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SPORODERM ULTRASTRUCTURE OF *OEDIPODIUM GRIFFITHIANUM* (OEDIPODIOPSIDA, BRYOPHYTA)

СТРУКТУРА СПОРОДЕРМЫ *OEDIPODIUM GRIFFITHIANUM* (OEDIPODIOPSIDA, BRYOPHYTA)

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Abstract

Spores of *Oedipodium griffithianum* are studied under SEM and TEM, revealing a unique combination of characters: distinct trilete laesura, distal surface densely covered by club-shaped papillae often fused by their distal parts, perine mostly eroded and fallen off in the mature spores, and layer between exine and intine strongly variable in size and texture between distal and proximal hemisphere. This layer is homogeneous or occasionally totally absent at distal pole, lamellose in equatorial region in sections of fully developed spores, while indistinctly lamellose to homogeneous in a slightly premature spores; in the proximal hemisphere and in laesura it is thick and has complex structure. In somewhat premature spores an electron dense perine is observed upon exine, but it seems to easily fall off during spore maturation, so fully mature spores almost lack perine like in *Bruchia brevifolia*. *Oedipodium* is similar to Sphagnopsida in distinct laesura, unstable perine and complex multilaminate innermost layer of exine, a remnant of tripartite lamella.

Резюме

Споры *Oedipodium griffithianum* изучены с помощью сканирующего и трансмиссионного электронных микроскопов, что позволило выявить уникальную комбинацию признаков, включающую: хорошо выраженную трилетную лезуру на проксимальной полусфере; столбиковидную скульптуру дистальной полусферы, вершины столбиков часто сливаются в небольшие фрагменты сетчатой скульптуры; перисporий нестойкий, легко стирающийся по мере высыпания спор из коробочки, представлен электронно-темными гранулами, изменчивыми по размерам и форме; срединный слой (производное трехчастной пластинки, TPL) между экзоспорием и эндоспорием, который слабо представлен на дистальной полусфере, где он гомогенный, хорошо выражен в месте перехода на проксимальную полусферу. Здесь срединный слой имеет четкую мультиламеллятную структуру, а далее, на проксимальной стороне он становится сильно утолщенным, размыто-волокнистым и особо сложно устроенным под лезурой. У недоразвитых спор срединный слой в экваториальной части может не иметь отчетливых ламелл, а перисporий может быть богато представлен особенно на проксимальной полусфере. Споры *Oedipodium* напоминают споры Sphagnopsida хорошо развитой лезурой, нестойким перисporием и сложным ламеллатным строением внутреннего слоя экзины, производного TPL. С другой стороны, по участию в формировании скульптуры экзоспория и отсутствию перисporия в зрелом состоянии *Oedipodium* имеет сходство с видами рода *Bruchia*.

KEYWORDS: exine, intine, perine, tripartite lamellae, spore wall, *Oedipodium*, Oedipodiopsida, TEM, SEM, mosses

INTRODUCTION

Moss spore studies with light microscopy and SEM are fairly numerous. Many taxonomic revisions nowadays use SEM, and in a number of genera spore characters are useful for taxonomy. Especially well-known and thoroughly studied are families Encalyptaceae (Horton, 1983), Polytrichaceae (Smith, 1971), and Bruchiaceae (McClymoth, 1955). The greater variation occurs in acrocarpous mosses, however in Hypnales the thorough studies in *e.g.* Plagiotheciaceae (Ireland, 1987) and Entodontaceae (Kungu *et al*., 2007) also found correlation between spore surface sculpture and taxonomy. These studies however do not cover all the families, as alete spores in many families are fairly uniform and have limited diagnostic value. Likely for this reason, comprehensive spore atlases are few. They include only regional species from Europe (Boros *et al*., 1993) and China (Zhang & Wu, 2005), and even then include not all the genera. No worldwide review of moss spores of all the families has been published so far.

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Ultrastructural studies of moss spores using-TEM were started before SEM technique became widely available (McClymoth & Larson, 1964), but the published results are much fewer and many groups have not been studied at all. The reason for that is likely the more difficult specimen preparation, requiring more complicated methods and less diverse structure, making results not so straightforward for discussion. However the study of the spore wall structure proved its usefulness both for systematic studies, as well as for understanding of its development (Brown & Lemmon, 1980, 1981, 1984, 1988, 1990; Brown *et al*., 1982a,b; Estébanez *et al*., 1997, Carrion et. al., 1990, 1995, Estébanez et. al., 1997, 2006; Filina & Filin, 1984, 1985; Luizi-Ponzo & Barth, 1998; Luizi-Ponzo & Melhem, 2006; Mueller, 1974; Rernzaglia *et al*., 1997). Brown *et al*. (2015) provided especially useful overview of the spore ultrastructure of the basal groups of mosses, liverworts, and hornworts. They included, among others, the genus *Oedipodium*, which was also simultaneously studied by me, as a moss recently assumed as having a basal and intriguing phylogenetic position. I am presenting here my observations which are in general congruent with data published in this paper, although expanding data on variation of its ultrastructure, mostly due to material used for Brown *et al*. (2015) observation was likely slightly premature when compared with ours.

The genus *Oedipodium* includes one species, *O. griffithianum* (Dicks.) Schwägr., with wide and strongly disjunctive distribution (Ignatov *et al*., 2006). It has been placed at first with *Tayloria* in Tayloriaceae of the order Splachnales (Schimper, 1860), but later segregated in monotypic family of the order Funariales (Schimper, 1876) and placed most commonly near Splachnaceae (Brotherus, 1924) or even within the latter family (Savicz-Lyubitskaya & Smirnova, 1970) which was at that time universally accepted as a member of Funariales. The placement in Splachnaceae was likely due to superficial similarity to some species of *Taylora* in obtuse leaves and long hypophysis of the capsule.

Already the first molecular studies found this placement to be erroneous. Its position was revealed among the most basal mosses of subclasses Takakiopsida, Sphagnopsida, Andreaopsida and Andreaobryopsida and the most basal group of peristomate mosses of Polytrichopsida (Newton *et al*., 2000; Cox *et al*., 2004, Tsubota *et al*., 2004).

The sporophyte development of *Oedipodium* has been studied by Shimamura & Deguchi (2008), who showed that its structure do not contradict the hypothesis of the primarily peristome absence, not its reduction as was thought before.

This fact deserved the segregation of *Oedipodium* in a separate subclass Oedipodiopsida, with a position in moss system previous to the Polytrichopsida (Goffinet *et al*., 2009; Frey & Stech, 2009). Ligrone & Ducket (2011) however challenged such placement basing on the placenta study, which indicates more similarity with Tetraphidales than with Polytrichales. It is worth mentioning that the similarity in the protonemal leaf structure between *Tetraphis* and *Oedipodium* has been outlined by Correns (1899).

MATERIAL AND METHODS

The study was based on two specimens of *O. griffithianum*. The first one was collected in the Russian Far East, in alpine belt of the Tardoki-Yani Mountain by V.A. Bakalin in the late August 2013 and still not completely dried (for herbarium) collection was put in a refrigerator with +4°C. Illustrations based on this specimen are in Figs. 1-12, 14-17, 23-24.

Second specimen was collected in 2014 in September in Olkhovaya Mountian in Primorsky Territory by V.E. Fedosov, and delivered in perfectly living condition. Capsules were opened likely long ago and almost empty, but at its bottom spores were observed lying near spore sac wall, allowing comparing surface of the latter with that of spore surface. Illustrations of this second specimen are in Figs. 13, 18-22, 25-29. Some differences were found between these somewhat premature spores from capsule bottom and fully mature spores in the first specimen, so below their differences are specially discussed.

