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ULTRASTUCTURE OF THE ASCUS AND THE ASCOSPORES IN PSEUDASCOZONUS (PEZIZALES, ASCOMYCOTINA)

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The ultrastructure of the ascus and the ascospores in *Pseudascozonus* is described. The top of the ascus opens by a small rather roughly delimited operculum. A circular furrow in the inner ascal wall causes a weakened zone. The development of the ascospore wall shows a simple homogeneous epispore and disappearance of all secondary wall material. In the epiplasm of the ascus a unique type of envelope, surrounding all eight ascospores, is described. Relationship with *Ascozonus* and Theleboli with 8-spored asci is suggested.

Since the first description of *Pseudascozonus* Brumm., based on a single species, *Pseudascozonus racemosporus* Brumm. (van Brummelen, 1985), fresh material of this fungus came to hand for a more detailed study.

The earlier study of *P. racemosporus* with the help of light microscopy already indicated the presence of an interesting structure of the opening mechanism of the ascus top and the peculiar way in which the ascospores are loosely united into fascicles or bunches.

The aim of this study is to reveal the fine structure of the ascus and the ascospores with the help of electron microscopy.

MATERIALS AND METHODS

The material of *Pseudascozonus racemosporus* was isolated from deer dung collected at the type locality of the species, at the 'Tourbière de Frasne', Frasne, dép. Doubs, France, 20 III 1985 (*van Brummelen 7398*).

In the laboratory fruit-bodies were taken from the substratum kept under humid conditions in petri-dishes for several weeks at 12°C in a conditioned growth chamber.

Fruit-bodies were fixed for 3 hours in 1% glutaraldehyde buffered at pH 7.2 with 0.2 M cacodylate at 4°C. To compensate for differences in osmotic value 1.5% sucrose was added. The material was post-fixed for 1 hour in 1% buffered OsO_4 at 4°C. Fixed material was spread in very thin layers of solidifying water agar, dehydrated in an ethanol series, and embedded in Epon.

Longitudinal median sections were cut with a diamond-knife on an LKB Ultratome III, contrasted with Reynolds' lead citrate and uranyl acetate, and examined with a Philips EM 300 electron microscope.

OBSERVATIONS

The ascus

Because of the rather small number of fruit-bodies available for this study, the methods were restricted to glutaraldehyde-OsO_4-fixation followed by Epon embedding.

In *P. racemosporus* the fruit-body consists for the main part of a fully exposed, relatively small bundle of asci.

The shape of the asci is broadly clavate to oblong obovoid, often slightly curved towards one side. They reach a length of 28–33 μm and a width of 11–15 μm (Figs. 1A, B, D).

The base is rather broad with a plugged narrow central pore in the basal septum. The top is regularly rounded. The walls of the young ascus, up to the moment of meiosis, are of rather uniform thickness (approximately 250–300 nm) and undifferentiated.

After meiosis the ascal wall thickens and becomes stratified. An inner, rather thick, more flexible, electron-dense layer is differentiated from an outer, rather rigid, relatively electron-transparent layer.

In the lateral region of the ascal wall the inner layer reaches a thickness of 250–340 nm and the outer layer 70–110 nm.

On further ripening neither a subapical ring, as observed in asci of *Ascozonus* (Renny) E.C. Hansen (van Brummelen, 1974, 1978; Samuelson, 1978), nor a subapical ring-shaped protrusion of the inner ascal wall layer, as reported for several members of the Pyronemataceae (Hung, 1977; Samuelson, l.c.), is found.

Structural differentiation in the top of the ascus is observed only shortly before the moment of spore discharge. Especially the inner ascal layer thickens. Slightly behind the tip it then measures 380–550 nm. At the extreme tip an irregular hemispherical or truncate-conical body is demarcated at the inner side of the wall (Figs. 2B, C). The diameter of this body is about 1780–2100 nm, in the centre it reaches a thickness of 800–900 nm. At the inner side of the ascal wall it is surrounded by a gradually deepening circular furrow. At this stage no stratification of the inner or the outer layer can be observed.

