

Spore morphology of the European species of *Phascum* Hedw. (Pottiaceae, Musci)*

by

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Abstract: A study of spore size, polarity and shape in the European species of *Phascum* has been made. The spore wall ultrastructure was also studied. Morphological and morphogenetical observations under LM, SEM & TEM are described. Afterwards the spore ornamentation is discussed from a taxonomic point of view.

Introduction

The genus *Phascum* Hedw. (Pottiaceae) is included in the subfamily Pottioideae (Saito 1975) together with *Pottia* Ehrh. (the two genera are taxonomically very close) and other genera such as *Acaulon* Müll.

Phascum is widely distributed throughout both hemispheres, but most of the species are represented in the Northern Hemisphere. Until recently only four species were known in Europe: *Phascum curvicolle* Hedw., *P. cuspidatum* Hedw., *P. floerkeanum* Web. & Mohr and *P. cunnetii* Biz. & Pierr. (Corley & al. 1981). However in the last two years another two species have been found in the Iberian Peninsula: *Phascum vlassovii* Laz. known until now from Asian and American localities (Jiménez, Ros & Guerra (1989) and *P. longipes* Guerra, Martínez & Ros from the southeast of Spain (Guerra et al. 1990).

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Detailed study of the structure and ornamentation of the spore wall has been scarcely used in the morphology and taxonomy of mosses, probably due to its difficulty. After the pioneer work of McClymont (1955), Erdtman (1957, 1965) and Afzelius (1957) other studies were made, in which electron microscopy was used regularly (Larson 1964, McClymont & Larson 1964, Gllvag 1967, Monroe 1968, Sorsa & Koponen 1973, Mueller 1974, Vitt & Hamilton 1974, Hirohama 1977a and b, Koponen 1977, Brown & Lemmon 1980a, b, 1981, 1984, 1985). Saito & Hirohama (1974) studied some Pottiaceae species with light and scanning electron microscopy. They found that spore morphology does not follow the same patterns of differentiation as those established in the traditional systematics of the family. They also observed in a recurrent way the verrucate, gemmate, multistalked verrucate, granulate, rugulate, baculate, convolute and extervermiculate sculptural pattern. No *Phascum* species were studied in this work. Lewinsky (1974) did not find a relation between spore ornamentation in *Tortula* Hedw. (Pottiaceae) and the taxonomy of the genus. McClymont & Larson (1964) studied sporoderm structure under transmission electron microscopy in three species of Pottiaceae: *Weissia viridula* (L.) Hedw., *Astomum phascoides* (Hook.) Grout and *Phascum cuspidatum* (Schreb.) Hedw. and observed that the rate of contribution of the exinic layer to spore ornamentation is always very small and that the processes are made up almost exclusively of a very electron-dense material of a perinic nature.

From a taxonomic point of view, spore attributes have been scarcely used in *Phascum*. Bruch, Schimper & Gmbel (1849-1850) illustrated some spores of the genus. Dixon (1924) described the spores of *Phascum floerkeanum* and *P. curvicolle* as smooth or hardly granulate. Smith (1978) used the spore size as a secondary character for the delimitation of some species. In the SEM study of Derrick (1978) a great variety of ornamental elements were found: verrucae, gemmae, pilae, echinae, etc. This author used them to differentiate between the subgenus *Phascum* (*Euphascum* Limpr., which includes *P. cuspidatum*) with echinate or baculate spores and the subgenera *Pottiella* Limpr. (including *P. curvicolle*) and *Microbryum* Schimp. (including *P. floerkeanum*), both with smooth or verrucate spores.

In this work the spore morphology of European species of *Phascum* has been studied. Light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used.

Materials and methods

All the material used in this study was removed from dried herbarium specimens. The various herbaria are mentioned in the list of moss material investigated. Identifications were confirmed before the studied material was chosen. The taxonomic nomenclature is that of Corley et al. (1981).

Before making this microscopic study, a methodological test was carried out. Mature spores of *P. cuspidatum* and *P. curvicolle* were subjected to different treatments: a) acetolysis (Erdtman 1960), b) heating in 10% NaOH for 5 minutes at 80 °C, c) consecutive washing in ethyl alcohol series (50%, 60%, 75% and 90%), d) washing in distilled water. Mounting and observation of the spores so treated showed that shaking in distilled water was the method that least altered the original shape, size and ornamentation. The acetolytic mixture damaged a large number of spores and removed a part of the perinic material; heating in NaOH was corrosive and washing in ethanol led to frequent sporoderm ruptures due to a very violent dehydration.

For LM observation the contents of three to ten capsules of each sample were shaken in distilled water and then 40-50 spores were examined. Measurements and observations were made in a light microscope LEITZ LABORLUX K and micrographs were made to 1250× in a camera WILD MPS 515. Spores for the electron microscopy studies were set aside from this initial material.

The preparation of material for SEM was as follows. The spores were: 1) fixed in 3% Glutaraldehyde with cacodilate buffer 0.1M at 4°C, 2) washed in cacodilate tampon and saccharose, 3) dehydrated in a graded acetone series (30%, 50%, 70%, 90% and 100%), 4) submitted to critical point in 100% acetone and liquid CO₂, 5) sputtered to get a gold layer 200-250 Å thick. Finally these were observed and photographed in a JEOL JSM P-300 TEM operated at 15-25 Kv.

For TEM the selected spores were treated as follows: 1) pre-fixed in 3% Glutaraldehyde in cacodilate buffer 0.1M at 4°C, 2) washed in cacodilate and saccharose, 3) post-fixed in 1% osmic acid at 4°C, 4) washed in cacodilate and saccharose, 5) uranyl acetate at 4°C, 6) dehydrated in a graded ethylic series (30%, 50%, 70%, 90% and 100%), 7) treated in absolute alcohol and SO₄Cu, 8) treated in propylen oxide, 9) impregnated with epoxy resin. Thin sections were made using a REICHERT JUNG ultramicrotome and stained with uranyl acetate and lead citrate, before observation and photography in a ZEISS EM 10c TEM operated at 60 Kv.

The terminology corresponds partially to the next authors: McClymont & Larson (1964), Moore & Webb (1978), Clarke (1979) and Mogensen (1983). The terms "clavate" and "psilate" have been considered in a very broad sense according to Iversen & Troels-Smith (1981).

Specimens investigated

Phascum floerkeanum Web. & Mohr. Spain: Zaragoza, Pallaruelo de Monegros, Casas, 6.3.1978 (BCB 4144). Lérída, La Segarra, Torà de Riubregós, Brugués, 3.1986 (BCB 20681).

Phascum longipes Guerra, Martínez & Ros. Spain: Almería, Sorbas, near Gafares, Lomilla de las Colmenas, WF8997, Martínez & Ros, 7.4.1988 (Typus, Bryoth MUB 3068). Almería, Lucainena de las Torres, Cerro de las Cuevas, WF8498, Martínez & Ros, 7.4.1988 (Bryoth MUB 3070).

Phascum curvicolle Hedw. Spain: Murcia, Bullas, El Carrascalejo, XH1313, Ros, 5.11.1983 (Bryoth MUB 2965). Zaragoza, Mequinenza, Casas, 11.4.1978 (BCB 4125). Alava, Navaridas, WN2910, Heras, 21.2.1986 (Herb. Heras 152/86).

Phascum cuynetii Biz. & Pierr. Spain: Almería, Lucainena de las Torres, Cerro de las Cuevas, WF8498, Martínez, Ros & Guerra, 7.IV.1988 (Bryoth MUB 3076). Alicante, Cabo de la Nao, Bizot & al., 15.7.1968 (Isotypus, Herb. Pierrot).

