PERSOONIA
Published by Rijksherbarium / Hortus Botanicus, Leiden
Volume 15, Part 2, pp. 129–148 (1993)

ULTRASTRUCTURE OF THE ASCUS AND THE ASCOSPORE WALL IN SCUTELLINIA (PEZIZALES, ASCOMYCOTINA)

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The development of wall layers in the ascus top and of ornamentation of ascospores is studied with the electron microscope in *Scutellinia pseudotrechispora, S. umbrorum, S. patagonica, S. trechispora,* and *S. scutellata.* In all species studied the ascus top shows a roughly delimited operculum and ascostome, a subapical ring, and special plasmatic structures just beneath the operculum. Details of these structures, as found with different methods of fixation and contrasting, are summarized. The structure and dehiscence mechanism of the ascus as described here for *Scutellinia* are considered to be characteristic of the family Pyronemataceae. The developmental pattern of the ascospore wall in *S.* pseudotrechispora is different from that found in the other species. The secondary wall in this species develops a complex structure not known from other representatives of the Pyronemataceae.

Recently, much attention has been paid to the taxonomy of the genus *Scutellinia* (Cooke) Lamb., a well characterized genus of the Pezizales with a great number of species. Most species possess ascospores with a characteristic pattern of ornamentation.

As shown by Svrček (1971), Kullman (1982), and Schumacher (1990) there is a wide variation in the ascospore ornamentation within the genus *Scutellinia.*

Earlier work on the ultrastructure of the ascospore wall in *Scutellinia scutellata* (L.: Fr.) Lamb. and *S. armatospora* Denison [= *S. trechispora* (B.& Br.) Lamb.] was carried out by Merkus (1974), while the ascan substructure was studied in *S. armatospora* by van Brummelen (1978) and in *S. scutellata* by Samuelson (1978).

Despite these investigations, several problems of the fine structure of asci and ascospore ornamentation remained unsolved. Especially some components of the apical ascoplasm, such as a tract, a funnel, and an apical globule, observed by Chadefaud (1942) with light microscopy in *Ciliaria* [*Scutellinia*] *hirta* (Schum.) Boud. and in some species of related genera, could not be traced in ultrastructural studies of *Scutellinia* and other representatives of the '*Otidea-Aleuria* complex' by Samuelson (1978).

In the present study five species of *Scutellinia* have been investigated with different conventional and advanced methods of fixation and contrasting for the development of their asci and ascopore ornamentation.

Unlike representatives of the families Pezizaceae, Ascobolaceae, and Sarcoscyphaceae, most of the differentiation of the ascus top in species of *Scutellinia* can only be observed in mature asci shortly before spore discharge.

Chemical fixation of mature asci poses two problems. The asci can easily collapse because of minor changes in the osmotic value of their contents and the primary wall of

mature ascospores becomes resistant to fixation, embedding, and thin-sectioning. Often the material is distorted and the structures difficult to interpret. In contrast material fixed by ultra-rapid freezing followed by freeze substitution proved to be much more suitable in this respect even though the dimensions of asci and ascospores are just at the limit of the possibilities of this technique. The shape and arrangement of cytological elements are found to be perfectly conserved. However, the good preservation of structure usually decreases rapidly in zones more distant from the surfaces that have been in direct contact with the freezing medium.

Different wall layers and even different wall regions of the ascus top show a different capacity to take up water. As this imbibition changes when turgor falls or disappears, the relative thickness of wall layers or regions may change considerably.

MATERIALS AND METHODS

The material used in the present study was collected in the Netherlands and in Sweden. The following list gives more details of the specimens and their localities.

Scutellinia pseudotrechispora (Schröt.) Le Gal — *van Brummelen 7861*, on the ground, Hägnen, Femsjö, Småland, Sweden, 4.VIII.1989 (L); *S. umbrorum* (Fr.) Lamb. — *Huyser*, on the ground, 'De Schouw' between Helmond and Someren, Noord-Brabant, the Netherlands, 31.V.1989 (L); *S. patagonica* (Rehm) Gamundi — *Huyser*, on the ground, 'De Schouw' between Helmond and Someren, Noord-Brabant, the Netherlands, 2.VI.1989 (L); *S. trechispora* (B. & Br.) Lamb. — *Piepenbroek*, on damp soil, Duursche Waarden between Olst and Wijhe, Overijssel, the Netherlands, 19.VIII.1973 (L); *S. scutellata* (L.: Fr.) Lamb. — *van Brummelen 4070*, on burnt wood, Nederhorst den Berg, Noord-Holland, the Netherlands, 12.V.1973 (L).