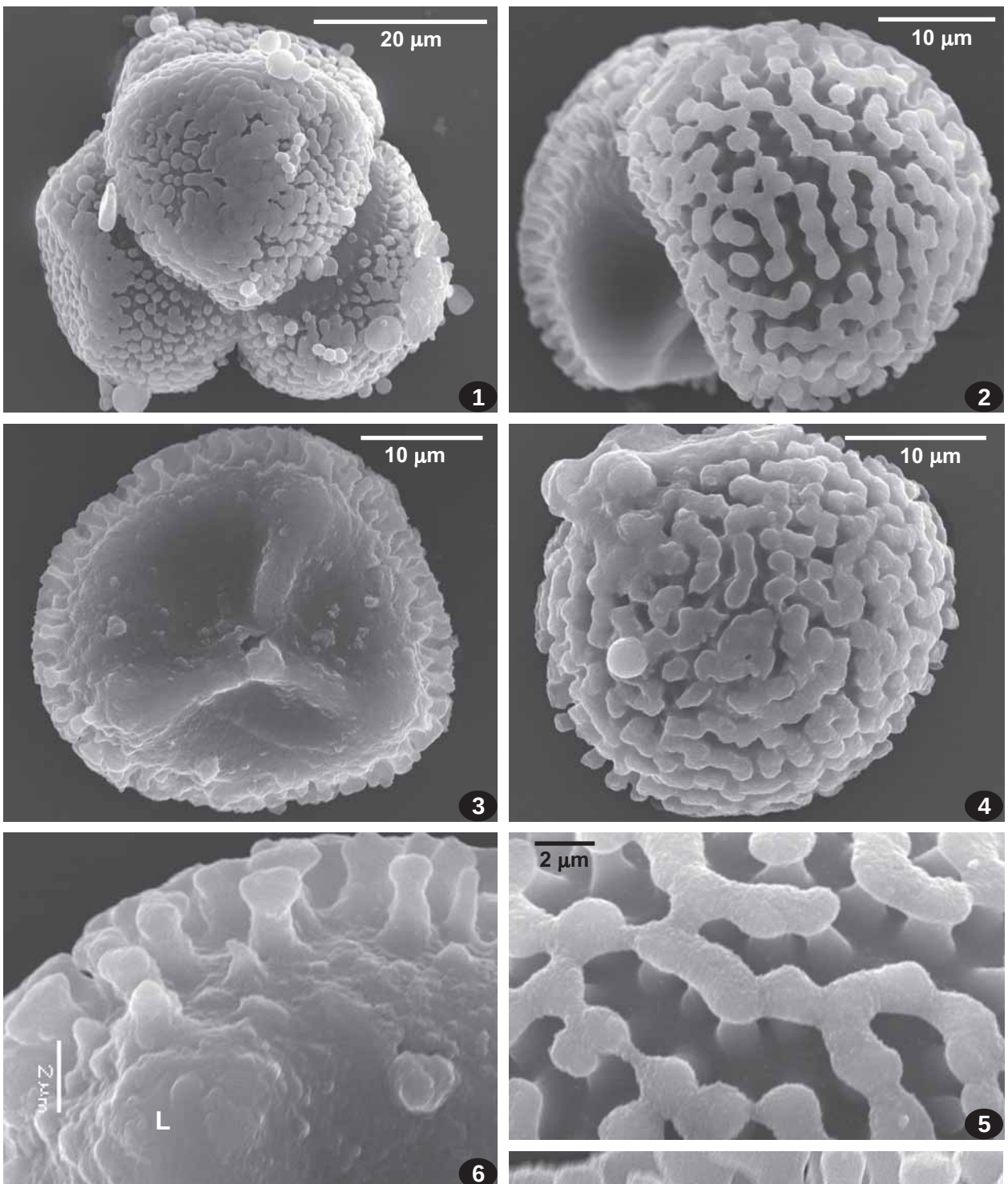
Specimens for SEM studies were coated by gold and studied under JSM-6380LA SEM (JEOL, Japan). TEM specimen preparation included wetting in cacodylate buffer for 1 hour, fixation in 2% glutaraldehyde (on the same cacodylate buffer for 1 hour at room temperature, washed in buffer and postfixed in 1% osmium tetroxide for 2 hours, room temperature. Then spores were dehydrated in ethanol series to 96%, moved to pure, acetone, acetone-epon and then epon-mix medium for 24 hours. After that, polymerization was conducted for 5 days at +62°C. The sectioning was done with a Leica-5 ultratome, for sections 50 nm thick. Specimens partly underwent contrasting with the uranyl acetate and lead citrate by the protocol at http://www.2spi.com/catalog/chem/lead_cit-addinfo.html, partly were studied without any additional treatment.

Sections were studied under JEM-1011 TEM (Jeol, Japan) at 80 kV and a CCD GATAN ES500W under control Digital Micrograph GATAN in Laboratory of electron microscopy at the Biological faculty of Lomonosov Moscow State University. The terminology follows Brown & Lemmon (1990) and Brown *et al*. (2015).