Phascum vlassovii Laz. Spain: Granada, Cortijo de Don Andrés, WG3972, 900 m, Guerra, Ros & Heras, 4.3.1988, (Bryoth MUB 2953). Lérída, Torà, E of Ponts, CG62, Brugués & During, 20.2.1986 (BCB 20325)

Phascum cuspidatum Hedw. (with spores type A): Spain: Córdoba, Sierra Morena, Trasierra, Oliva, 25.11.1979 (Herb Oliva 638). Navarra, Miranda de Arga, Fuertes & García, 24.2.1975 (MA MUSCI 99). Belgium: Meise, Domaine de Bouchout, Lawalrée, 20.2.1981 (G). Hungary: Comit Pest, in agris arenosis prope pag. Budaörs, Szurák, 31.3.1912 (H). Italy: Toscana, Firenze, viale dei Colli, Fiori, 7.2.1904 (F).

Phascum cuspidatum Hedw. (with spores type B). Spain: Toledo, Navahermosa, Jordi & al., 19.3.1982 (BCB 7212). Portugal: Faro, Solms, 1866 (WIEN 10757). Denmark: Zealand, Storkskoven, Wood at Hvalsø, Jensen, 4.1905 (H). France: Eure-et-Loir, auf Ackerboden bei Dangeau, Douin, 3.1910 (GZU). Vendée, St. Michel, Mont Mercure, Charrier, 4.1919 (MACH). Italy: Emilia, Bologna, nei parterres del Giardino Margherita, Fiori, 11.1892 (F).

Results

General observations

Shape and polarity

The spores of *Phascum* species ranges from suborbicular or elliptic to plano-convex in equatorial view and from subcircular or elliptical to subtriangular in polar view. The polarity of the spores of *Phascum* species, like other mosses, is determined by structural differences which are not always externally clear (Clarke 1979). However the proximal pole usually shows a less noticeable convexity with sculptural elements unevenly distributed.

Size

The equatorial diameter has a very broad range of variation: 15-50 μm . This dimensional variability is greatest in *P. cuspidatum*. Nevertheless most of the studied spores range from 20 to 45 μm .

Sporoderm

1. Under LM a thin homogeneous layer can be recognized on which more intensely contrasted projections can be arranged, whose form, size and density are variable. Outside this basic organization, characteristic for each species, irregularly distributed, heterometric, globular or membranous elements, can be found. Since we do not have any information about the sporoderm ontogeny, we do not know the exact importance of these structures as determinants of the final ornamentation of the sporocite. However, we can state that: a) in all studied species, they form something like a matrix inside the mature capsule, not always uniformly opaque, b) their incorporation rate into the surface of some mature spores can be very high.

2. SEM allows us to see the spore ornamentation, which ranges from almost completely psilate to baculate, echinate or clavate. There are many intermediate types and a very great irregularity in the form of the processes, so that its description according to classic palynological types is very difficult.

3. Under TEM the structural pattern of the sporoderm in *P. cuspidatum* and *P. curvicolle* was studied (Plates X and XI). In *P. cuspidatum* several layers can be recognized. From the cytoplasm to the outside of the spore they are: a) a homogeneous and very densely fibrillar intinic layer, 0.5-0.7 μm thick in the distal pole and more than 2 μm in the proximal pole, b) a very thin separating layer, which is found only in the proximal pole, observed only by chance as a thin electro-dense layer, sometimes with lamellae, c) a homogeneous, non stratified low or medium electrodense exinic layer, 0.7-1.0 μm thick in the distal pole and thinner (to 0.2 μm) in the proximal pole. Usually the face in contact with the former layer is smooth, although the outermost one has small undulations orientated towards the everlying

layer, d) an irregular, discontinuous perinic layer made up of a very electrodense material, which gives rise to very variable projections, ca. 3 μm long, along its vertical axis. In the spores we designated as type A (see particular observations) there is a tendency for the apex processes to be wider and more markedly lobed. In the spores designated type B, the apex processes are mostly acute. Outside, there are globular structures arranged in a discontinuous way. All of them have the same electrodensity but they are variable form and size. Sometimes other globular structures can be seen on the exinic layer; these have the same electrodensity as this layer and are placed in the clear spaces between the radial projections of the perinic layer. Thus the sculptural processes of the spore of *P. cuspidatum* are almost exclusively made up of the perinic layer, whereas the contribution of the exinic layer is restricted to a small basal part of these processes.

In *P. curvicolle*, following the same order as in *P. cuspidatum*, the following layers can be recognized: a) a homogeneous intinic layer of a fibrillar nature but less electrodense than the equivalent in *P. cuspidatum*. Usually it is not more than 0.6 μm thick, except for some points in the proximal pole, where it is thicker than 1 μm , b) a homogeneous, as thick as the intinic layer, non stratified, low or medium electrodense exinic layer, c) a very thin, more electrodense perinic layer, which forms a very slight superficial and irregular cover to the outermost face of the exinic layer. Outside and in very discontinuous way there are multilayered laminar structures and small globular structures, both of them medium or highly electrodense. Differences in thickness between the exinic and intinic layers can also be observed, showing a polarity similar to that of *P. cuspidatum*. Thus, the spore sculpturing of *P. curvicolle* is also mainly marked by non exinic processes, although the base of the small projections has a exinic nature.

Particular observations

***Phascum floerkeanum* Web. & Mohr**

Plate I

Spores 20-32 μm in diam. Under LM a smooth and scarcely granulate surface can be seen, on which darker membranous or globular depositions are frequent. Under SEM the spore is psilate to slightly scabrate. The incorporation of globular material can cause granules or isolated verrucae, 0.7-1.6 μm in diam.

***Phascum longipes* Guerra, Martínez & Ros**

Plate II

Spores 19-30 μm in diam. Under LM they are very similar to those of the former species. Occasionally the external depositions are more abundant, which is proportionally somewhat thicker. Under SEM the spore is psilate, slightly scabrate or scarcely granulate. Isolated granulae or verrucae 0.5-1.6 μm in diam. can be present.

Phascum curvicolle Hedw.

Plate III

Spores 15-29 μm in diam., usually sub triangular in polar view. Under LM they cannot be differentiated from those of the two former species. Under SEM the spore is basically scabrate-rugulate or granulate-rugulate, with some overlying isodiametric particles 0.5-1.7 μm in diam. In the proximal pole of some spores there is a psilate, probably apertural, area.

Phascum cuynetii Biz. & Pierr.

Plates IV and V

Spores 15-23 μm in diam. Under LM a superficial, a well defined layer of more or less related granules and another granular suprabaasal layer with bigger and irregularly distributed elements are present. Thus, the spores seem to be verrucate-granulate. The sporoderm is thicker than in the three former species. Under SEM the spore has a densely granular surface, with granules usually anastomosed, which give rise to rugulae, striae or irregular reticula. In addition, the observations made under LM are confirmed, namely that there is a coexistence of globular elements, which form granules, verrucae or gemmae, ca. 2 μm in diam., sometimes also anastomosed. The surface of some areas is slightly granulate or psilate.

Phascum vlassovii Laz.

Plate IV

Spores 16-35 μm in diam. Under LM they appear rugulate, striate or even partially reticulate. Under SEM stipitated processes can be observed. They are very different from the processes of the former species. The surface is covered by radial oblique projections, like thin, intermingled claws, irregularly fused in different way. They give rise to an open reticulum with electrodense apex. The processes are 0.3-1.8 μm long (usually about 1 μm). When the stipe is cylindrical it is approximately 0.3 μm wide. Sometimes degree of anastomosis is less in the proximal pole. The perisporeal depositions produce a variable appearance in the process capitae.

Phascum cuspidatum Hedw.

Plates VII-IX

This is the taxon in which spore morphology is the most variable and were the sporoderm processes are the most developed. Despite this variability we distinguish two different ornamental types:

Type A (Plates VII and VIII). Spores 23-50 μm in diam. Under LM and SEM most of the sculptural elements can be seen. The most frequent projection is like a claw. There seems to be a relationship between the form and the length of the processes: the shortest elements are usually spinae, gemmae or verrucae, the middle-length elements are spinae or baculae and the longest one baculae or clavae. The predominant elements vary from one population to another. There is a percentage of irregular or intermediate elements. Under SEM the apex projections can be irregularly lobed, anastomosed or partially covered by detached intracapsular tissues. The processes

are 0.5-2.7 μm long and its stipe 0.5-1 μm wide. Their distribution on the spore surface can be less dense in some areas.