The apothecia were collected along with the substratum on which they were growing. At the laboratory they were removed from the substratum and subsequently fixed for electron microscopy, using different methods. Material of all species was fixed using chemical fixation procedures with glutaraldehyde and potassium-permanganate (van Brummelen, 1986). In addition, part of the material of *S. patagonica* and *S. umbrorum* was fixed by using the ultra-rapid freeze fixation method followed by freeze substitution.

For rapid freeze fixation of the asci very small parts of the hymenium, containing a few asci only, were spread out as thinly as possible, quickly brought into the narrow space of a doubled 100 mesh copper grid, and then rapidly shot with the help of an injector into liquid propane at -180°C. The frozen material was quickly placed in liquid nitrogen for transfer to precooled small metal cylinders each containing 2 ml of a solution of about 1% OsO_4 in anhydrous acetone.

Freeze substitution was carried out in a Reichert KF80 freeze substitution apparatus at -80°C for about 72 hours. After this, the containers were allowed to warm up slowly to about 10°C for two hours. After several rinses in dry acetone the material was infiltrated with increasing concentrations of Epon over a period of 24 hours.

Longitudinal median ultrathin sections of asci were cut with a diamond-knife on a LKB Ultratome III. Sections were usually stained with Reynold's lead citrate and uranyl acetate, while others were stained with this and barium permanganate.

Selected sections of material fixed in glutaraldehyde or by rapid freezing were placed on gold grids and treated with the Thiéry technique for polysaccharides (Thiéry, 1967). The ultrathin sections were viewed with a Philips EM 300 electron microscope.

Legends to Figures 1–10 (on pages 4–13)

Abbreviations used in figures: AG, apical globule; AS, ascostome; AW, ascus wall; CM, condensed material; E, epiplasm; EN, endospore; EP, epispore; ER, endoplasmatic reticulum; FU, funnel; IL, inner layer; IM, investing membrane; IS, inner stratum; M, mitochondrion; N, nucleus; O, operculum; OL, outer layer; OS, outer stratum; P, periascus; PM, plasma membrane or plasmalemma; PW, primary spore wall; S, ascospore; SP, sporoplasm; SR, subapical ring; SW, secondary spore wall; T, tract or funiculus; TS, tubular structure; V, vacuole; WZ, weakness zone. – The scale markers in all figures equal approximately 0.5 µm. Unless stated otherwise, the illustrated material was fixed in 1% $KMnO_4$, post-fixed in 1% OsO_4 and contrasted with uranyl acetate and lead citrate.

Fig. 1. *Scutellinia pseudotrechispora*, electron micrographs of young and ripening asci. a, c. Young asci, showing early development of subapical ring. b, d. Details of lateral ascan wall with subapical ring. e. Idem, showing ripening wall, fixed in 1% glutaraldehyde and stained with uranyl acetate and lead citrate.

Fig. 2. *Scutellinia pseudotrechispora*, development of ascus top. a–c. Median sections showing details of apical ascoplasm. d. Detail of ascus wall of almost mature ascus, fixed in 1% glutaraldehyde and contrasted with Thiéry technique.

Fig. 3. *Scutellinia umbrorum*, development of ascus top. a, c, d. Median sections showing apical ascoplasm, fixed by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique. b. Idem, but contrasted with uranyl acetate and lead citrate.

Figs. 4a–e. *Scutellinia patagonica*, development of ascus top, fixed by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique. a. Top of emptied ascus. b. Operculum of the same ascus. c–e. Details of ripening ascus. — Fig. 4f. *Scutellinia umbrorum*, detail of apical ascoplasm, fixed by ultra-rapid freezing and freeze substitution and contrasted with uranyl acetate and lead citrate.