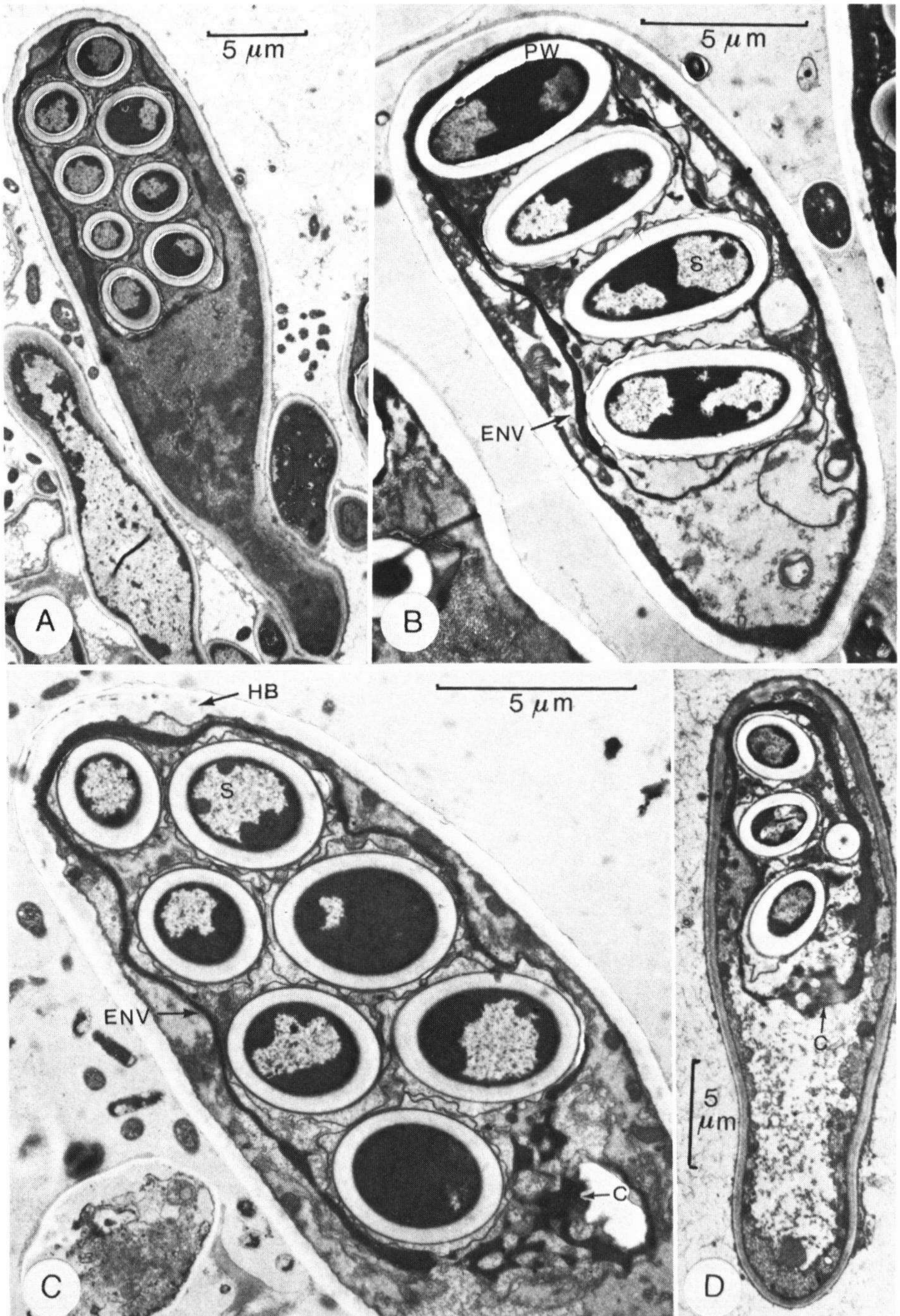
Abbreviations used in figures.—AS, ascostome; AW, ascus wall; C, complexes of electron-dense material of the envelope surrounding all ascospores; E, epiplasm; EN, endospore; ENV, envelope; EP, epispore; ER, endoplasmatic reticulum; F, furrow; HB, hemispherical body; IL, inner layer; IM, investing membrane; O, operculum; OL, outer layer; PW, primary spore wall; S, ascospore; SW, secondary spore wall; WZ, weakened zone.

