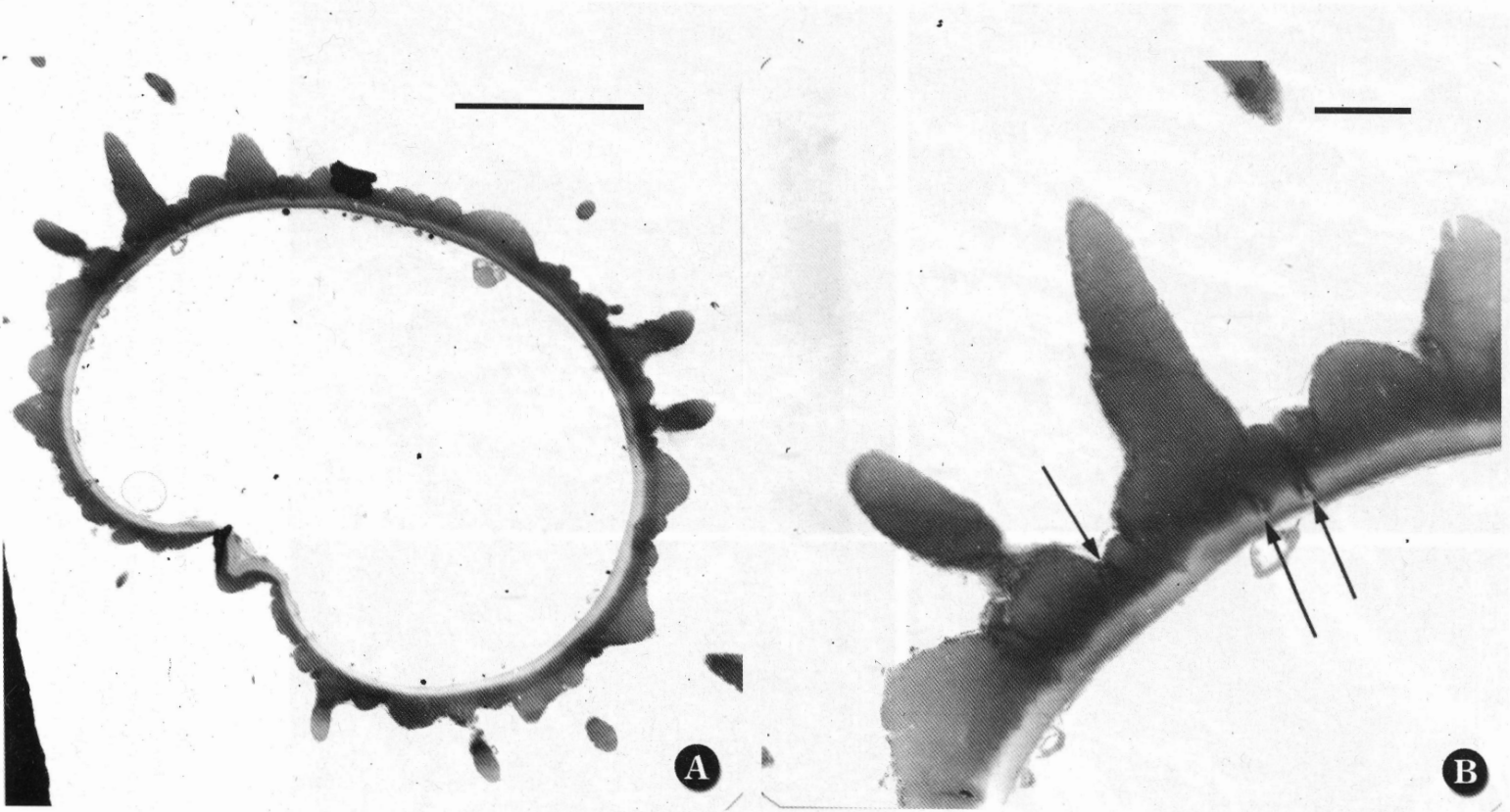
**Fig. 38. A - B:** TEM micrographs of cross sections of the exospore of *S. sulcata* megaspores. **A:** The ordered middle portion of the exospore grading downward in the figure to less wide unordered units. Stain: UA-Pb. Bar: 10  $\mu$ m. **B:** The outer portion of the exospore. The outermost levels of the exospore have a very open unordered structure. Inwards from these open structures the units are densely packed and arranged in a highly ordered pattern. Granules of silica fill spaces between units in the outer portion of the ordered exospore (see Fig. 37 A). Stain: PA - PTA-C. Bar: 1  $\mu$ m.