Figs. 5a–c. *Scutellinia trechispora*, development of ascus top. a. Detail of emptied ascus near ascostome. c. Idem, lower part of lateral wall. b. Detail of mature ascus wall with zone of fracturing. — Figs. 5d–f. *Scutellinia pseudotrechispora*, ascospore development. d. Early differentiation of secondary wall, fixed in 1% glutaraldehyde and contrasted with Thiéry technique. e, f. Idem, fixed in 1% $KMnO_4$ and 1% OsO_4 and contrasted with uranyl acetate and lead citrate.

Fig. 6. *Scutellinia pseudotrechispora*, ascospore development. a–c. Advanced differentiation of secondary wall. d. Idem, fixed in 1% glutaraldehyde and contrasted with Thiéry technique.

Fig. 7. *Scutellinia umbrorum*, ascospore development, fixed by ultra-rapid freezing and freeze substitution and contrasted with uranyl acetate and lead citrate. a, b. Ripening ascospores. c, d. Details of secondary wall.

Figs. 8a–d. *Scutellinia patagonica*, ascospore development, fixed by ultra-rapid freezing and freeze substitution and contrasted with uranyl acetate and lead citrate. a, b. Differentiation of the primary wall. c, d. Details of mature secondary wall. — Fig. 8e. *Scutellinia trechispora*, detail of nuclear segregation in very young ascus.

Fig. 9. *Scutellinia trechispora*, ascospore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and contrasted with uranyl acetate, lead citrate, and barium permanganate. a. Primary wall and very early secondary wall. b. Condensation of secondary wall material. c, d. Development of secondary wall. e. Detail of advanced state of spore development.

Fig. 10. *Scutellinia scutellata*, ascospore development (a, c, d, f, fixed in 1% glutaraldehyde and 1% OsO_4; b, e, fixed in 1% $KMnO_4$ and 1% OsO_4). a. Primary wall and early secondary wall. b–d. Condensation of secondary wall material. e. Development of secondary wall. f. Detail of advanced state of ascospore development.