Type B (Plate IX). Spores 20-32 μm in diam. The most frequent projection is the echina, sometimes folded or rounded at the tip. These processes are 0.5-3 μm long and 0.3-0.7 μm wide in the middle part. As in type A, the density of these processes can vary, sometimes appearing very spaced or entangled. This ornamental type can be considered as a variant of the former, in which the basic form of the spinae it is not affected by its growth.

The ornamental type A has been found in many specimens which were previously named *Phascum cuspidatum*. The ornamental type B has been found in samples originally identified as *P. papillosum* Lindb. or *P. cuspidatum* var. *mitraeformis* Limpr.

Discussion

Morphological and morphogenetical aspects

The spore size and shape of *Phascum* species have a very high intraspecific and intrapopulation variability. Nevertheless, it is very interesting to note that the immature spores are usually subtriangular and become spherical when mature. This morphological change has been very often observed in pollen (Ferguson & Müller 1976). They proposed that the initial shape of the spore is due to the physical pressures before it detaches itself from the tetrad. Brown & Lemmon (1980a) think there is a cytoplasmic polarity in bryophyte sporogenesis, which becomes more important than any mechanical force.

Under TEM spores of *Phascum cuspidatum* and *P. curvicolle* are shown to have a structural polarity: the exinic layer in the proximal pole become thinner and the intinic layer thicker. This polarity has been previously observed in mosses (McClymont & Larson 1964, Sorsa 1976, Brown & Lemmon 1981), although it was mostly internal and only observed in thin sections. In the genus *Phascum* there are differences in the density, form and size of the processes, which can be externally observed.

The exact origin of this polarity is not yet known. Brown & Lemmon (1985) observed in the genus *Archidium* that at first the exine covers the distal surface and later the proximal pole, which means that polarity is partly defined by an exinic ontogenic delay.

The polarity in the studied spores of *Phascum* species is intimately related to the possible existence of an apertural area, that is, a more or less defined region in the proximal pole in which the protonema would emerge during germination (Brown & Lemmon 1981). TEM studies on *P. cuspidatum* and *P. curvicolle*, and the former observations under SEM, lead us to think that this apertural area is probably present in all the *Phascum* species. Whether or not this area should be named an

aperture is open to discussion (Erdtman 1957, 1965). Nevertheless, the studies of Olesen & Mogensen (1978) and Reighard (1967) have revealed its role in germination. Those of Brown & Lemmon (1980a, 1980b, 1981, 1985) on the ultrastructure of sporogenesis also emphasize that the minimal morphological, ontogenic and functional requisites are present for it to be considered as an aperture. In any case, we agree with Blackmore (1983), who stresses that "aperture needs to be a very flexible term equally applicable to widely differing types of aperture and so it must still be defined in the broadest possible terms". Other nomenclatural problems arise when the sporodermic layers are studied under TEM. The named intine and exine layers seem to correspond respectively to the intine and exine in the sense of Afzelius (1957), whereas the perinic layer seems to be equivalent to the perine of Erdtman (1957). Also they would be equivalent to the so-called endosporium, exosporium and perisporium of Faegri & Iversen (1964) and Gllvag (1967). The use of both terminology raises problems because they compare structures whose equivalent morphogenesis has been not demonstrated. In consequence we prefer not to use them. The so-called separating layer seems to coincide with the observations of Olesen & Mogensen (1978). They observed an exinic lamellar layer in the contact area with the intine layer.

From the TEM studies, the most notable phenomenon is the low contribution of the exinic layer to spore ornamentation. The observations of McClymont & Larson (1964) are confirmed for *P. cuspidatum*. If the results of the SEM studies are also born in mind, it seems to be probable that the structural pattern of *P. cuspidatum* is similar to *P. vlassovii* and *P. cuynetii*, whereas that of *P. curvicolle* could be applicable to *P. floerkeanum* and *P. longipes*. In the first case, the perinic layer would produce projections more or less defined, whereas in the second case it would give rise only to an irregular scabrous surface. Most of the ultrastructure studies on moss spores present similar results on the high contribution of the perine to spore sculpturing, but in some cases the exine acquires more relevance (McClymont & Larson 1964, Gllvag 1967).

The presence of globular or membranous elements on the outermost sporoderm of *Phascum* could be linked to the process of sporogenesis. Jarvis (1974) observed in *Funaria hygrometrica* that globular elements from the sporal fluid remained adherent to the spore surface. Afterwards they came away from the spore but always some particles remained on it. Brown & Lemmon (1980a) established some similarities between the exine and intine development in *Ditrichum pallidum* (Hedw.) Hamp.: both layers began to form after deposition of two types of vesicle of differing electrodensity. This observation supports the revelation of Neidhart (1979), who saw that both layers were acetolysis resistant but that there were very clear histochemical differences. After that Brown & Lemmon (1984, 1985) thought that tapetal cells of mosses and nutritive cells of *Archidium* were involved with exine and perine formation. The secretion of these cells (rich in mitochondria, dictyosomes and smooth endoplasmic reticulum) gives rise to two vesicle types: some very electrodense from which the perine is originated and others of medium electrodensity which give rise to the exine.

These observations could explain the presence of two types of globular particles of differing electrodensity on the spore surface of *P. cuspidatum* (Plate X, g1 and g2).

The presence of lamellar structures is more difficult to understand, because: (1) many of them are sensitive to acetolysis, unlike the globular structures, (2) it seems there is no doubt that they contribute to the final spore ornamentation, at least in *P. floerkeanum*, *P. longipes* and *P. curvicolle* and (3) hardly anybody has found them (Güllvag 1967, Heckman 1970 and Sorsa 1976). Due to the lamellar composition of these structures it is possible to think they are membrane remains from the lysis of the tapetal cells, which might adhere to the young spore surface, usually with granular material too.

Taxonomic aspect

The spore shape and size of *Phascum* species are so variable that they are not useful from a taxonomic point of view. Nevertheless, it is very interesting to verify that the size variability is particularly great in *P. cuspidatum*, a species distributed all over the world, whereas it is very small in *P. cuynetii*, a endemic species from south-eastern Spain.

From a general point of view it must not be forgotten that many of the spores are bigger than 25 μm in diam. According to Mogensen (1983) that is the size after which the dispersal range is considerably reduced.

The most valuable sporal feature for the taxonomical delimitation of *Phascum* species is the ornamentation. The differences in size and pattern of distribution of the perinic elements justify the delimitation of two main groups, confirming the conclusions of Derrick (1978). It seem to be clear that: (1) *P. longipes* must be included in the subgenus *Microbryum* because of its affinity with *P. floerkeanum*, (2) *P. vlassovii* must be in the subgenus *Euphascum* and (3) *P. cuynetii* should be afforded an independent infrageneric status. Accordingly we have elaborated the following keys:

- a. Spore surface psilate, scabrate, granulate or verrucate-granulate. Group 1
- b. Spore surface echinate, baculate or clavate. Group 2

Group 1

- 1. Spore surface thickly granulate or verrucate-granulate. *P. cuynetii* type
- 1. Spore surface with other characters. 2
- 2. Spore surface scabrate-rugulate or granulate-rugulate. *P. curvicolle* type
- 2. Spore surface psilate, scabrate or slightly granulate. *P. floerkeanum* type
(includes *P. longipes*)