The scale markers in all figures equal approximately 0.5 μm.

Fig. 1. *Pseudascozonus racemosporus*, electron micrographs of asci. — A, B, D. Longitudinal sections of almost mature asci. — C. Distal portion of an ascus.

Fig. 2. *Pseudascozonus racemosporus*, electron micrographs of ripening and emptied asci. — A. Detail of a ripening ascus. — B. Detail of the apex of an almost mature ascus just before dehiscence. — C. Median section of the apex of a ripening ascus. — D. Median section of an emptied ascus showing the operculum.

Fig. 3. *Pseudascozonus racemosporus*, electron micrographs of advanced ascospore development. — A. Ascospores showing investing membranes and a portion of the envelope. — B. Detail showing investing membranes. — C, D. Details showing investing membranes and portions of the envelope.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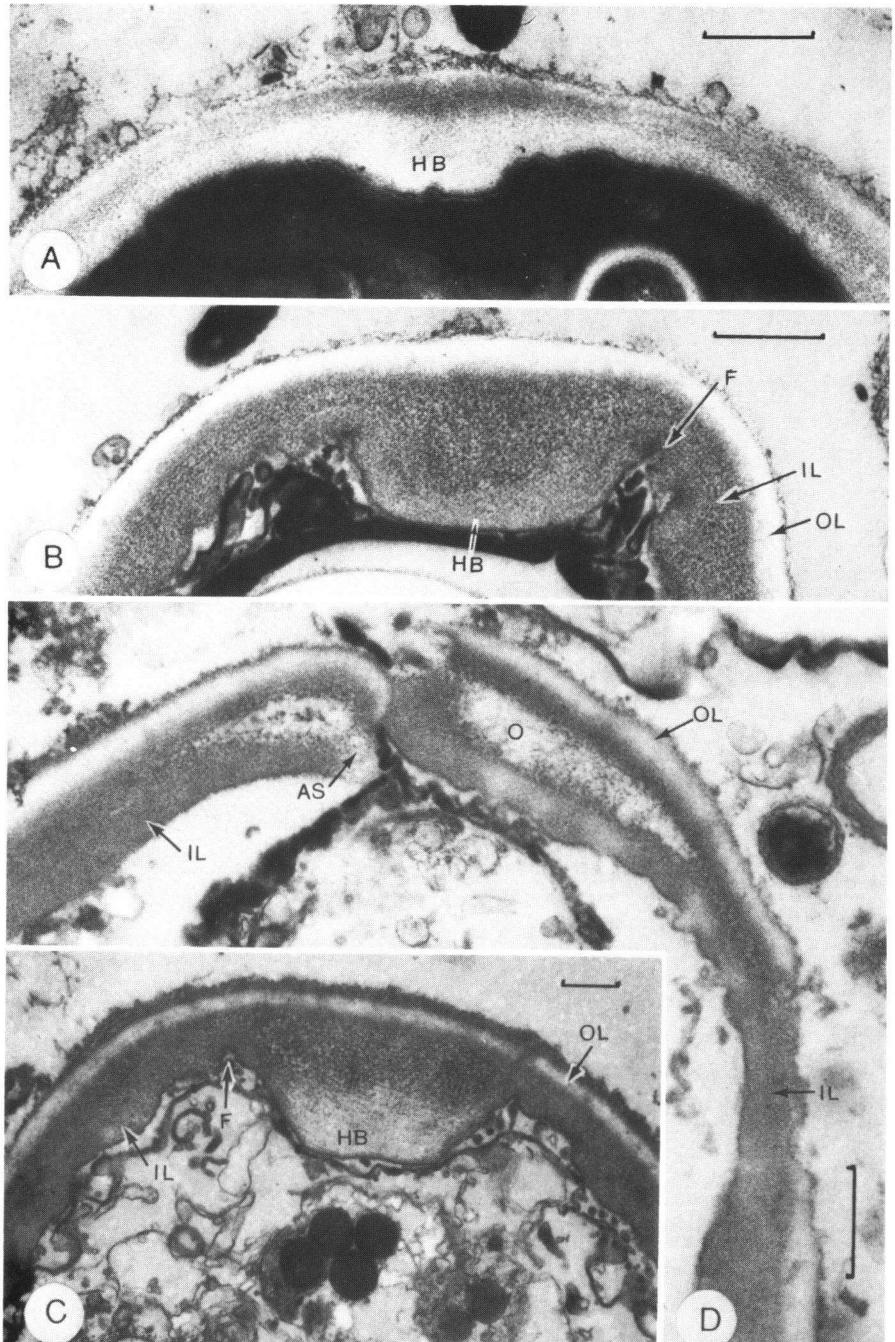