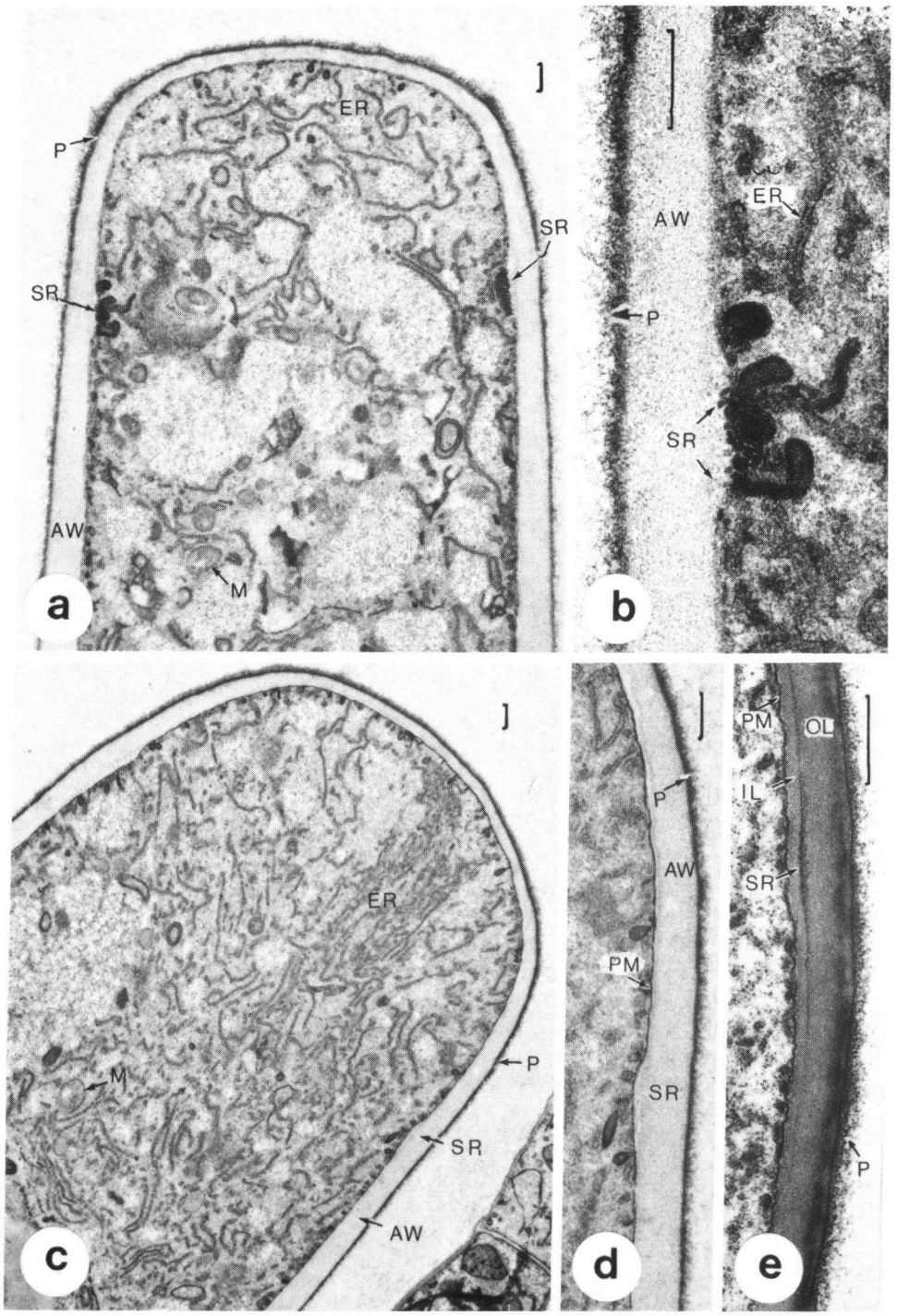
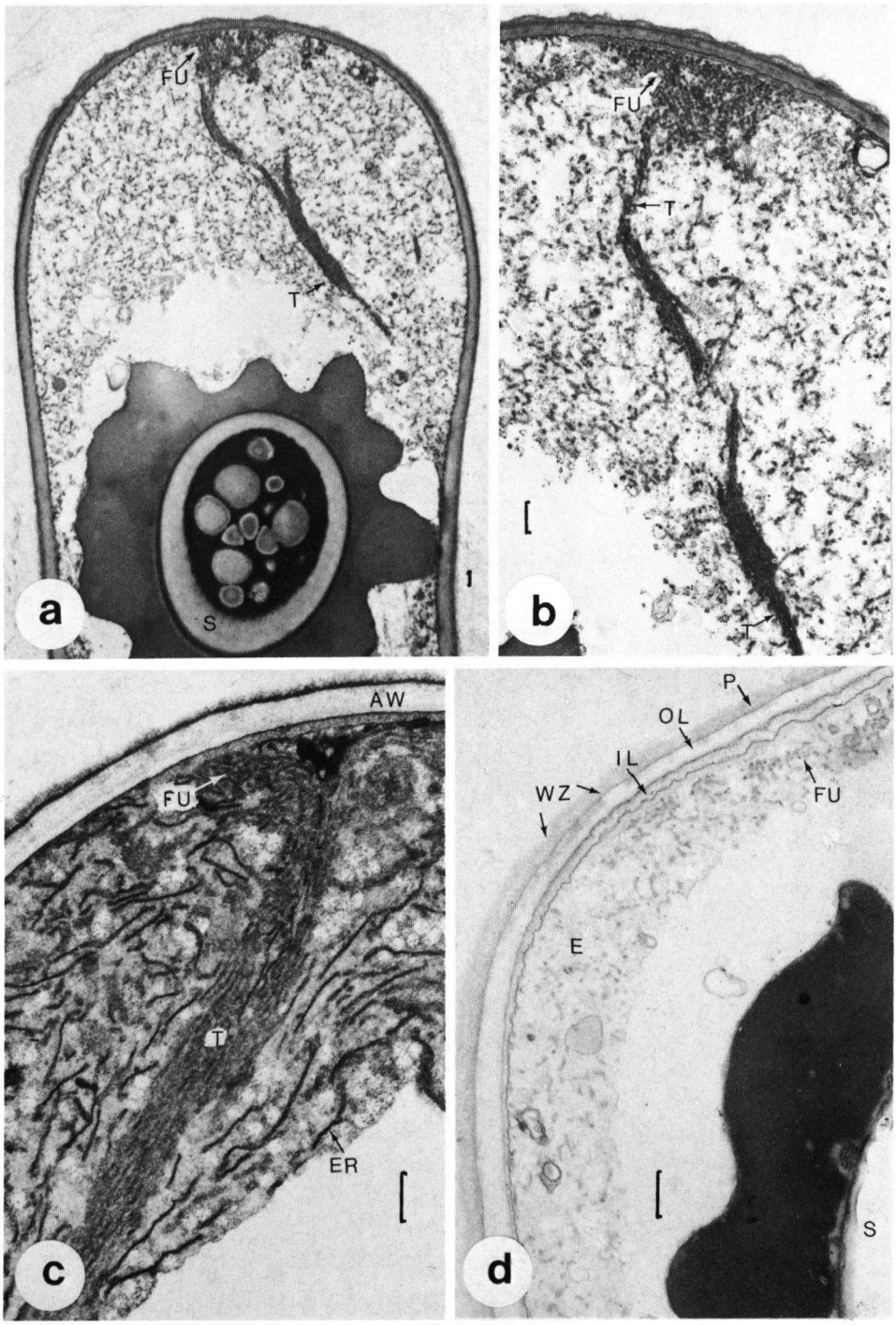