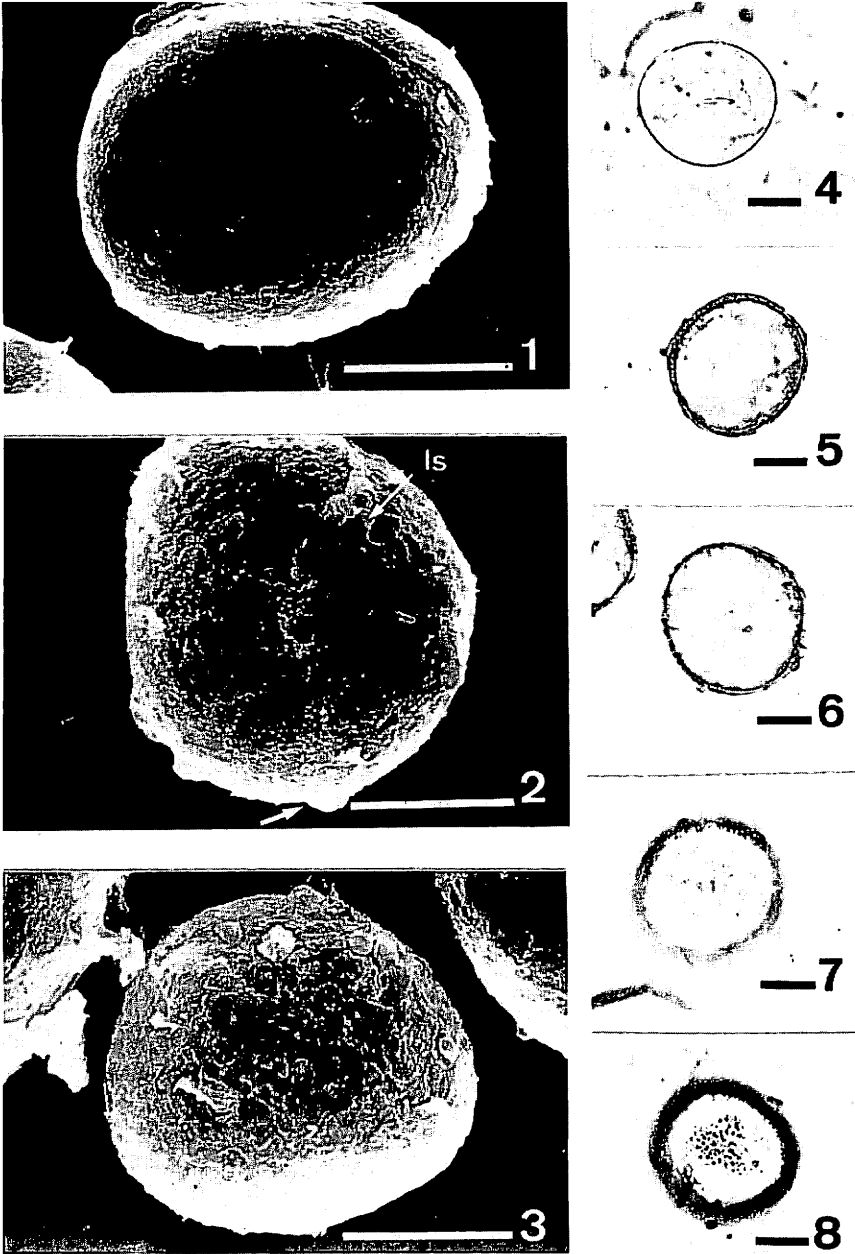


Plate I: *Phascum floerkeanum*. Figs. 1-3 SEM, Figs. 4-8 LM. Fig. 1: spore with psilate surface. Fig. 2: psilate-scabrate surface with verrucae (arrow) or laminar structures (ls) coming from the sporal fluid. Fig. 3: scabrate surface with many laminar remains and some isodiametric globular structures (gs). Fig. 4: immature spore with very thin sporoderm. Figs. 5 and 6: mature spores showing the irregularity of the external depositions. Fig. 7 and 8: superficial view showing the scabrate ornamentation. (Scales: 10 μ m.)

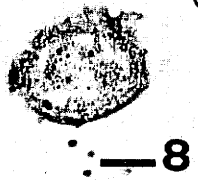
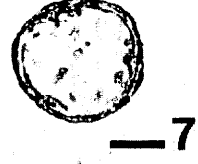
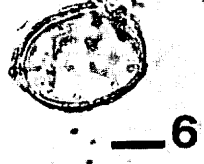
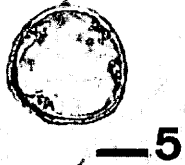
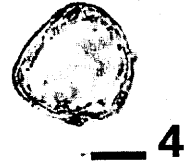
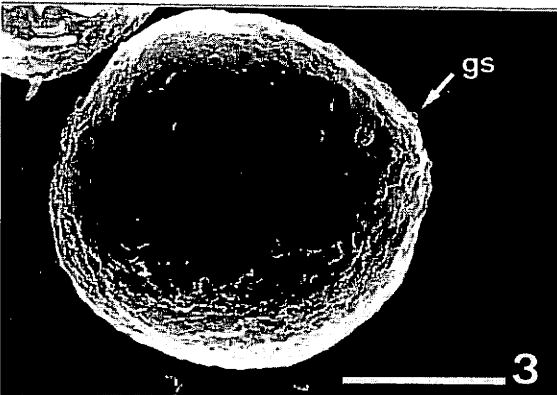
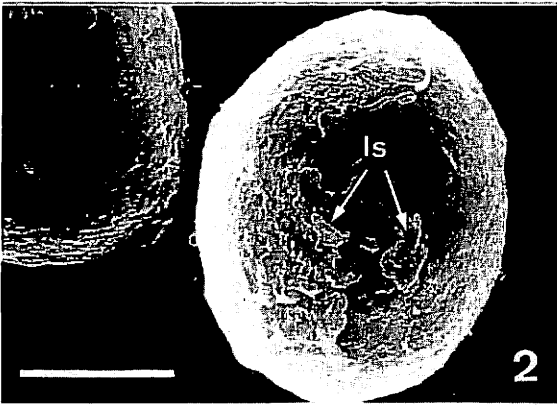
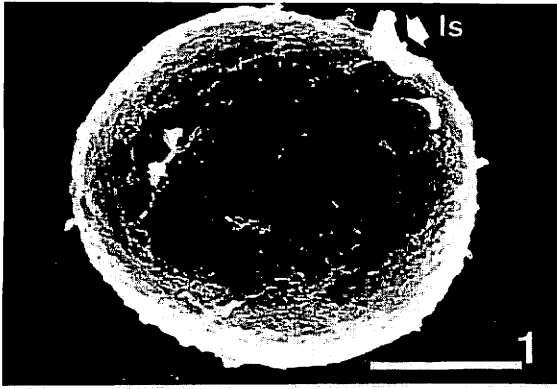


Plate II: *Phascus longipes*. Figs. 1-3 SEM, Figs. 4-8 LM. Figs. 1 and 2: spores with psilate-scabrate surface and variable depositions like laminar structures (ls). Fig. 3: slightly granulate surface with small globular structures (gs). Figs. 4, 5 and 7: subtriangular and irregular outline in polar view. Fig. 6: lateral view. Fig. 8: superficial view showing small granulae. (Scales: 10 μ m).