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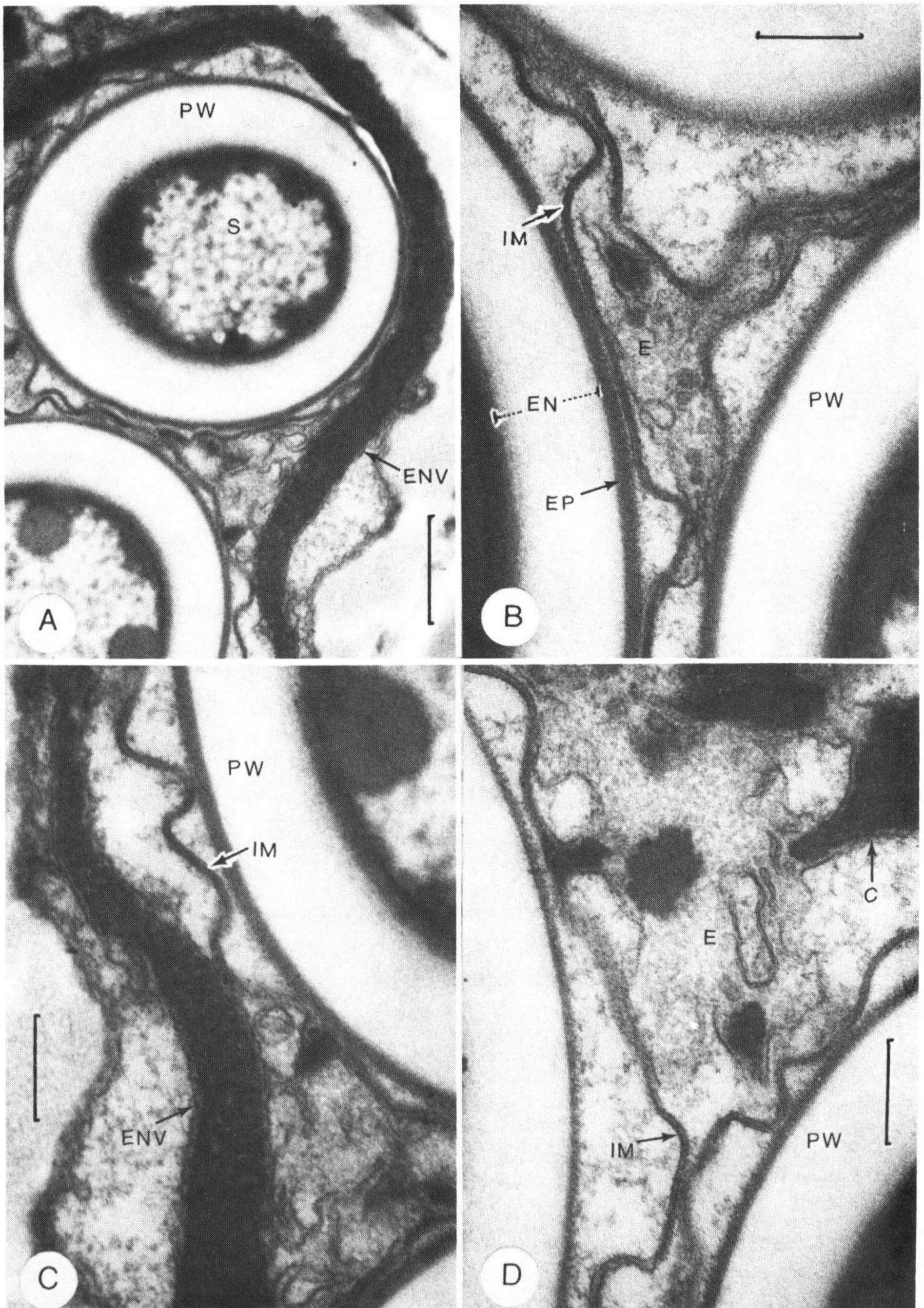
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The circular furrow in the inner layer forms a weakened zone in the top of the ascus. From observations of sections of dehisced asci it is clear that the initial fracturing starts very close to this circular furrow. A small operculum of about 2 μm diameter and 1 μm thick opens forcibly by a fracture in this zone of the inner layer and a very irregular rupture in the outer layer (Fig. 2D). As a result the margins of the operculum and the ascostome are very irregular and rough (Fig. 2D). Because of strong tearing towards the base, often a more or less tongue-shaped operculum is formed. Also rather deep cleavage of the ascal wall from the top downwards can be found in emptied asci. After dehiscence of the ascus, swollen remnants of the inner layer can be found at the inner face of the operculum (Fig. 2D).