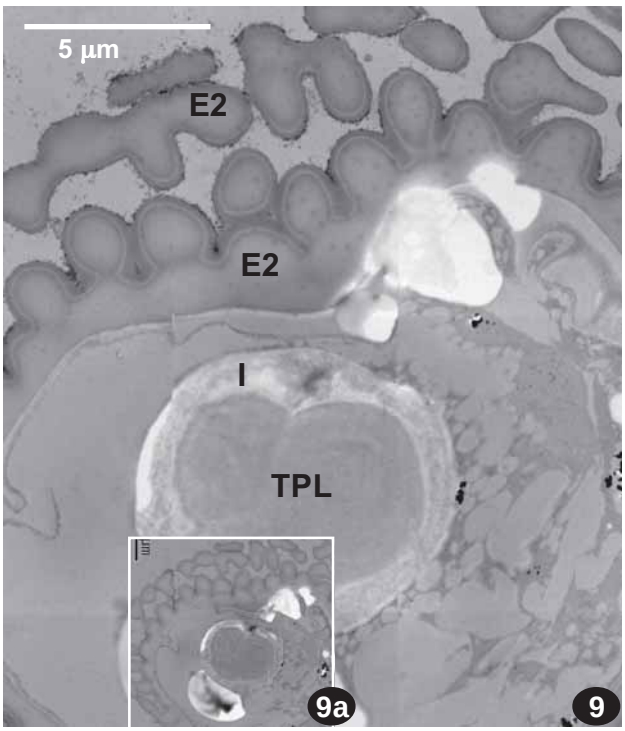
RESULTS

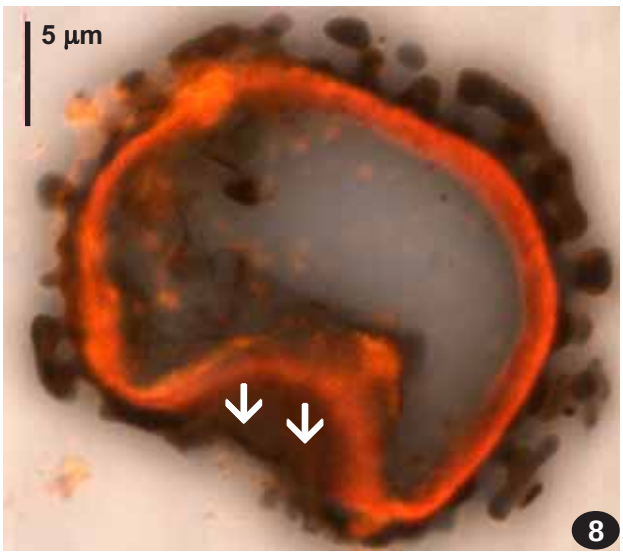
General morphology of mature spores

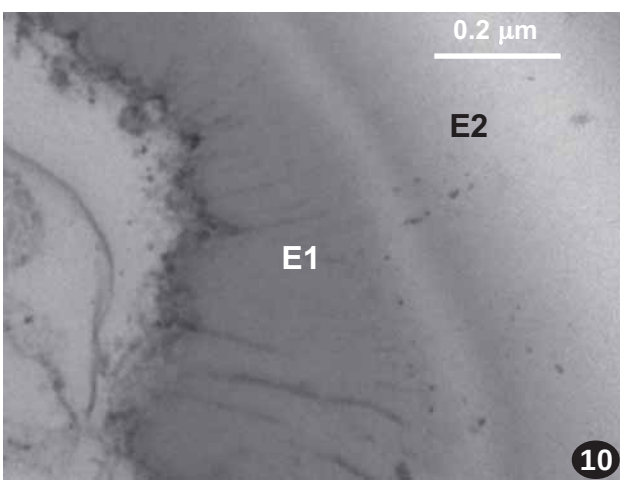
Spores are trilete, convex-hemispheric and trihedral, round in polar view, hemispheric to convex-hemispheric in equatorial view, 24–25 μm in polar axis and 29–35 μm in equatorial diameter. **Laesurae** are straight, with labrum and never reach the sporoderm thickness; suture is 13.3–16.7 μm long. **Sporoderm** thickened by wall sculpture on distal hemisphere and thickened under laesura on proximal hemisphere (Figs. 1-4).

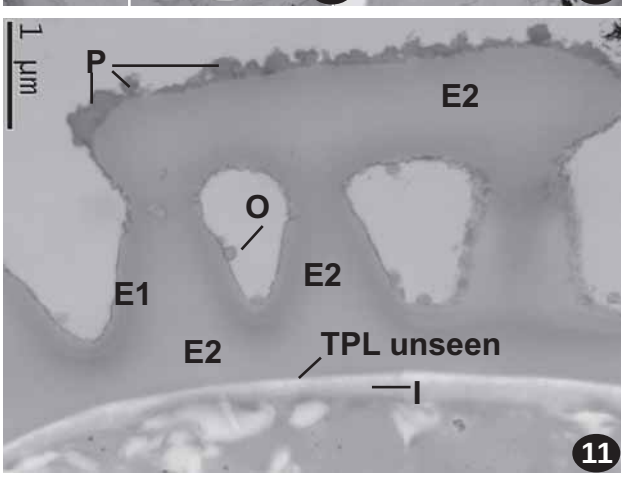


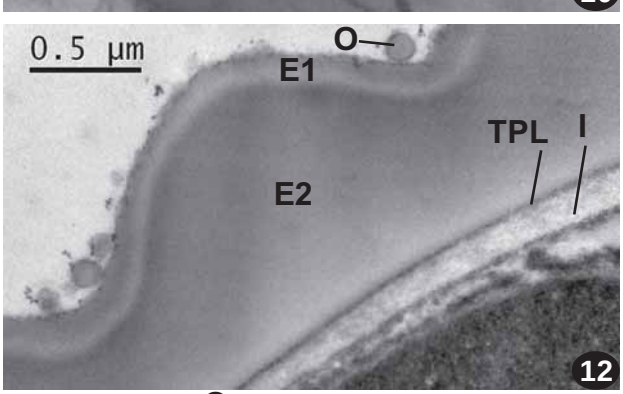
Figs. 1-7. SEM micrographs of *Oedipodium griffithianum*. 1: spores in tetrads; 2: two spores of disintegrated tetrad, still adjoining by their equatorial edge (above); 3: spore proximal hemisphere showing micro-waved sculpture, small globular orbicules (Ubish bodies) and trilete laesura; 4: spore distal hemisphere, clavate sculpture and large globular orbicules (Ubish bodies) are visible; the conglomerate heads of clavae are fused in reticulum at places; 5: sculpture of distal spore surface, showing scabrate-microverrucate tops of clubs coalesced in the reticulum; 6: equatorial region, a view from proximal side, showing edge of area covered by clavae and end of triradiate laesura, enlarged from Fig. 3; 7: sculpture of spore surface at transition from distal side to equatorial region.

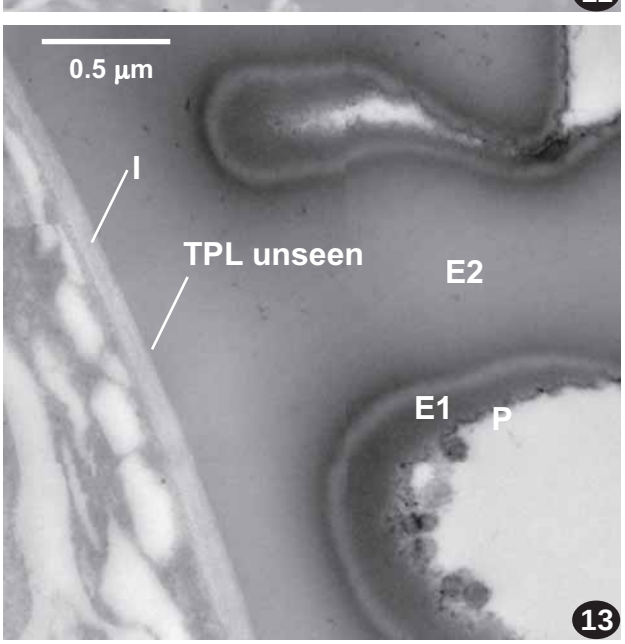

5 µm
E2
E2
I
TPL
9a
9

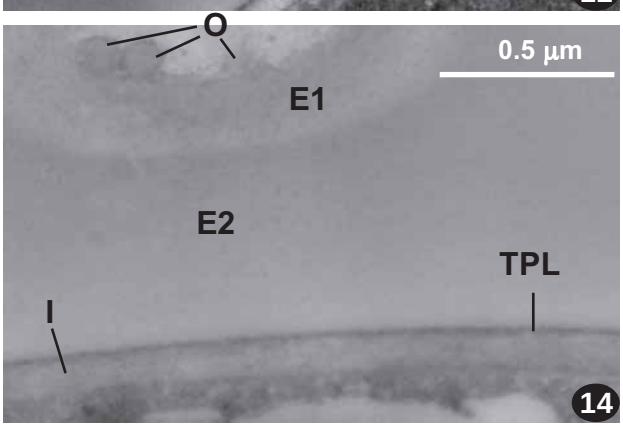

5 µm
8


0.2 µm
E2
E1
10


1 µm
P
E2
O
E1
E2
E2
TPL unseen
I
11


0.5 µm
O
E1
TPL
I
E2
12


0.5 µm
I
TPL unseen
E2
E1
P
13


O
0.5 µm
E1
E2
TPL
I
14

Spores appeared to be difficult for impregnation, thus the spore content was observed only partly. At the same time invagination of laesure was commonly observed (Figs. 8-9).