Fig. 1 (legend on page 131)



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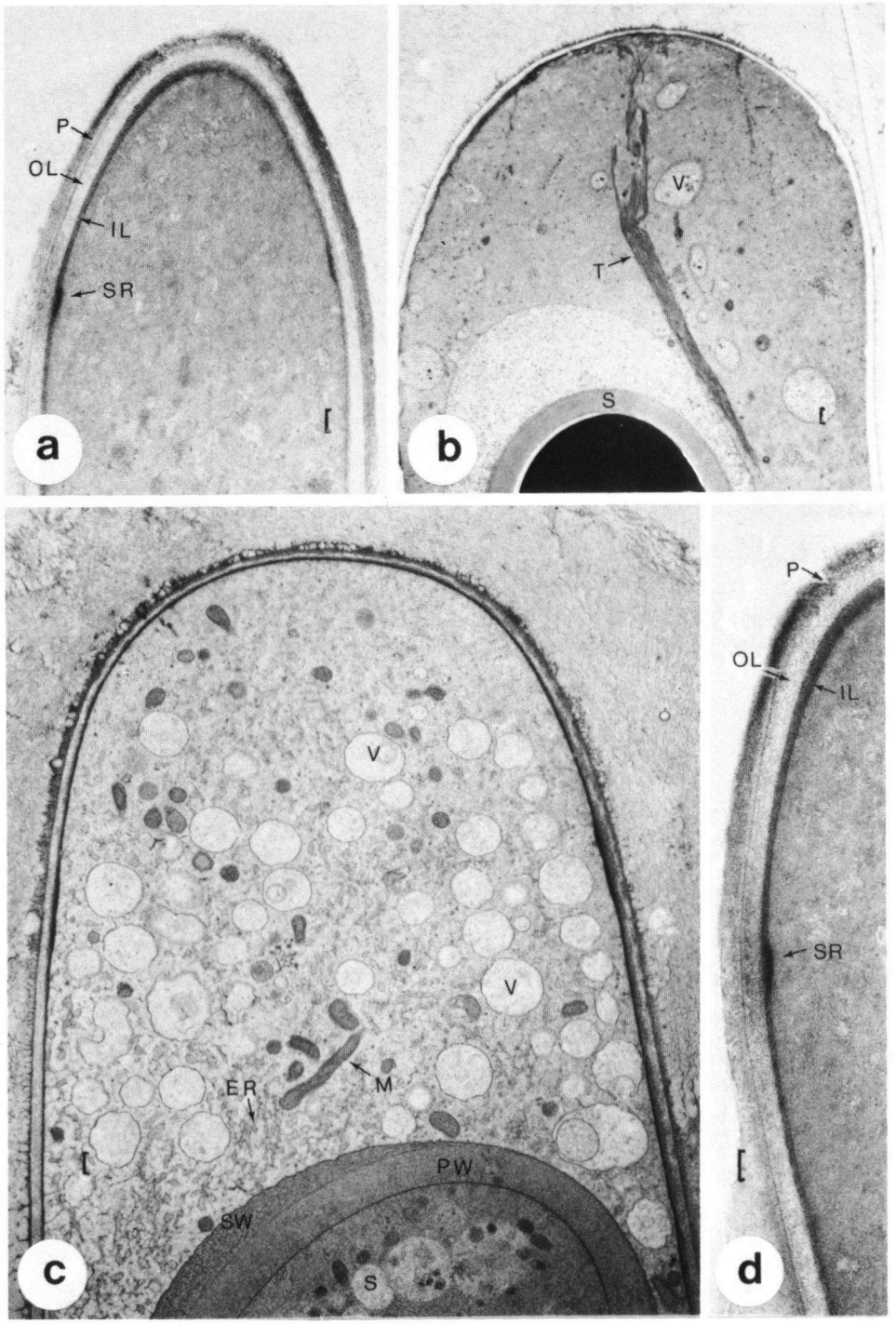