Neither a funnel nor a tract is present in the apical part of the epiplasm at any stage of development.

The ascospores

The initial development of ascospores in *Pseudascozonus* closely confirms the general process described for Ascomycetes (e.g. Reeves, 1967; Wells, 1972; Merkus, 1973, 1974, 1975, 1976).

In the young ascus eight ascospores are formed. The primary wall develops between the two delimiting unit membranes and consists of homogeneous electron-transparent material. At a later stage two layers can be distinguished in the primary wall, an inner endospore and an outer epispore. The endospore varies in the thickness from 230–340 nm and is always fairly electron-transparent, with only some vague sublayering at full maturity. The epispore is 33–38 nm thick and seems to consist only of a single fairly electron-dense layer without finer striation (Figs. 3A–D).

While the inner spore delimiting membrane becomes the sporoplasmalemma, the outer spore delimiting membrane changes into the investing membrane (or perisporal sack).

Between the investing membrane and the primary wall new material is deposited on the outside of the epispore. This secondary wall material is floccose and fairly electron-transparent. Its thickness varies with the strong undulations of the investing membrane (Figs. 3A–D).

The investing membrane remains till the last stages, has a constant thickness of 14–18 nm, and consists of two very thin electron-dense layers separated by a transparent one.

The secondary wall material shows no condensation on the primary wall and becomes fully electron-transparent in the end (Fig. 3A). The mature ascospores are perfectly smooth.

During development all eight ascospores become surrounded by a more or less complete envelope (Figs. 1A–D). This envelope includes all ascospores with their investing membranes and some epiplasm. It is formed by very thin cohering membranes of what seems to be endoplasmatic reticulum. Between these membranes an almost homogeneous, very electron-dense material is deposited as an irregular often very incomplete layer. Its thickness varies usually from 100 to 370 nm, but reaches even 1000–2000 nm in greater complexes (Figs. 3C, D).

With light microscopy only the thick parts and the larger complexes can be observed as a yellowish or pale brownish amorphous substance (van Brummelen, 1985).

To a certain degree the arrangement of the ascospores in the fully mature ascus is influenced by the presence of this envelope. In spore shots, the ascospores are often found to be more or less cohering in clusters (van Brummelen, 1985: fig. 2). On ripening, the amorphous substance tends to concentrate at one side of the spore-cluster.