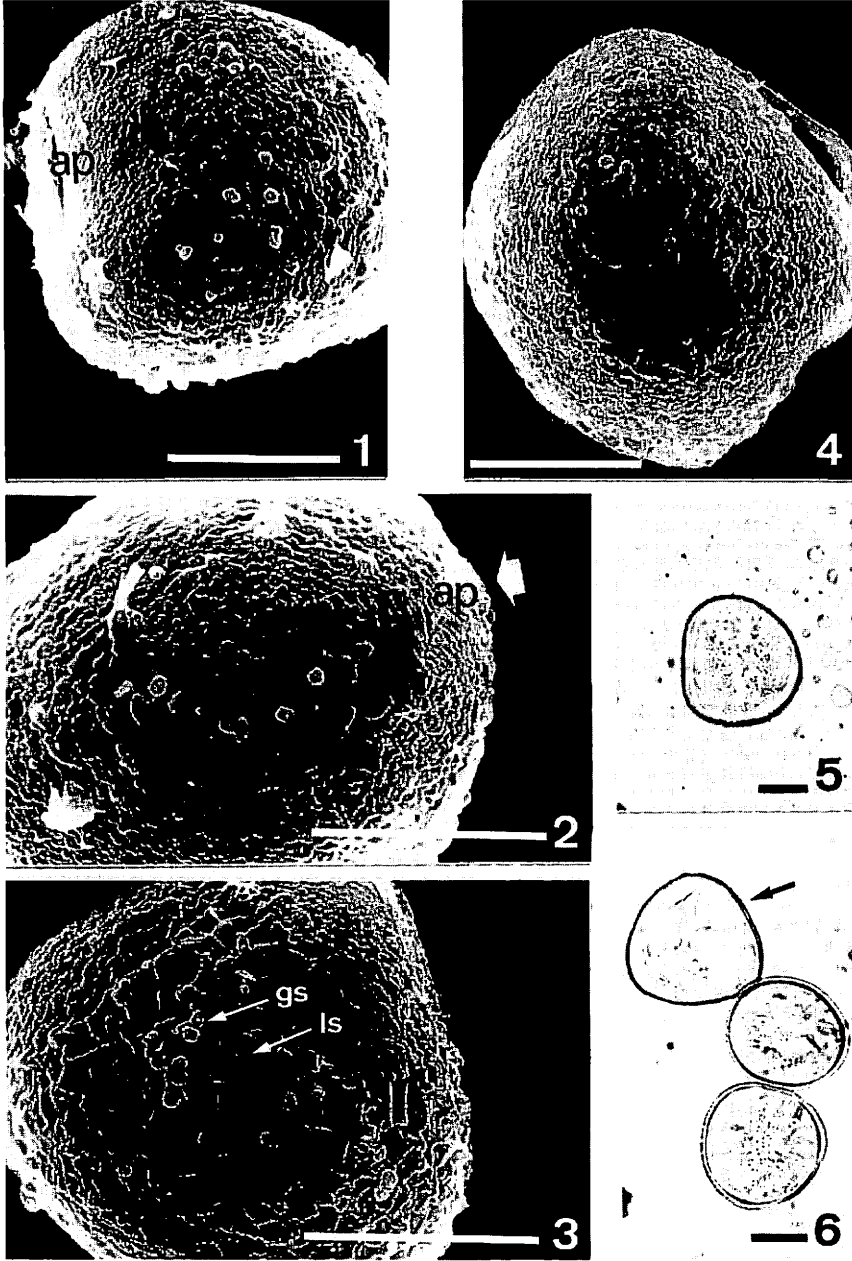


Plate III: *Phascum curvicolle*. Figs. 1-4 SEM, Figs. 5 and 6 LM. Figs. 1 and 2: spores with granulate-rugulate surface, heterometric globular particles and a psilate or perforate apertural area (ap, arrows). Fig. 3: rugulate spore surface consists of larger angular ridges, on which laminar (ls) and globular structures (gs) can be recognized. Fig. 4: spores with less distinct ornamentation showing up the scabrate sculpture. Figs. 5 and 6: spores showing slightly granulate surface and subtriangular shape (arrow). (Scales: 10 μ m).

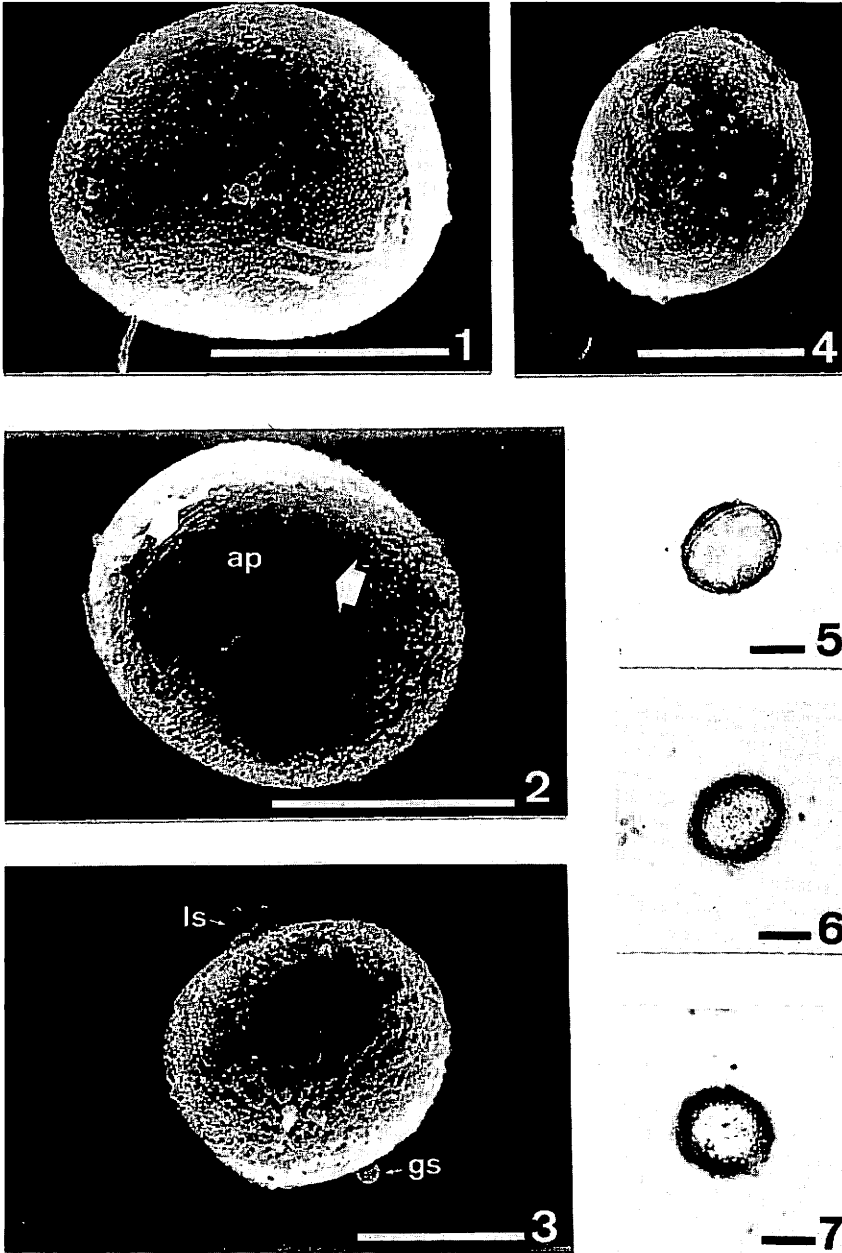


Plate IV: *Phascum cuynetii*. Figs. 1-4 SEM, Figs. 5-7 LM. Fig. 1: spore with granulate ornamentation. Fig. 2: spore with striate-granulate surface and a psilate apertural area (ap, between arrows). Fig. 3: reticulate-granulate surface with many laminar (ls) and globular structures (gs). Fig. 4: spore surface rugulate-granulate with few small peripheral globules. Fig. 5: spore with elliptical shape in side view. Figs. 6 and 7: superficial view showing the verrucate-granulate ornamentation. (Scales: 10 μ m.)