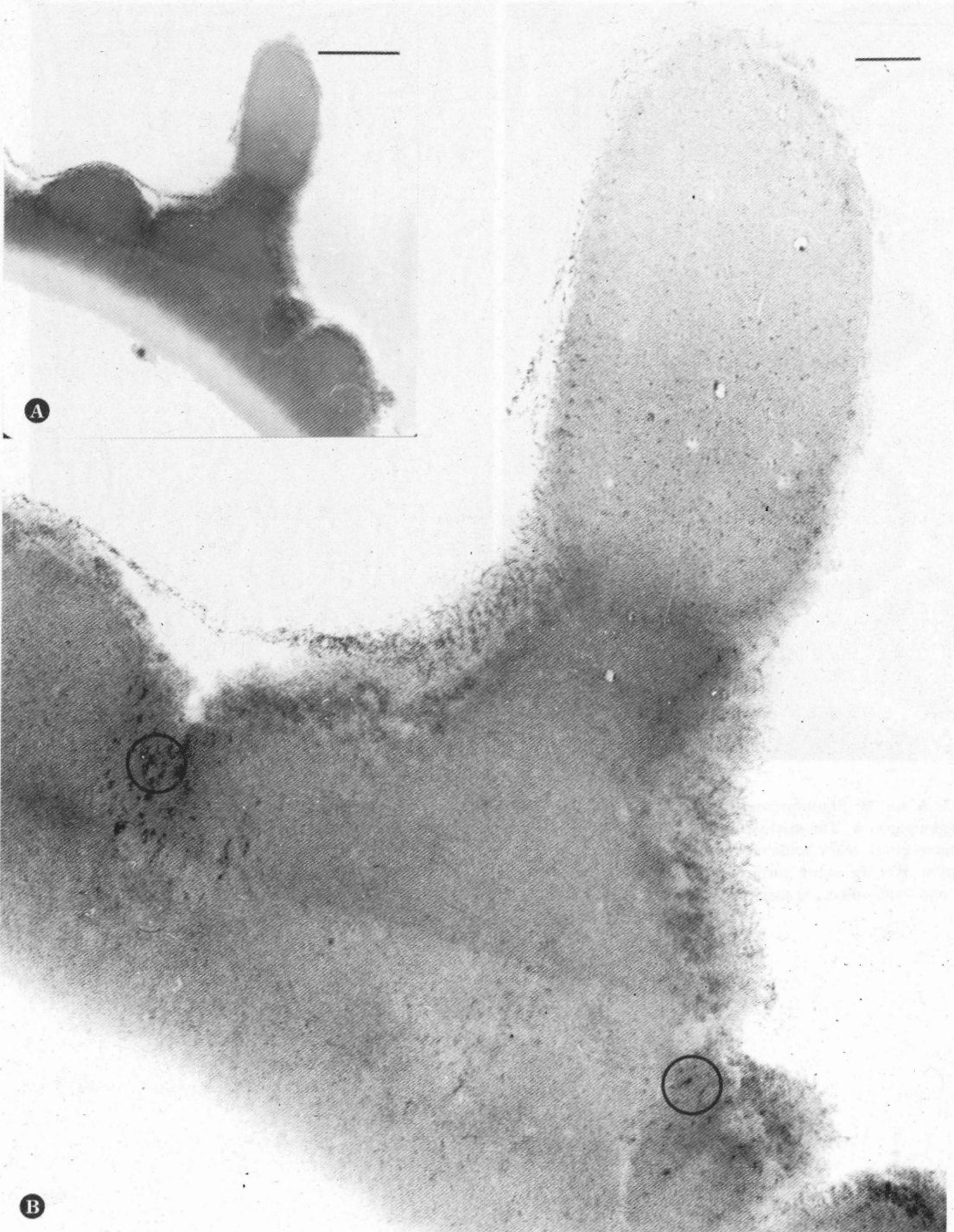
**Fig. 39. A:** TEM micrograph of the *S. sulcata* megaspore exospore at the ordered/unordered transition. The unordered zone (lower half of figure) has wider spaces between units. The units in the ordered part are circular in cross section while the units of the unordered zone are circular to ellipsoidal (flattened). Mini-gaps (asterisks) are present within both zones. Stain: Sp. Bar: 1  $\mu$ m.