Sporoderm ultrasculpture

Distal hemisphere is covered by clavate ornamentation and bacula. The sporoderm surface and the top of clubs are scabrate-microverrucate, while their lateral surface is smooth. The height of clubs vary from 2.3 to 3.5 μm, while bacula often intermingled with them are shorter, to ca. 1.5 μm. The diameter of the club heads is 1.5–2.0 μm in average, while the distance between them is 3-4 μm, so larger heads of the clubs are partly fused forming a reticulum (Figs. 2, 4, 5, 9, 11). Ultrasculpture type is changing abruptly to finely wavy one at the transition to the proximal hemisphere (Figs. 2, 3, 6).

Sporoderm ultrastructure

In general, Sporoderm of *Oedipodium* consists of a two-layered exine (exosporium), intine (endosporium), separated along most of spore surface by a well-differentited innermost layer of exine, called here TPL-layer, the derivate of tripartite lamellae, the structure crucial for exine formation in mosses (Brown & Lemmon, 1990; Brown *et al*., 2015). As spore walls mature, the lamellae are cemented with sporopollenin and obscured, but a distinctive multilamellate layer is often seen in the innermost exine. TPL recently has been studied at developmental level (Wallace, 2013).

Outside the sporopollenin wall a layer of perine occurs at places, more pronounced in spores from capsule bottom, apparently somewhat premature ones.

Exine in most cases is stratified into the outer exine (E1) and inner exine (E2). These two layers are differentiated mainly in electron-density: the inner exine is lighter inside and gradually changed to somewhat darker, so at the border of the inner and outer exine the outer exine appears to be lighter than inner exine, although in average both layers of exine can be of about the same color (Figs. 12-13). However the outer exine is darker (Fig. 26). Within laesura exine structure can be even more complicated, as in the peripheral zone of inner exine two slightly differentiated layers may be recognized, being differentiated in electron density and in fine texture (Fig. 26).

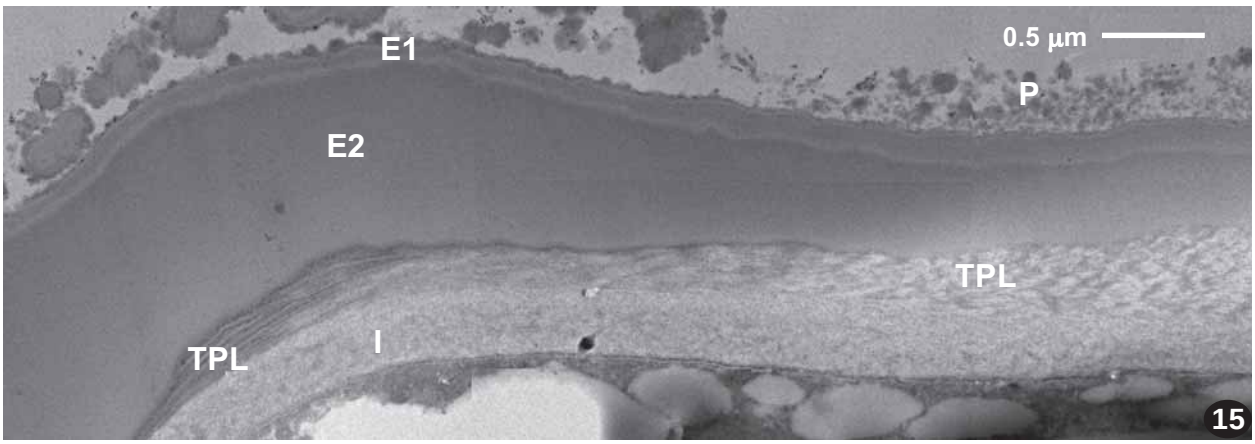
Outer exine is also grading in color, being lighter in its innermost part, which additionally contrasting border with the inner exine (Figs. 12, 13, 16). Inner exine is fairly homogeneous in most places (Figs. 11, 15, 17), and forms sculpture of clavae and bacula on the distal hemisphere (Fig. 11). The outer exine has at places apparent stratification in color (Figs. 16), however in most cases is electron grey, grading to lighter zone towards the border with inner exine (Figs. 14, 15, 22). Upon the heads of sculpture elements outer exine may be thinning up to the total absence (Figs. 11, 27).

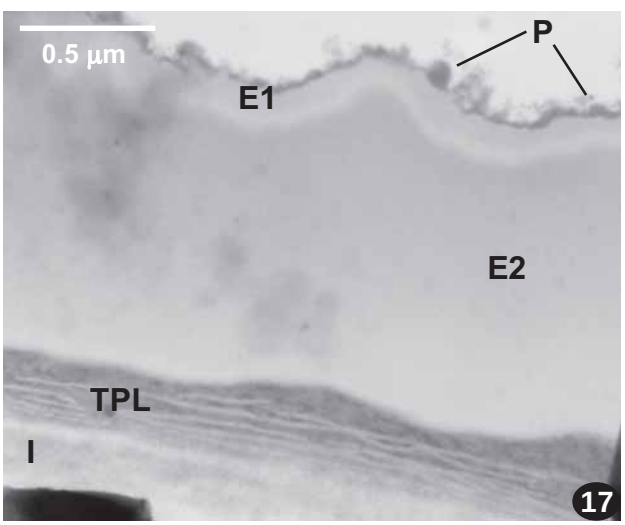
The layer between intine and exine in the distal hemisphere is thin, dark and homogeneous and occasionally totally absent, in the equatorial region it is widened, distinctly multilamellar to almost homogeneous, while in proximal part of spore and in laesura this median layer has complex structure. The transitions of this median layer from one variant of structure to another was observed in numerous spore sections, ensuring that the structure of the common origin is at hand and it is called and denoted in figures as TPL layer following Brown & Lemmon (1990) and Brown *et al*. (2015).

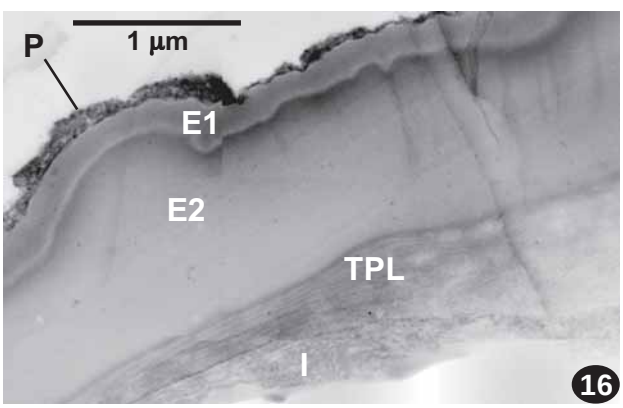
Electron-dark perine coats exine, but in *Oedipodium* it is quite unevenly developed. It forms more or less solid layer in a quite few places (Fig. 18), more commonly it is thin and fragmentary (Fig. 16), mixed with electron-light material (Fig. 20), strongly eroded and appeared as unconnected immediately to exine (Fig. 15) or almost absent (Figs. 9, 12, 14). It is granulose, with electron-dark granules of 0.1 μm or smaller. In the capsule bottom, perine upon the spore wall (Fig. 28) is quite similar to the electron dense layer upon the spore sac (Fig. 29), just near the corresponding spore (cf. Fig. 27), which indicates a putatively common origin of this material.