DISCUSSION

The structure of the ascus top in *Pseudascozonus* is summarized in a diagrammatic scheme (Figs. 4B–C).

This type of ascus is characterized by a relatively thin, electron-transparent, rather rigid outer layer and a thicker, more electron-dense, rather flexuous inner layer. At the inner face of the latter an apical hemispherical or truncate-conical part is segregated, surrounded by a narrow circular furrow. Here disintegration of the inner wall takes place and causes a circular weakened zone at the top. Dehiscence of the ascus starts in this weakened zone of the inner layer, inciting rather irregular tearing at the corresponding places of the outer layer.

This causes a small roughly delimited operculum and ascostome. Further tearing of the ascal wall from this initial opening downwards is caused by the forcible release of the more or less cohering ascospore cluster.

Although such a type of ascus top is not known from the genera of Pezizales studied thus far, there is a certain resemblance with the tops of asci described for species of *Ascozonus* and *Thelebolus* (van Brummelen, 1974, 1978).

In *Ascozonus* a minor operculum or an apical disk (van Brummelen, 1974) is also found at the extreme top of the ascus, and the layers in the top are similar. The prominent ring in the ascal wall of *Ascozonus* is absent in *Pseudascozonus*. A somewhat similar type of ascus top is found in *Thelebolus* Tode, where a roughly delimited operculum occurs in some of the species with 8-spored asci.

In the development of the ascospore wall in *Pseudascozonus* it should be noted that the epispore consists of only one homogeneous electron-dense layer, that all secondary wall material disappears, and that the investing membrane remains persistent till the latest stages of development. This is very similar to the structure and development of the ascospore wall as described by Merkus (1976) for *Ascozonus woolhopensis* (Berk. & Br. apud Renny) E.C. Hansen. Rather resembling are also the ascospore walls in *Thelebolus crustaceus* (Fuckel) Kimbr. apud Y. Kobay. & al., *T. stercorarius* Tode, and '*Ascophanus*' *coemansii* Boud. (a representative of *Thelebolus* with 8-spored asci), but here it is reported (Merkus, l.c.) that the secondary wall condenses on the epispore to form a smooth, persistent, extra layer.

The envelope surrounding all ascospores is very reminiscent of the common investing membrane of the ascospores in *Saccobolus glaber* (Pers.: Fr.) Lamb., as described by Carroll (1967, 1969, as *S. kerverni*). But, while the common investing membrane in *Saccobolus glaber* is continuous and replaces the individual investing membranes of the asco-