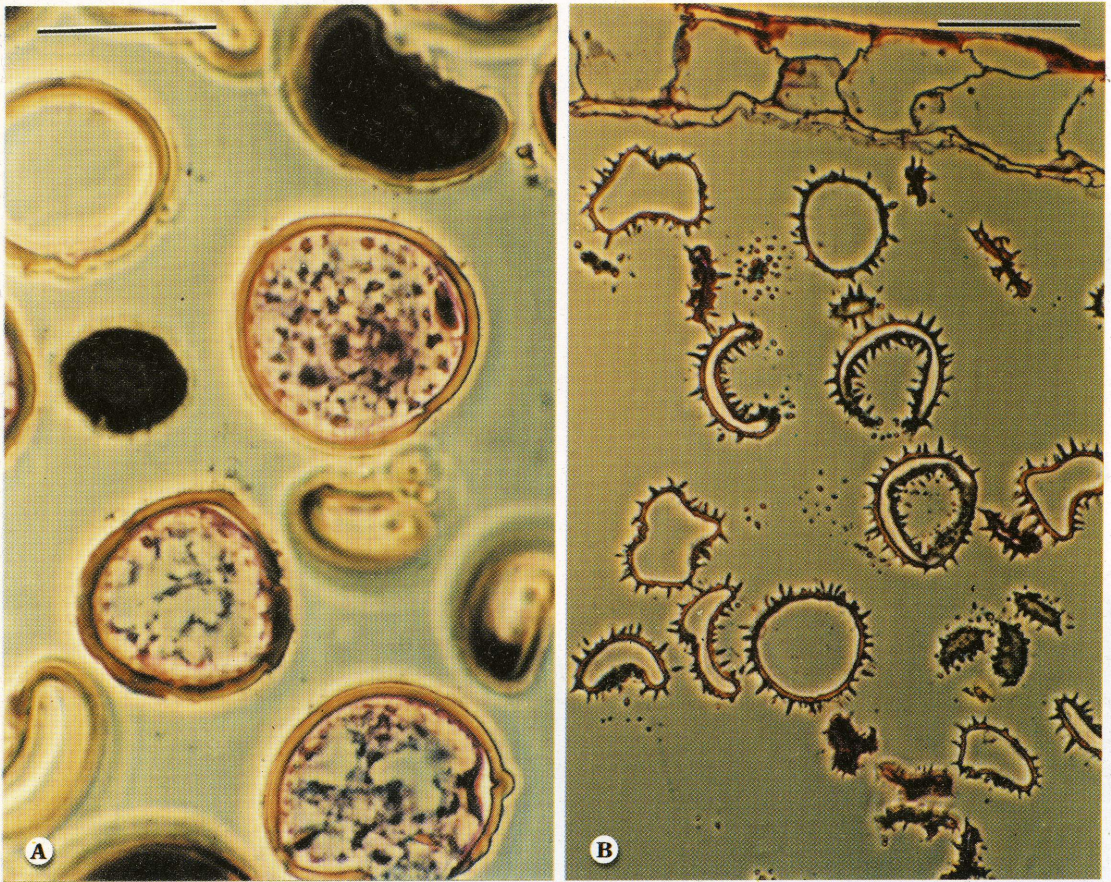
**Fig. 40.** A - D: SEM micrographs of fractured mature *S. sulcata* megaspores. **A:** The exospore at the distal pole. The outer perforated (arrow) surface is at the top. The units show different planes of orientation. Bar: 10  $\mu$ m. **B:** Exospore units as seen laterally. The attachment between units seem to be stronger laterally since they tend to remain together in that orientation. Bar: 1  $\mu$ m. **C:** The highly orientated arrangement of units is evident in this fracture. The transition to the unordered (inner) zone is at the bottom of the figure. Units in the unordered zone can be seen to be slender (arrow). Bar: 5  $\mu$ m. **D:** The ordered zone of the exospore showing groups of units in different orientations. Gaps are evident where groups of units change orientations. Bar: 3  $\mu$ m.



**Fig.41. A - B:** TEM micrographs of *S. sulcata* microspores. Stain: UA-Pb. **A:** A section through an entire microspore. The processes show several shapes as seen in thin section (See Fig. 43, B). The inner part of the exospore is only weakly contrasted. Bar: 5 µm. **B:** The section includes two tall spines, one that is club-shaped, and several verrucae. There are conduits (arrows) that traverse the wall. Bar: 1 µm.



**Fig. 42. A - B:** TEM micrographs of *S. sulcata* microspores. Stain: UA-Pb. **A:** Section of the exospore. Bar: 0.5  $\mu\text{m}$ . **B:** Same section but at a higher magnification and printed lighter. There is a complex exospore surface coating that is shown best in the thin part of the section at the base of the club-shaped spine. There are regularly arranged dark (ca 10 nm wide) spots near the left margin of the spine. In thicker parts of the sectioned spine there are such spots throughout but without any apparent regular order. Dark spots and radially oriented dark files (circled areas) of the above size are common in the inner part of the exospore below the spine. These are considered to be subunits that are 10 - 15 nm in diameter. Bar: 0.1  $\mu\text{m}$ .



**Fig.43. A and B:** Photomicrographs of ca 3  $\mu\text{m}$  thick sections of *S. novae-hollandiae* and *S. sulcata* microspores within microsporangia. **A:** The microspores of *S. novae-hollandiae* have a relatively smooth surface. Part of the laesura appears on two microspores. Stain: toluidine blue. Bar: 20  $\mu\text{m}$ . **B:** The microsporangial wall is evident at the top. The spiny ornamentation is similar over the entire surface. In TEMs it can be seen that the microspore wall is composed by two layers and that the outer one, with spines, is denser to electrons (e.g., Fig. 42 A) than the inner layer. Bar: 20  $\mu\text{m}$ .