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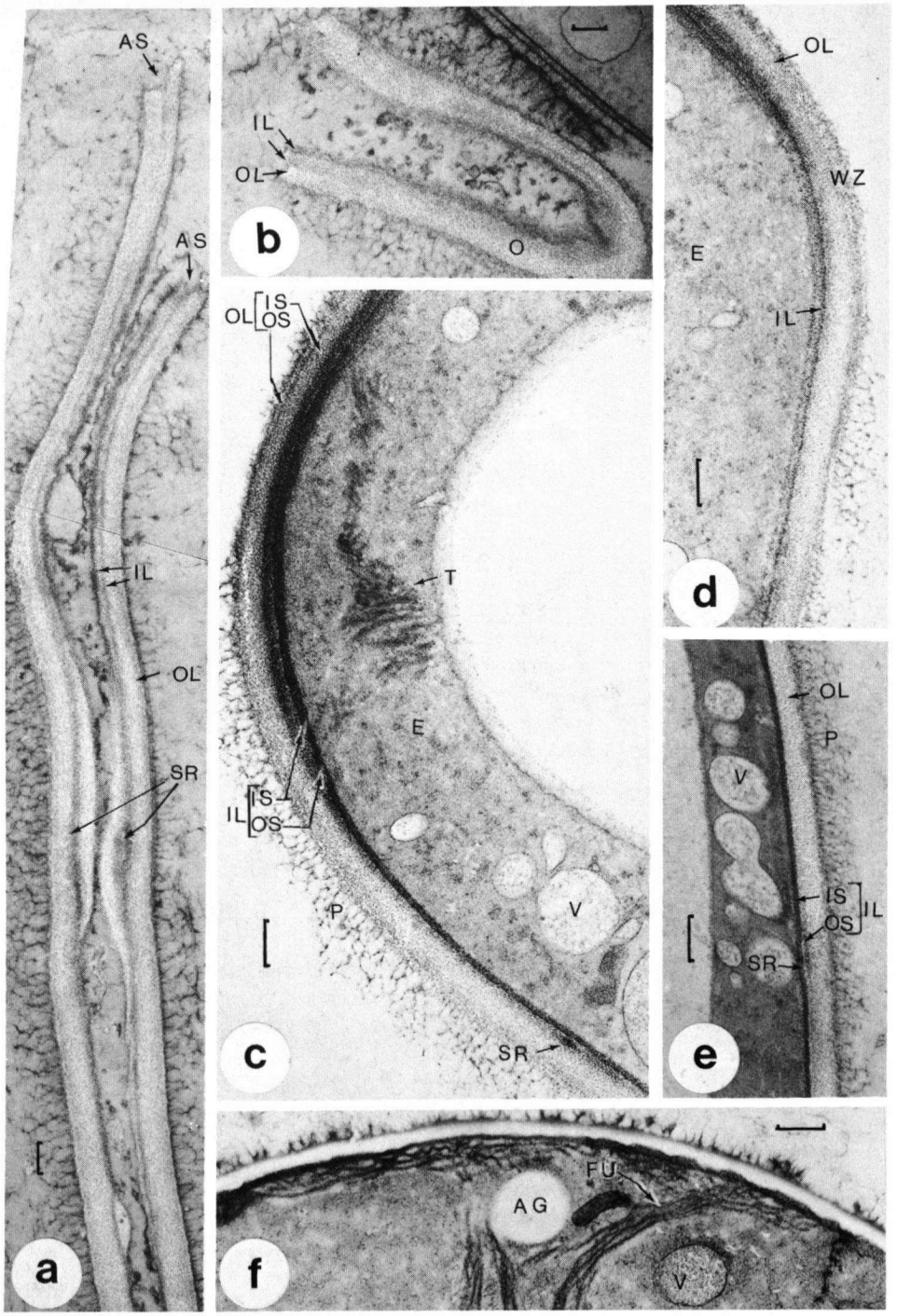