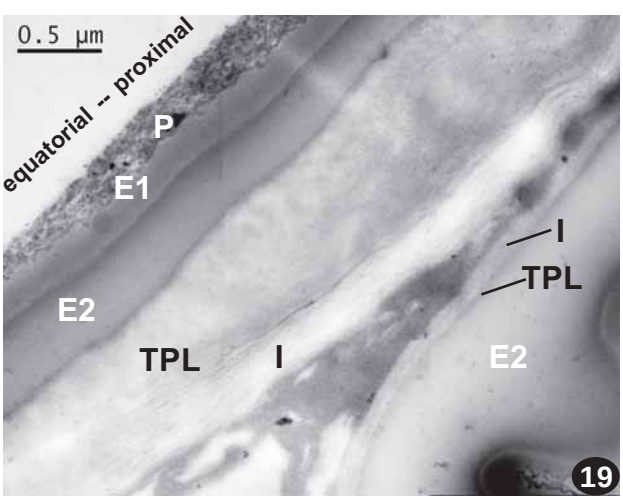
Distal hemisphere and equatorial region. The spore outer surface is moderately smooth (Figs. 12, 14) to at places wavy and channeled (Fig. 10). It is covered by electron-light spheroidal granules of orbicules (Ubish bodis) partly mixed with electron-dark, or occasionally only moderately electron-dark microgranules of perine. In mature spore perine is poorly represented on distal hemisphere, often almost absent, while in the capsule bottom, likely a somewhat premature spores, the poorly structured mass is seen between clavae of the distal hemisphere (Figs. 11, 25) and close to equatorial region the perine layer is sometimes quite apparent (Fig. 15).

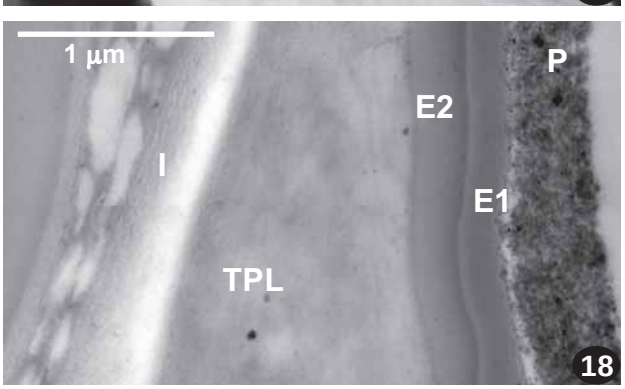
Figs. 8-14. Spores of *Oedipodium griffithianum* with details of sporoderm ultrastructure on the distal hemisphere, TEM (9-14) and LCSM (8). 8: spore section in distal/proximal direction, showing in orange color fluorescence of cellulose in intine stained by berberine, in contrast exine is dark brown; clavate bacula cover all surface of the distal hemisphere; the intine is thickened on the proximal hemisphere; the middle layer is arrowed on the proximal hemisphere; 9: total spore section in parallel to proximal side, which is somewhat invaginated, thus the laesura near proximal pole is cut (in the middle of the figure); 10: part of sporoderm between bacula in a spore younger than one in Fig. 14; the filled with the electron-dark substance, narrow channels pierce the outer exine, it is possible that this exine layer is formed by granule aggregation; 11: ultrastructure of distal sporoderm, showing thin, electron-light intine (I), an almost unseen TPL layer, darked outer exine (E1), lighter inner exine (E2) and electron-dark micro-granules of perine (P) on club tops; 12: distal sporoderm between clavae, with weak differentiation into E2 and E1, and orbicules (O); TPL-layer is seen as dark line between exine and intine. 13: distal sporoderm in base of clavae, TPL unseen, E1 somewhat striolate, E2 homogenous, thin electron-light intine (I), with surface covered by orbicules (O) and mass of fine particules of perine (P); 14: ultrastructure of distal sporoderm: thin, spotted, electron-light intine, the electron-dark TPL-layer, homogenous inner exine, the thin electron-lighter outer exine with orbicules on its surface are visible;

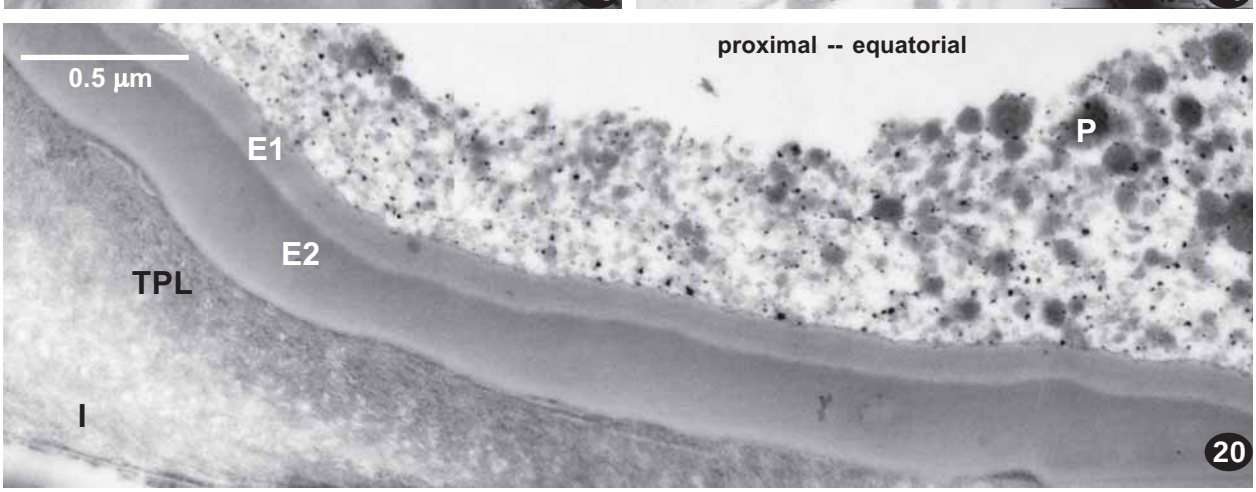

0.5 µm
E1
P
E2
TPL
I
TPL
15


0.5 µm
P
E1
E2
TPL
I
17


P
1 µm
E1
E2
TPL
I
16


0.5 µm
equatorial -- proximal
P
E1
I
TPL
E2
TPL
I
E2
19


1 µm
P
E2
I
E1
TPL
18


0.5 µm
proximal -- equatorial
P
E1
E2
TPL
I
20

Thin electron-dark TPL-layer of to ca. 0.05 μm thick occurs between the exine and the intine in distal hemisphere in most spore sections (Figs. 12, 14), although sometimes it is totally indiscernible (Figs. 11, 13). TPL-layer is more apparent towards the equatorial region where it changes into thicker and multilamellar, especially distinct at the bend to the proximal hemisphere (Fig. 15). The multilaminar structure, however, is not apparent in all sections: Figs. 16, 17 illustrate rather electron light TPL with non contrast lamellae while no traces of lamellae are seen in Fig. 19. Further on from equator to proximal side, such TPL appears to be more homogeneous than intine, which in the distal hemisphere and in transition from equator to proximal hemisphere, is often distinctly fibrillose.

The total exine thickness is 0.58–0.67 μm between the sculpture elements on the distal hemisphere. The outer exine ranges from 0.12 to 0.17 μm, and the inner exine being 0.42–0.52 μm between the sculpture elements on the distal hemisphere. The intine thickness on the distal hemisphere varies at 0.08–0.21 μm.