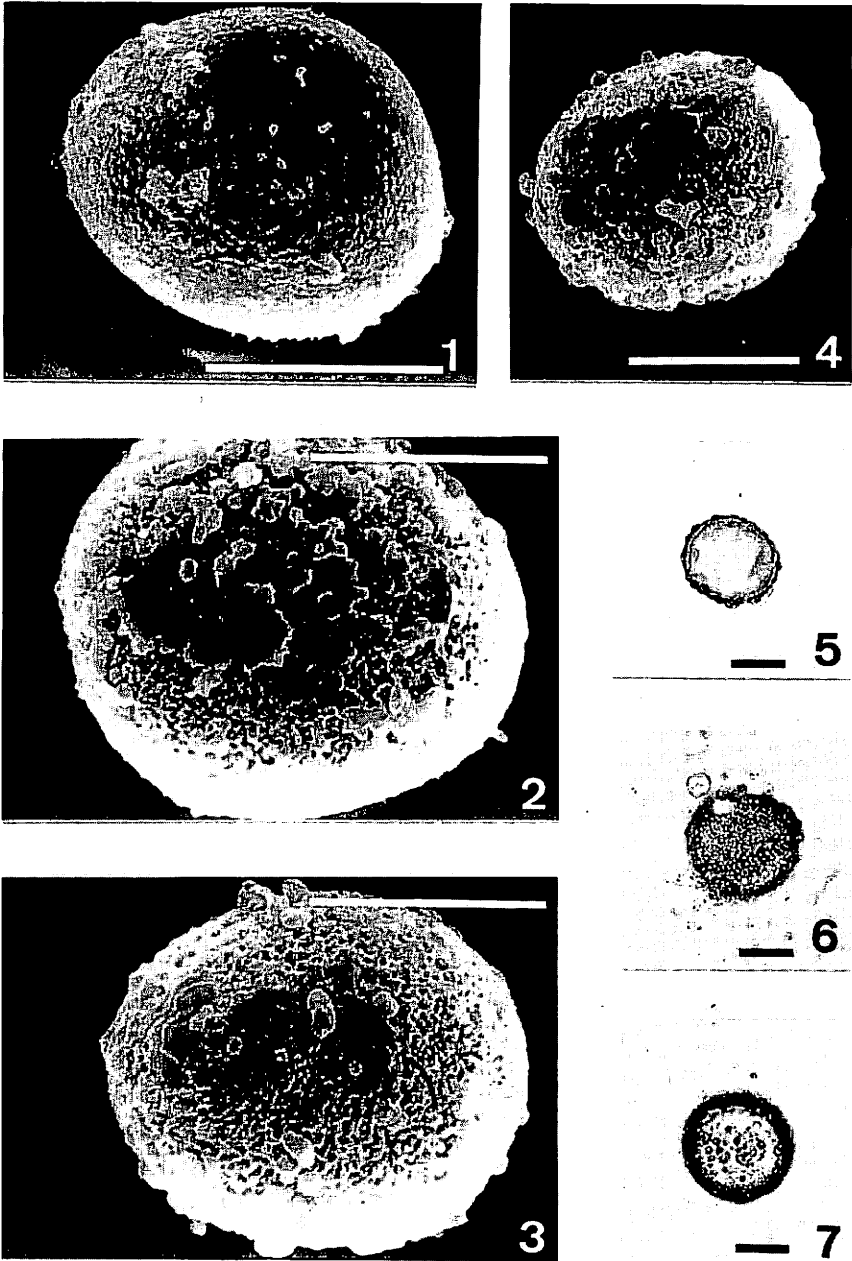


Plate V: *Phascum cuynetii*. Figs. 1-4 SEM, Figs. 5-7 LM. Fig. 1: spore with rugulate-granulate ornamentation and small globular particles. Fig. 2: rugulate-granulate surface and abundant globular material, which causes suprabasal and sometimes anastomosed granulae, verrucae or gemmae. Figs. 3 and 4: reticulate-granulate surface with many verrucae and gemmae as membrane rests. Fig. 5: verrucate outline. Figs. 6 and 7: superficial view. (Scales: 10 μ m.)

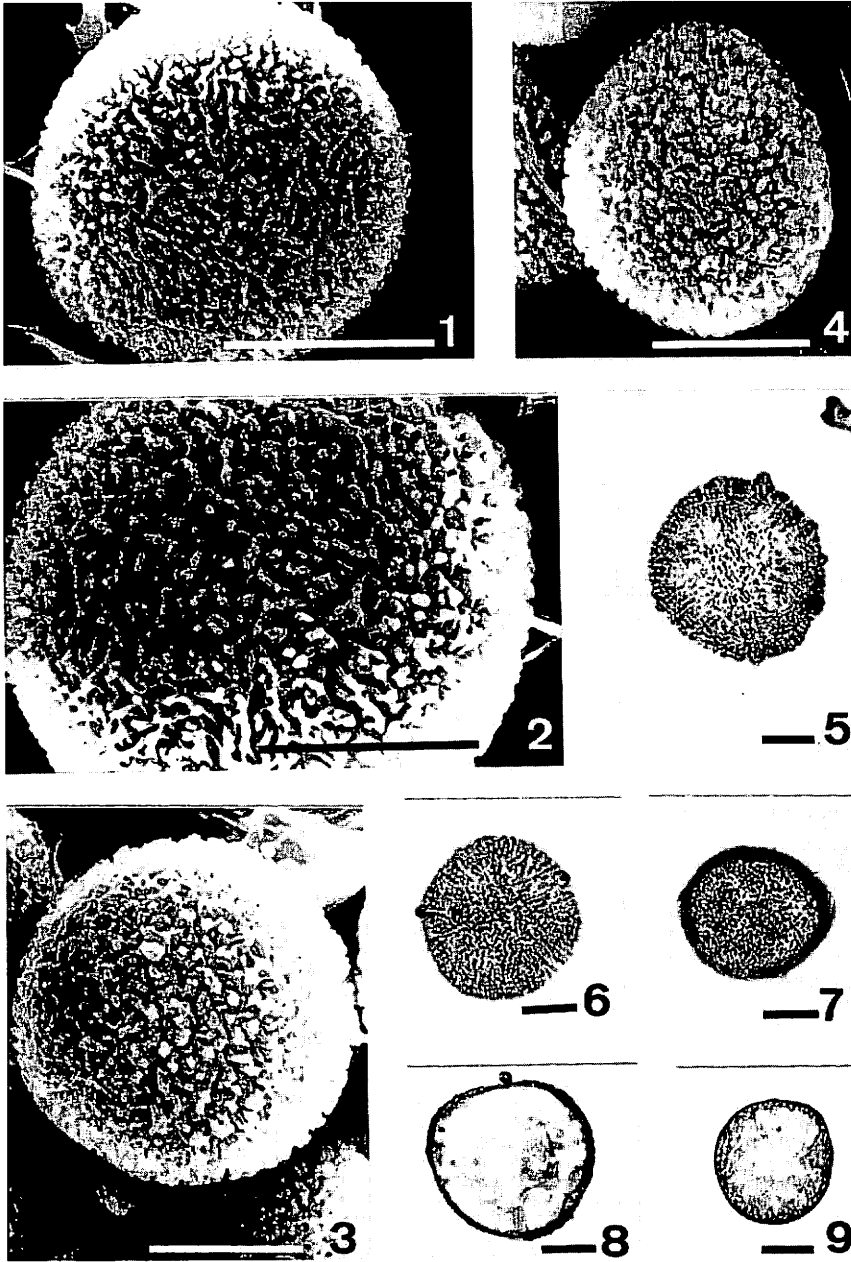


Plate VI: *Phascum vlassovii*. Figs. 1-4 SEM, Figs. 5-9 LM. Figs. 1 and 2: spore surface showing anastomosing thin processes which are fusing at different levels and topping of capitae. Figs. 3 and 4: processes with bigger capitae. Figs. 5, 6 and 7: superficial view showing the irregularly aggregated projections. Fig. 8: subtriangular shape in polar view. Fig. 9: subcircular shape. (Scales: 10 μm .)