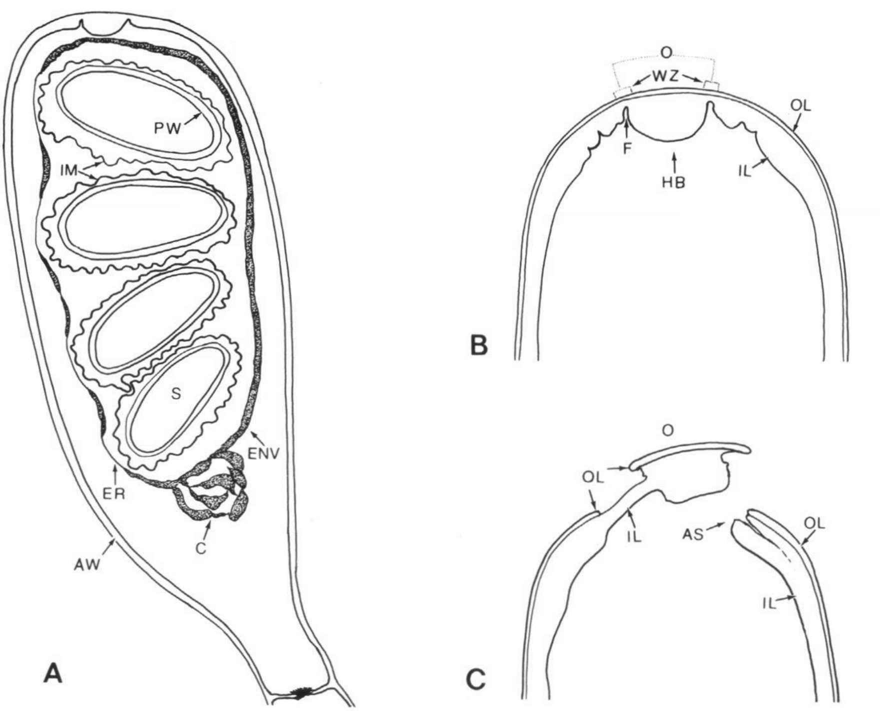

Fig. 4. Diagrammatic longitudinal, median sections of the ascus and the ascus top as seen with electron microscopy. — A. Mature ascus. — B. Top of mature ascus. — C. Top of ascus after spore discharge.

spores, the envelope in *Pseudascozonus* is not formed by fusion of these membranes, since here they are very persistent.

In the epiplasm of *Pseudascozonus*, cohering elements of the endoplasmatic reticulum form the inner and outer clothing of a common envelope consisting for the main part of rather homogeneous electron-dense material. Such an envelope has not previously been observed in other representatives of the Pezizales (Fig. 4A).

Peculiar epiplasmatic structures have especially been recorded in some species of the Thelebolaceae. A pale brownish net-shaped structure, holding all eight ascospores is known from *'Ascophanus' coemansii* (Boudier, 1869: pl. XXX, figs. 7, 8; Merkus, 1976: pl. 5F, G). Large globules with a net-work of plaited units occur in the epiplasm of *Thelebolus stercoreus* (Merkus, l.c.: pl. 4F, 5A, B).

The ultrastructure of the ascus top and of the ascospore wall strongly support the view that *Pseudascozonus* is related to *Ascozonus*, while it also shows affinity to representatives of *Thelebolus* with 8-spored asci. There are, however, sufficient differences to maintain it as a separate genus within the Thelebolaceae.

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REFERENCES

BOUDIER, J. L. E. (1869). Mémoire sur les Ascobolacés. In Annls Sci. nat. (Bot.) V, 10: 191–268, pls. 5–12.

BRUMMELEN, J. van (1974). Light and electron microscopic studies of the ascus top in *Ascozonus woolhopensis*. In Persoonia 8: 23–32.

—— (1978). The operculate ascus and allied forms. In Persoonia 10: 113–128.

—— (1985). *Pseudascozonus*, a new genus of Pezizales. In Proc. Indian Acad. Sci. (Pl. Sci.) 94: 363–367.

CARROLL, G. C. (1967). The ultrastructure of ascospore delimitation in *Saccobolus kerverni*. In J. Cell Biol. 33: 218–224.

—— (1969). A study of the fine structure of ascosporogenesis in *Saccobolus kerveni*. In Arch. Mikrobiol. 66: 321–339.

HUNG, C.-Y. (1977). Ultrastructural studies of ascospore liberation in *Pyronema domesticum*. In Biol. Rev. 40: 52–92.

MERKUS, E. (1973). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)–I. *Ascodesmis microscopica* (Crouan) Seaver and *A. nigricans* van Tiegh. In Persoonia 7: 351–366.

—— (1974). Ultrastructure in the ascospore wall in Pezizales (Ascomycetes)–II. Pyronemataceae sensu Eckblad. In Persoonia 8: 1–22.

—— (1975). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)–III. Otideaceae and Pezizaceae. In Persoonia 8: 227–247.

—— (1976). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)–IV. Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae; general discussion. In Persoonia 9: 1–38.

REEVES Jr., F. (1967). The fine structure of ascospore formation in *Pyronema domesticum*. In Mycologia 59: 1018–1033.

SAMUELSON, D. A. (1978). Asci of the Pezizales. II. The apical apparatus of representatives of the *Otidea-Aleuria* complex. In Can. J. Bot. 56: 1876–1904.

WELLS, K. (1972). Light and electron microscopic studies of *Ascobolus stercorarius*. II. Ascus and ascospore ontogeny. In Univ. Calif. Publ. Bot. 62: 1–93.