Proximal hemisphere. Sporoderm has the same layers without sculpture of clavae and bacula. Perine is also fragmentary in fully mature spores (*e.g.* Fig. 24), but is much better developed in spores from capsule bottom (*e.g.* in Figs. 18, 20). The outer exine in such places has more rough surface than in other places and somewhat narrow channeled (Fig. 24 and 24a).

The total exine thickness (E1+E2) is 0.31–0.55 μm between laesurae, thinning to 0.21–0.24 μm on the laesura side. The outer exine is 0.10–0.18 μm and almost constant throughout proximal hemisphere, while the inner exine varies from 0.11–0.15 μm on the laesurae side and to 0.21–0.42 μm in between laesurae. The TPL-layer between exine and intine is very different in thickness and structure (see below). The intine ranges in thickness from 0.10–0.17 μm at equatorial zone to 0.54–1.52 μm on the proximal pole.

Sporoderm ultrastructure of laesura is formed of thin outer exine, thin inner exine, thick TPL-layer and intine. Both exine layers are as apparent as in distal hemisphere, becoming abruptly thin in the laesura centre (Figs. 21-24). A narrow canal in exine provides a contact between the intermediate layer and environment (Fig. 24a).

TPL-layer being often distinctly multilamellar in the equatorial zone, abruptly changes towards of the proximal pole: apparent lamellae are disappearing, although a weakly discernible lamellose patterns can be seen (Fig. 20). The overall color of the layer is quite similar to those of intine or only slightly darker, however the wavy texture allows delimitation of the TPL layer and intine without difficulty. In many cases (*e.g.* Fig. 15), the TPL-layer has "hatched" appearance, due to short irregularly arranged dark lamella clusters spreading among the light matrix. These clusters being darker provide a wavy appearance of the layer, due to darker clusters of lamellae are arranged at a narrow angle, 20-30°, with the inner exine border.

Ring-like structures are visible on the transverse cross-sections of TPL-layer (middle layer) in laesura (Fig. 21-23). It is probable that these structures looking like a ring or slits on cross-sections are tubulose being formed by lamellae. However details of the connections between sharp continuous lamellae in the spore equatorial zone and structures within the median part of laesura require special studies.

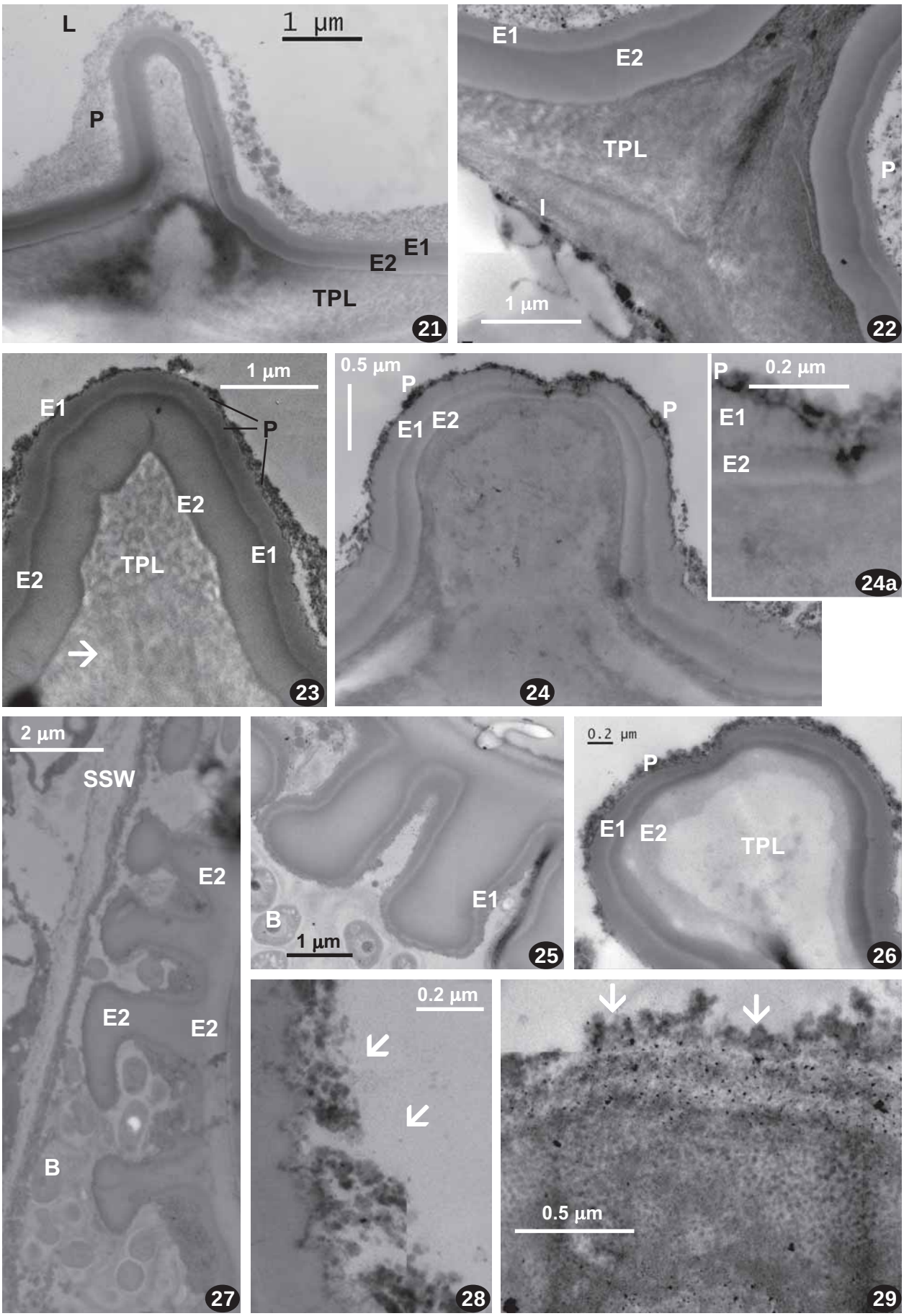
Discussion

Spores of *Oedipodium* are somewhat larger than average in mosses, considering that in many families 20 μm is the maximal spore size. In the moss spore atlas of Boros *et al*. (1993), the mean size is below 20 μm in 151 species out of 210 species described.

The reason for such an upper limit is likely related to the peristome, an organ specifically designed for optimizing moss spore release. The ventral trabeculae on the inner side of peristome teeth are spaced usually at 15-20 μm, which is the average cell size in moss sporophyte. As they work as the mechanism carrying spores outside, it can be assumed that the spore size in peristomate mosses is under the pressure of natural selection, which adjusts spore size to the distance between ventral trabecule. A partial proof of such a correlation was published by Huttunen *et al*. (2004) for pleurocarpous mosses, where the enlarged spore size is associated with the peristome reduction/modification.

Thus 24–35 μm spore size in *Oedipodium* well coincides with eperistomate *Sphagnum,* 15–41 μm, *Andreaea,* (10–)20–50(–110) μm; *Andreaeobryum,* (50–)90–100(–120) μm, and *Takakia,* 25–36 μm.