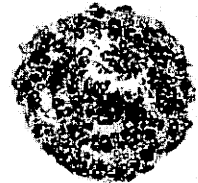
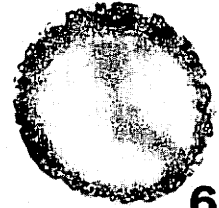
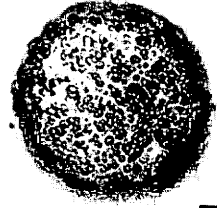
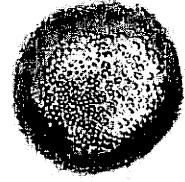
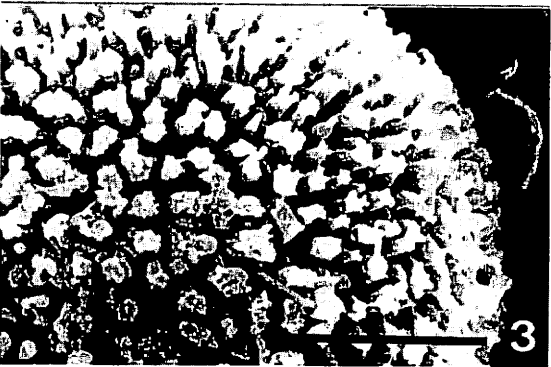
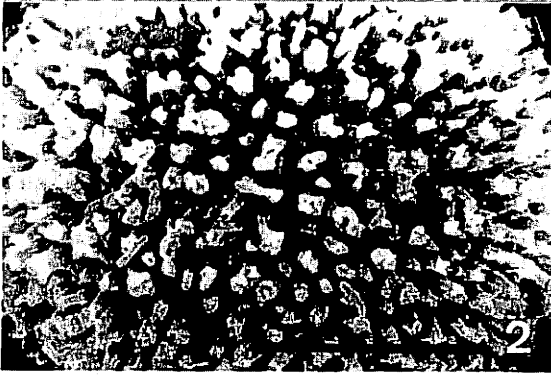
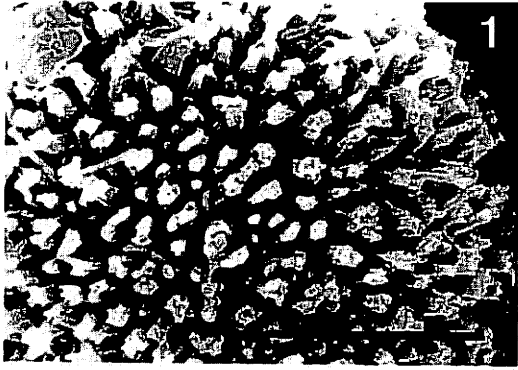


Plate VII: *Phascus cuspidatum* (spores type A). Figs. 1-3 SEM, Figs. 4-7 LM. Figs. 1-3: typical spore surface showing clavate processes with irregularly lobed apex; between them there are some shorter echinae. Fig. 4: spore with irregularly distributed, small projections. Figs. 5 and 7: spores with larger projections, irregularly distributed. Fig. 6: subequatorial view. (Scales: 10 μm .)

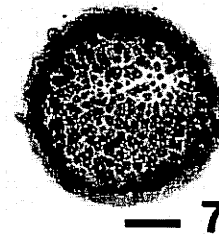
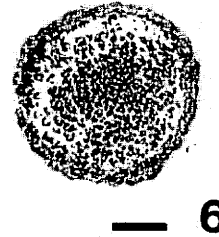
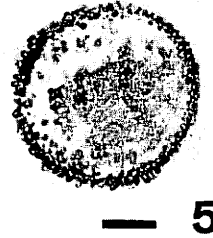
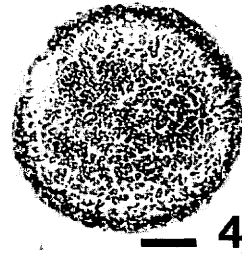
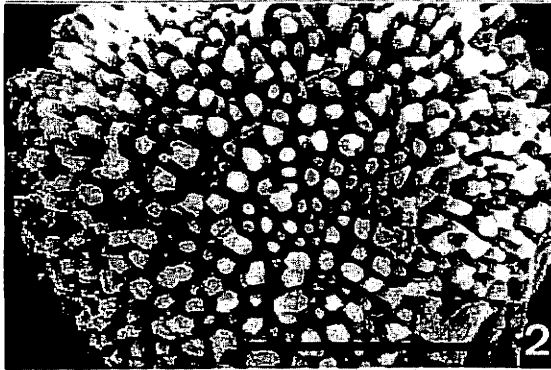
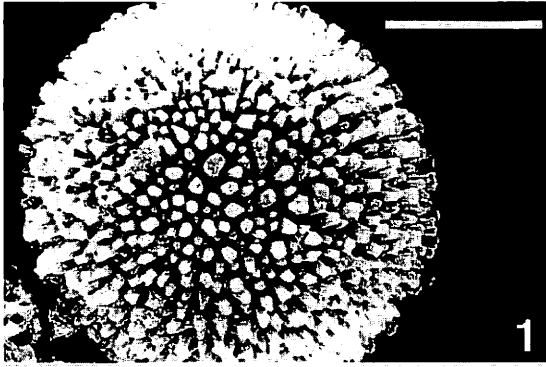


Plate VIII: *Phascum cuspidatum* (spores type A). Figs. 1-3 SEM, Figs. 4-7 LM. Figs. 1 and 2: spores with predominant baculate or clavate, non lobed apex processes. Fig. 3: spore with predominant clavate, lobed apex processes occasional anastomosis at the capitae (arrow). Fig. 4: spore with predominant short and wide echinae. Fig. 5: echinate-baculate spore. Fig. 6: superficial view of the former ornamentation. Fig. 7: superficial view with wide capitae. (Scales: 10 μ m.)

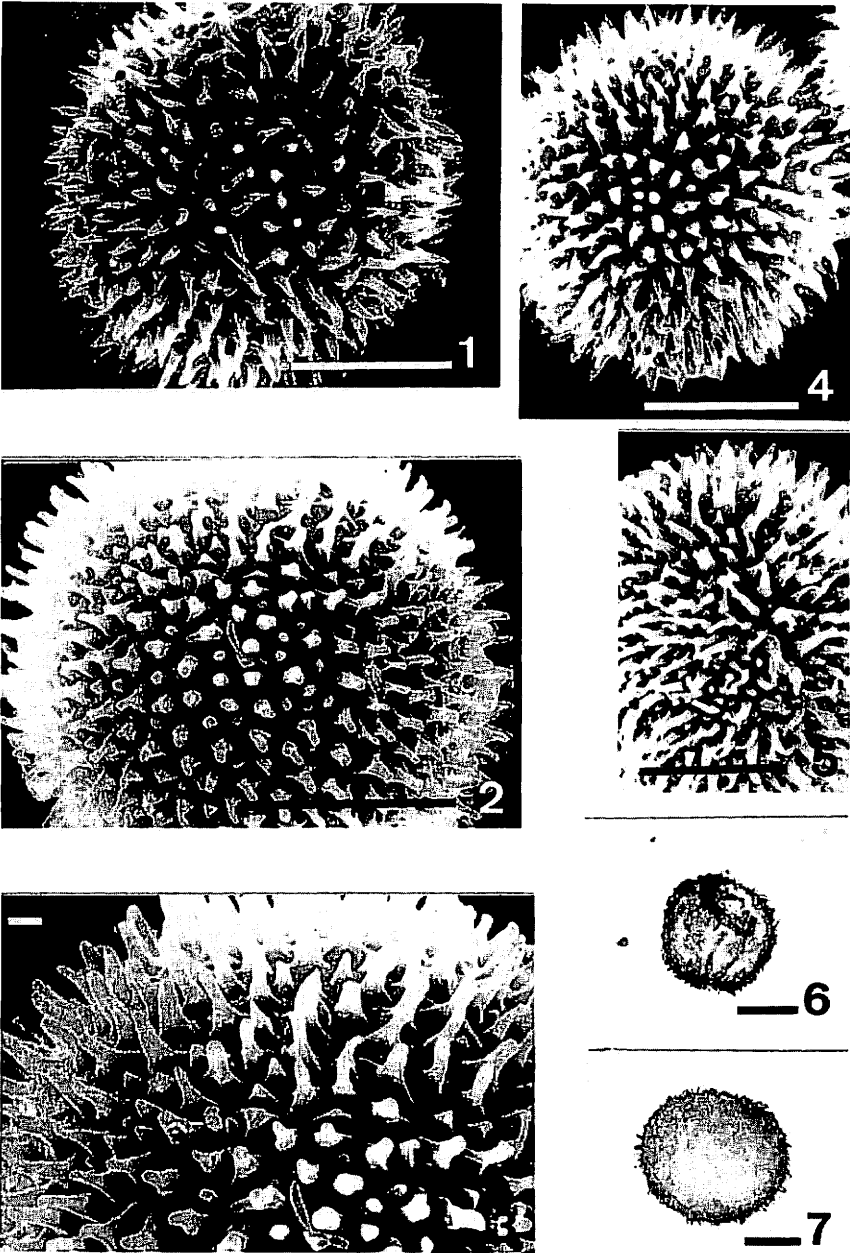


Plate IX: *Phascum cuspidatum* (spores type B). Figs. 1-5 SEM, Figs. 6 and 7 LM. Figs. 1 and 4: spore surface covered by sharp echinae. Figs. 2 and 3: rounded apex in the longest processes. Fig. 5: densely covered surface with very long and sometimes curved echinae. Fig. 6: spore with differently orientated echinae. Fig. 7: spore with rounded apex echinae. (Scales figures 1, 2, 4, 5, 6 and 7: 10 μm ; figure 3: 1 μm .)