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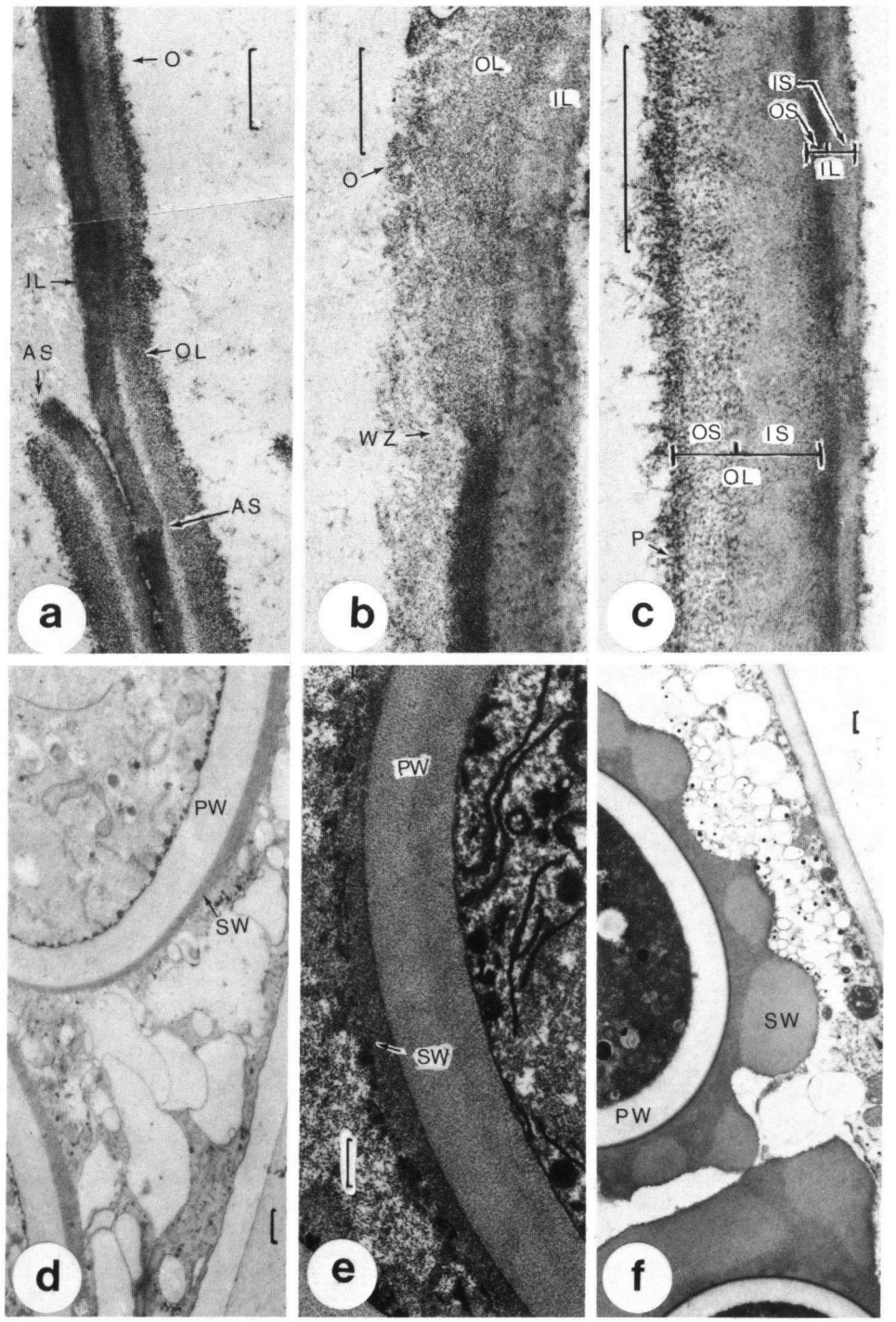