Figs. 15-20. Details of equatorial and proximal sporoderm ultrastructure in *Oedipodium griffithianum*, TEM. 15: Equatorial area with transition to proximal hemisphere: perine is fragmentary, outer exine is layered, while the inner one is homogeneous; distinctly lamellar at equator TPL-layer is broadened towards the proximal pole, with short dark 'hachures'; 16: ultrastructure of equatorial sporoderm, magnified: electron-grey intine, the lamellate more electron-dark middle layer, homogenous inner exine, the thin more electron-light outer exine, and the microgranulate, electron-dark perine are visible; 17: section of equatorial sporoderm, showing spotted, two exine layers with diffuse perine material, multilaminar TPL at the equator, continued into oblique-hatched layer between exine and intine, thus treated homologous to TPL, although quite distinct structurally. 18: proximal surface between laesulae, where the perine is remained, exine layers are homogeneous, broadened TPL-layer with 'cloudy' heterogeneity, and fibrillose intine. 19: section of a collapsed spore where both proximal (above left) and distal (below right) surfaces are seen; note thicker inner exine on distal side (outer exine is not shown on distal hemisphere), but much thicker TPL-layer and intine on proximal side. 20: proximal surface section, from plane surface to laesura; note well retained perine, two-layered exine, and much broadened TPL-layer and intine towards laesure; TPL includes darker and short 'hatchures' and few light lamellae, similar to those in equatorial zone; intine is finely fibrillose and indistinctly two-layered.


L
1 µm
P
E1
E2
TPL
21
E1
E2
TPL
P
I
1 µm
22
1 µm
E1
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E2
TPL
E1
E2
23
0.5 µm
P
E1
E2
P
24
P
0.2 µm
E1
E2
24a
2 µm
SSW
E2
E2
E2
B
27
B
1 µm
E1
25
0.2 µm
P
E1
E2
TPL
26
0.2 µm
28
0.5 µm
29

Even more apparent (although never statistically supported) is the trend to large and heavily ornamented spores in eperisomate ephemeral acrocarpous mosses, *e.g. Physcomitrium, Physcomitrella, Weissia, Bruchia, Ephemerum, Archidium*. It seems that the advantages of spore enlargements are almost universal, as there are a rather few groups where gymnostomous sporophytes are associated with the spores less than 20 μm.

In the spore wall structure, *Oedipodium* has a number of characters that are rare in other mosses. These are: (1) clear trilete laesura; (2) the lack of perine in the fully mature spores; (3) the column-like sculpture on the distal hemisphere contrastingly different from the proximal side which is only slightly wavy and the fusion of clavae heads with reticulum formation on distal spore surface.

(1) The **clear trilete laesura** occurs in many groups of hepatics and hornworts (Boros *et al*., 1993, Brown & Lemmon, 1990; Brown *et al*., 2015), characterized by a rather passive spore spreading. A clear trilete laesura is never observed in mosses with well-developed peristomes, being restricted to basal lineages, which are primarily eperistomate: *Sphagnum, Takakia, Oedipodium*. However the facts that some rather advanced groups, like Hedwigiaceae, Helicophyllaceae and Rhacocarpaceae may develop at least a superficially very similar structure indicate that this developmental pathway still remains. Note however that the laesura in *Hedwigia* is much less distinctly armed compared with that in *e.g. Sphagnum* (Brown *et al.*, 1982a,b), as well as in *Oedipodium*.

(2) An unusual character of *Oedipodium* spores is the **absence of perine in the fully mature spores**, although slightly premature ones are covered by electron-dark granulose mass. The perine presence was indicated and illustrated for *Oedipodium* by Brown *et al*. (2015), who likely described somewhat premature spores (given that spores were still in tetrads and clavate elements were lower, more scattered and not fused by their heads). Perine forms spore surface sculpture in almost all reports of mosses, with the only exception of *Bruchia brevifolia* (McClymoth & Larson, 1964), however Rushing (1985) supposed that in other species of this genus with ca. 25 species, spore walls may be formed with the more contribution of perine.

Among the other moss genera studied with TEM for the spore wall structure, a series of proportion on exine and perine contribution to spore wall formation occur. Most mosses have distinct perine sitting on the smooth surface of exine. Loose and fragmentary deposition of perine is typical to basal lineages of mosses including Sphagnopsida, Andreaeopsida and Oedipodiopsida, although it varies from species to species (Table 1).

There are also examples where the base of sculpture elements is formed by electronically transparent layer of exine (*Trematodon longicollis, Ditrichum ssp., Polytrichum commune*), while the main part of papillae are evenly dark to almost black, thus composed of material referred to for this reason as perine. Further, the spore wall in *Astomum phascoides* has a sculpture formed both by exine and perine, while in *Ephemerum spinulosum* perine forms a thin (but continuous) layer upon sculpture formed principally by exine.

The perine particles in mosses are usually larger than observed here in *Oedipodium*, except *Andreaea* (Brown & Lemmon, 1984).

The record of the electron-light perine (Estébanez *et al*., 1997) likely corresponds to orbicules (Ubish bodies) that are seen also on the sporoderm of *Oedipodium*, as well as of *Bruchia brevifolia* (Fig. 6 in McClymont & Larson, 1964). However the problem of referring to these electron-light structures as perine require a specific study of spore development.

(3) The **column-like clavae** on the distal spore surface is not a unique feature in mosses, but the fusion by their distal parts has never been reported except for *Oedipodi-*

Figs. 21-29. Details of laesura and inner wall of spore sac ultrastructure of *Oedipodium griffithianum*, TEM. 21: Transversal section of laesura, showing greatly thickened middle layer, TPL intruding in laesura, which raised over the proximal surface (cf. Fig. 3); the uniform two layered exine covers the middle layer on and between laesurae, the micro-granulate moderately electron-dark perine remains between laesurae; 22: inner part of laesura with granulose perine and homogeneous inner and outer exine; intine thickened immediately under laesure, while TPL-layer is much broadened, filling the main body of inner part of laesura; hachures in TPL is directed towards the top of laesura; 23: upper part of laesura beside proximal pole; perine granulose, inner and outer exine homogeneous; TPL included dark structures directed towards the laesura top; ring-like structure in TPL is arrowed; 24: outer parts of ultrastructure of laesura nearly a place of convergence of two rays (cf. Fig. 3), showing narrow hole in two-layered exine on the laesura center (cf. Figs. 3) and the loose micro-granulose perine; the thickened TPL layer contacts the spore environment, likely obtaining liquid water and conducting it through the intine and to a protoplast; 24a: close up of 24: note porose exine near hollow, that are likely also may contribute to water uptake; 25: distal sporoderm of spore at open capsule bottom; the bacula are composed of the homogeneous inner exine and heterogeneous outer exine, some bacteria are located between bacula; 26: transverse section of laesura top, somewhat beside from the proximal pole; exine fully covers the surface and the main body is filled by TPL-layer. 27: Spore sac wall (SSW) with nearby spore distal surface, in open capsule bottom; numerous bacteria are located between the spore sac and spore; electron-dark micro-granules covers bacula, and especially space in between them, as well as spore sac wall (cf. Figs. 25, 28-29); 28: part of outer exine with the electron-dark microgranules of perine; 29: part of spore sac wall with the electron-dark microgranules similar to those in Fig. 28; cell wall and intracellular structures of spore sac not visible; it is possible this granules are composed of the same material as the perine. Abbreviations: B: bacteria, C: clava, E1: outer exine, E2: inner exine, I: intine, O: orbicule (Ubish body), ML: middle layer, P: perine, PM: plasma membrane; SSW: spore sac wall.