Group 2

1. Spore surface covered by thin, clavate, radial or oblique processes, 0.3-1.8 μm long, irregular or openly connected. *P. vlassovii* type
1. Spore surface with other characters. 2
2. Spore surface covered by clavate or baculate processes, isolated or welded at the tip, 0.5-2.7 μm long; sometimes there is intercalated spinae. *P. cuspidatum* A-type
2. Spore surface clearly echinate; the longest processes with sharp or blunt apex, usually more than 2 μm long. *P. cuspidatum* B-type

The described taxonomical variability might basically be due to variation in development of the perinic layer. But the reasons for these differences and their concomitant biological consequences are unknown. Usually it is accepted that ornamental elements and sporoderm thickness affect spore drought resistance (Mogensen 1983). Sometimes it has been suggested that the presence of specialized apertures is linked to the need for spore germination in changeable habitats (Brown & Lemmon 1981). During (1979) thinks that *P. cuspidatum* is a clear case of a life strategy typical of annual plants (short reproductive cycle, frequent sporophytes, big spores and scarce asexual reproduction). Nevertheless, these characteristics seem to be common to all the studied species of *Phascum*.

The observed ornamentation patterns have a infrageneric variability low enough to consider them unusual in bryophytes (Clarke 1979).

This work clearly illustrates that new research lines should be initiated in order to clarify the ecological differences linked to the physiology of germination. In this way the variability of sculptural types, which recur among spores of bryophytes, might be understood.

Acknowledgements

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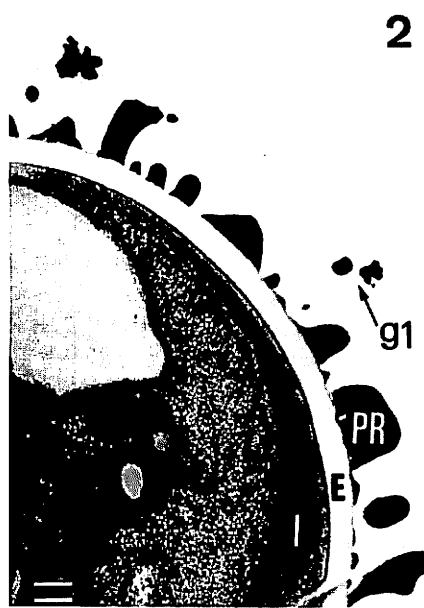
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Plate X: *Phascum cuspidatum* (spores type A in median section, TEM). Figs. 1-4: parts of spores cutted down the middle under TEM. See that the ornamentation is mainly due to a very electrodense, discontinuous perinic layer (PR), irregularly lobed at the apex of the longest elements. The medium electrodense exinic layer (E) is continuous and homogeneous, its external surface has small undulations. The intinic layer (I) is also continuous. The globules g1 are as electrodense as the perinic layer but the globules g2 are as electrodense as the exinic layer. Fig. 1: separating layer (SL) with lamellar structure. Figs. 1 and 2: the intine turns thicker at the proximal pole. (Scales: 1 μ m.)

1



2



3

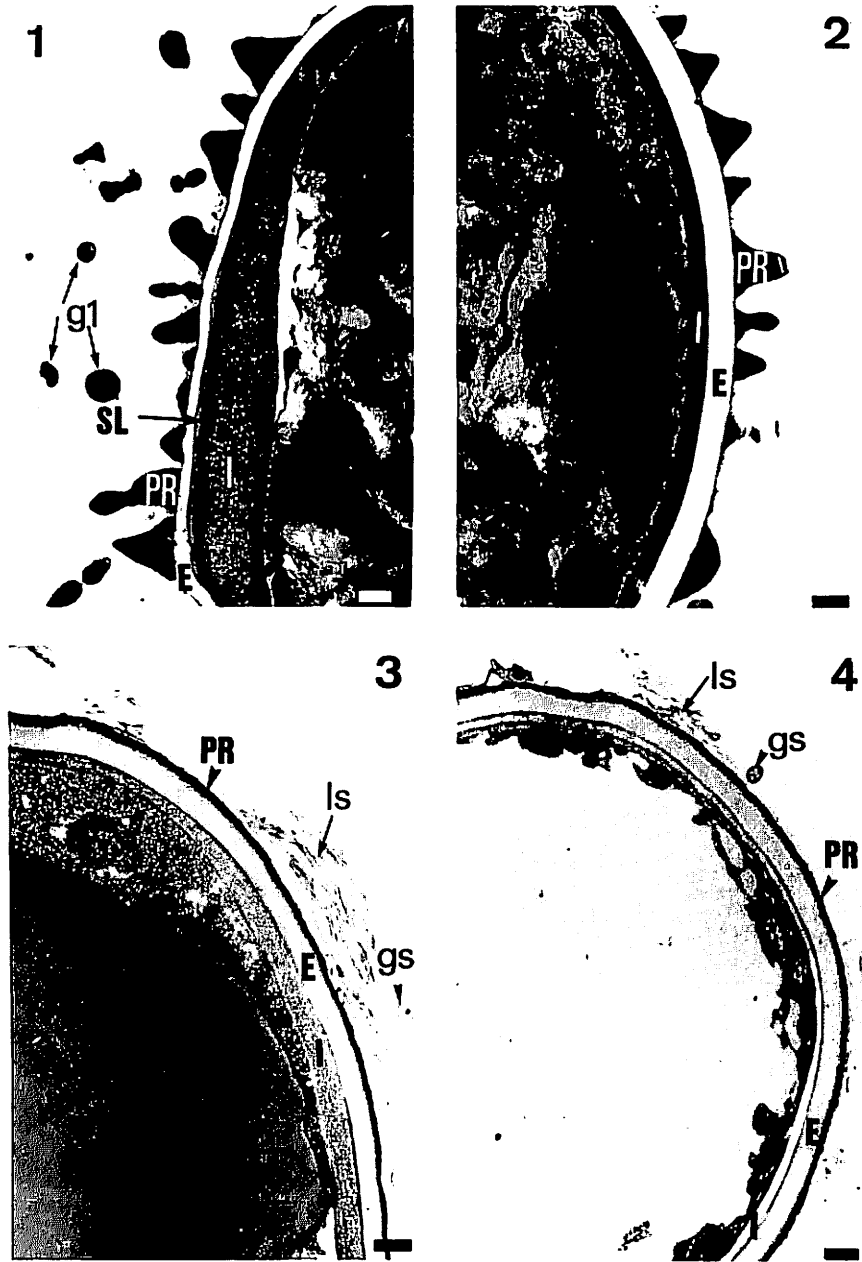


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Plate XI: *Phascum cuspidatum* (spores type B), figs. 1 and 2. *Phascum curvicolle*, figs. 3 and 4. Spores in median section, TEM. Figs. 1 and 2: projections of perinic nature (PR) and not branched at the sharp apex. Fig. 1: proximal pole with several electrodense globules (gl), thin exinic layer (E), lamellar separating layer (SL) and a thickened and fibrillar intinic layer (I). Fig. 2: distal pole with less globules, thicker exinic layer (E) and thinner intinic layer (I). The separating layer is not observed. Figs. 3 and 4: the



perinic layer (PR) consists of a superficial thin stratum more electrodense than the overlying exinic layer (E). On the perinic surface there are medium electrodense laminar (Is) and globular structures (gs); both of them form irregular depositions. Fig. 3 and 4: respectively proximal and distal pole. At the proximal pole the intinic layer (I) turns thicker than at the distal pole and the exinic layer (E) a bit thinner. (Scales: 1 μm .)