Table 1. A comparison of sporoderm layers in mosses

Systematic position	Species	Perine	Exine	Layer between exine and intine	Intine
Sphagnopsida: Sphagnaceae	*Sphagnum lescurii* (Brown *et al*., 1982a,b)	Loose and fragmentary	Sculpture forming	Lamellar exine A	Homogeneous or not specified
Sphagnopsida: Sphagnaceae	*Sphagnum capillifolium* (Filina & Filin,1985)	Loose and fragmentary	Sculpture forming	Lamellar exine	Homogeneous or not specified
Takakiopsida: Takakiaceae:	*Takakia ceratophylla* (Renzaglia *et al*., 1997; Brown *et al*., 2015)	Sculpture forming	Thin , electron-light	Lamellar exine	Homogeneous or not specified
Andreaeopsida: Andreaeaceae	*Andreaea rothii* (Brown & Lemmon, 1984)	Loose and fragmentary	Loose	Not observed	Homogeneous or not specified
Andreaeopsida: Andreaeaceae	*Andreaea rupestris* (Filina & Filin,1984)	Absent	Loose	Not observed	Fibrillose
Oedipodiopsida: Oedipodiaceae	*Oedipodium griffithianum* (Brown *et al*., 2015)	Present	Sculpture forming	Lamellar TPL at places	Fibrillose
Oedipodiopsida: Oedipodiaceae	*Oedipodium griffithianum* (present paper)	Loose and fragmentary	Sculpture forming	Lamellar, homogeneous to not observed at places	Fibrillose
Polytrichopsida: Polytrichaceae	*Polytrichum commune* (McClymont & Larson, 1964)	Sculpture forming	Forming bases of sculpture elements	?	Homogeneous or not specified
Bryopsida: Funariaceae	*Physcomitrium turbinatum* (McClymont & Larson, 1964)	Sculpture forming	Thin	?	Homogeneous or not specified
Bryopsida: Funariaceae	*Physcomitrella patens* (Schuette *et al*., 2009)	Sculpture forming	Two layered	Fibrillate-granulate	Fibrillate in aperture region
Bryopsida: Encalyptaceae	*Encalypta rhabdocarpa* (McClymont & Larson, 1964)	Sculpture forming	Thin	?	Homogeneous or not specified
Bryopsida: Archidiaceae	*Archidium alternifolium* (McClymont & Larson, 1964)	Sculpture forming	Thin	?	Lamellar in aperture region
Bryopsida: Grimmiaceae	*Grimmia* ssp. (Estebanez *et al*., 1997)	Sculpture forming	Two layered	Lamellar in distal hemisphere	Different intine in different species
Bryopsida Helicophyllaceae Lamellate in aperture region	*Helicophyllum torquatum* (Luizi-Ponzo & Melhem, 2006)		Sculpture forming	Thin	?
Bryopsida Ptychomitriaceae	Ptychomitrium ssp. (Estebanez et. al., 2006)	Sculpture forming	Thin or thick in different species	Lamellar or non-lamellar in different species	Bilayered
Bryopsida Bruchiaceae	*Bruchia brevifolia* (McClymont & Larson, 1964)	Almost absent	Sculpture forming	Opaque	Homogeneous or not specified
Bryopsida Bruchiaceae	*Trematodon longicollis* (Brown & Lemmon, 1981)	Sculpture forming	Forming bases of sculpture elements	Lamellar	Fibrillose and lamellar
Bryopsida Fissidentaceae	Fissidens limbatus (Muellier, 1974)	Sculpture forming	Thin	Electron-gray	Homogeneous or not specified
Bryopsida Ditrichaceae	Ditrichum pallidum (Brown and Lemmon, 1980)	Sculpture forming	Forming bases of sculpture elements	Electron-dark in proximal hemisphere	Homogeneous or not specified
Bryopsida: Ephemeraceae (or Pottiaceae s.l.)	*Ephemerum spinulosum* (McClymont & Larson, 1964)	Thin	Sculpture forming	Opaque	Homogeneous or not specified
Bryopsida Pottiaceae	*Astomum phascoides* (McClymont & Larson, 1964)	Sculpture forming	Forming bases of sculpture elements	Opaque	Homogeneous or not specified
Bryopsida: Pottiaceae	*Phascum cuspidatum* (Carrion *et al*., 1990)	Sculpture forming	Thin	Separating layer in proximal hemisphere	Homogeneous, gray
Bryopsida: Pottiaceae	*Phascum cuspidatum* (McClymont & Larson, 1964)	Sculpture forming	Thin	?	Homogeneous, light
Bryopsida: Pottiaceae	*Pterygoneurum ssp*. (Carrion et. al., 1995)	Sculpture forming	Thin	Separating layer in proximal hemisphere	Fibrillose
Bryopsida: Pottiaceae	*Weissia viridula* (McClymont & Larson, 1964)	Sculpture forming	Thin	Opaque	Homogeneous or not specified
Bryopsida: Orthotrichaceae	*Orthotrichum* (Medine & Estebanez, 2014)	Sculpture forming	Thin, with inner lamellae	Expanded and with labyrinth-like intrusions	Bilayered

um. At the same time it appears to be quite similar to ectexine of angiosperms (Hesse *et al*. 2009). A somewhat similar pattern has also been described in *Haplomitrium* (Brown & Lemmon, 1986; Brown *et al*., 2015) and *Apotreubia* (Brown & Lemmon, 1990; Brown *et al*., 2015).

Contribution of spore wall layers in surface sculpturing

Perine is a layer forming the surface ornamentation in most mosses. More rarely exine participates in the spore surface ornamentation, but most commonly only slightly participates in forming of basal parts of sculpture elements, *e.g.* in *Astomum phascoides, Trematodon longicollis, Ditrichum* spp., *Polytrichum commune*. Two latter species are rather ephemeral haplolepideous mosses of Dicranales (incl. Pottiales). A considerable contribution of exine to the sculputre element formation has been observed in *Bruchia brevifolia* and *Ephemerum spinulosum*. Although not specially studied, exine is the layer most likely responsible for stellate 'armature' on spores of *Encalypta* sect. *Ciliatae*, in a way similar to hepatic genus *Fossombronia* (Brown & Lemmon, 1993).

The layer between exine and intine treated here as **TPL-**layer (Figs. 12-29) was recognized in many mosses, although its terminology varies, as shown in Table 1. The reason for various evaluation is likely, at least partly, related to the variation within the individual spores and also to the stage of development. *Oedipodium* shows almost all known states of this character, although some being expressed only at specific places and moreover appear not in all spores. Equatorial region with the maximally developed lamellar structure formed by 5 alternations of dark and light zones (Figs. 15-17) is approaching to *Sphagnum*, where however multilamellar layer is performed along almost whole border of exine and intine. Lamellar structure is poorly expressed in the same place in spores from capsule bottom (Figs. 18-19), however their putatively premature state is hardly a reason. Brown *et al*. (2015) illustrated a very distinct multilamellar structure in *Oedipodium*, in spores which are assumed to be somewhat premature (by reasons explained above).

In the aperture region, there are a number of common trends in mosses, including thinning of perine, and more rarely thinning of exine, like it is seen in *Oedipodium* laesura near proximal pole (Fig. 24). Splayed TPL fills most volume of laesura, forming the aperture plug and favoring sporeling. Electron-dark 'hachures' are arranged along the proximal spore wall in the proximal hemisphere outside laesura and radially within laesura itself (Fig. 22). The short lamellar plates are arranged in direction from the pore where water uptake by spore is possible. The obtained images allow suggestion that the lamellae of the TPL-layer in proximity to aperture are functioning as a wicks or even at places tubes, accelerating water conduction from the aperture to other spore regions (cf. Figs. 15 & 20 with Figs. 21-23).

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