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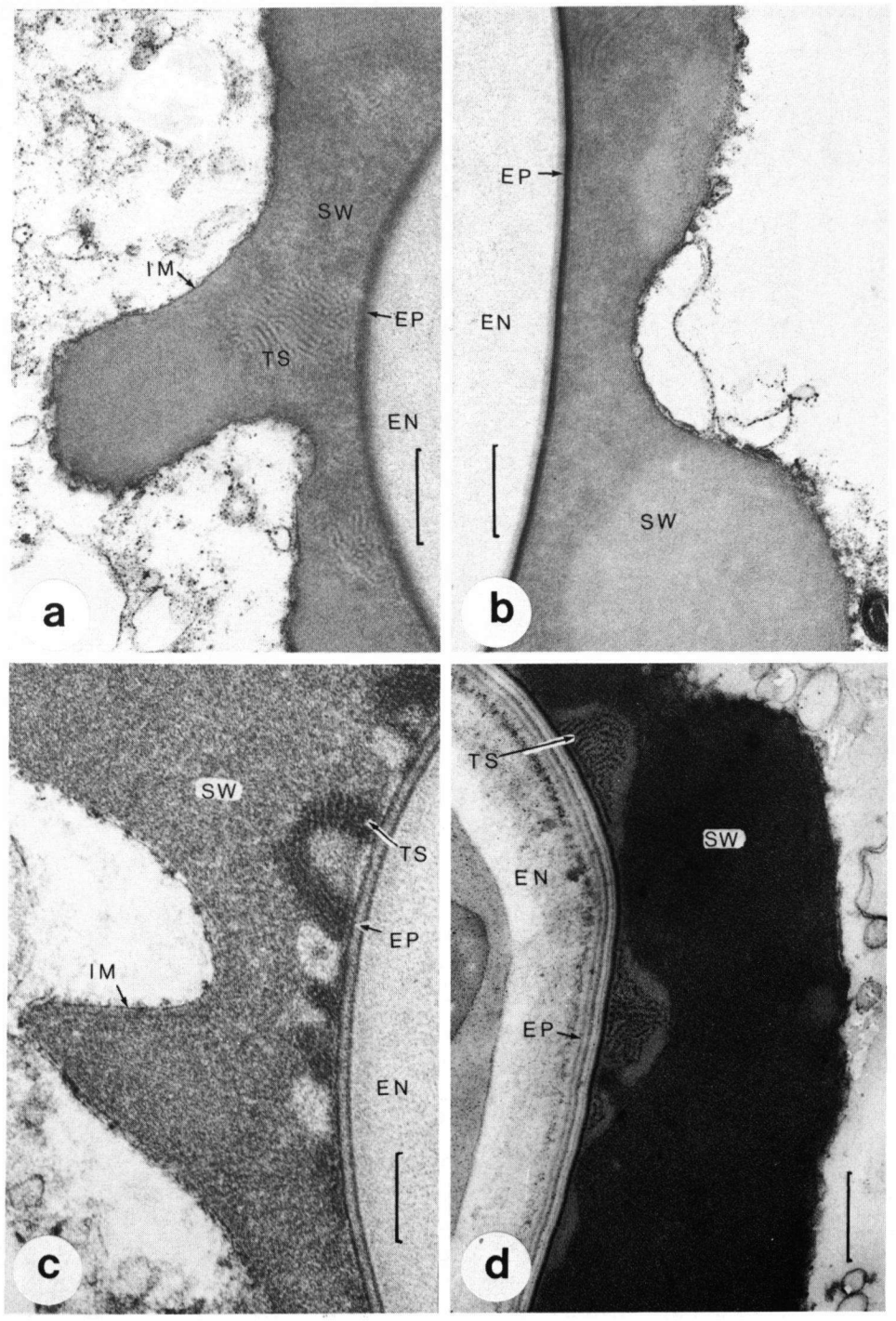

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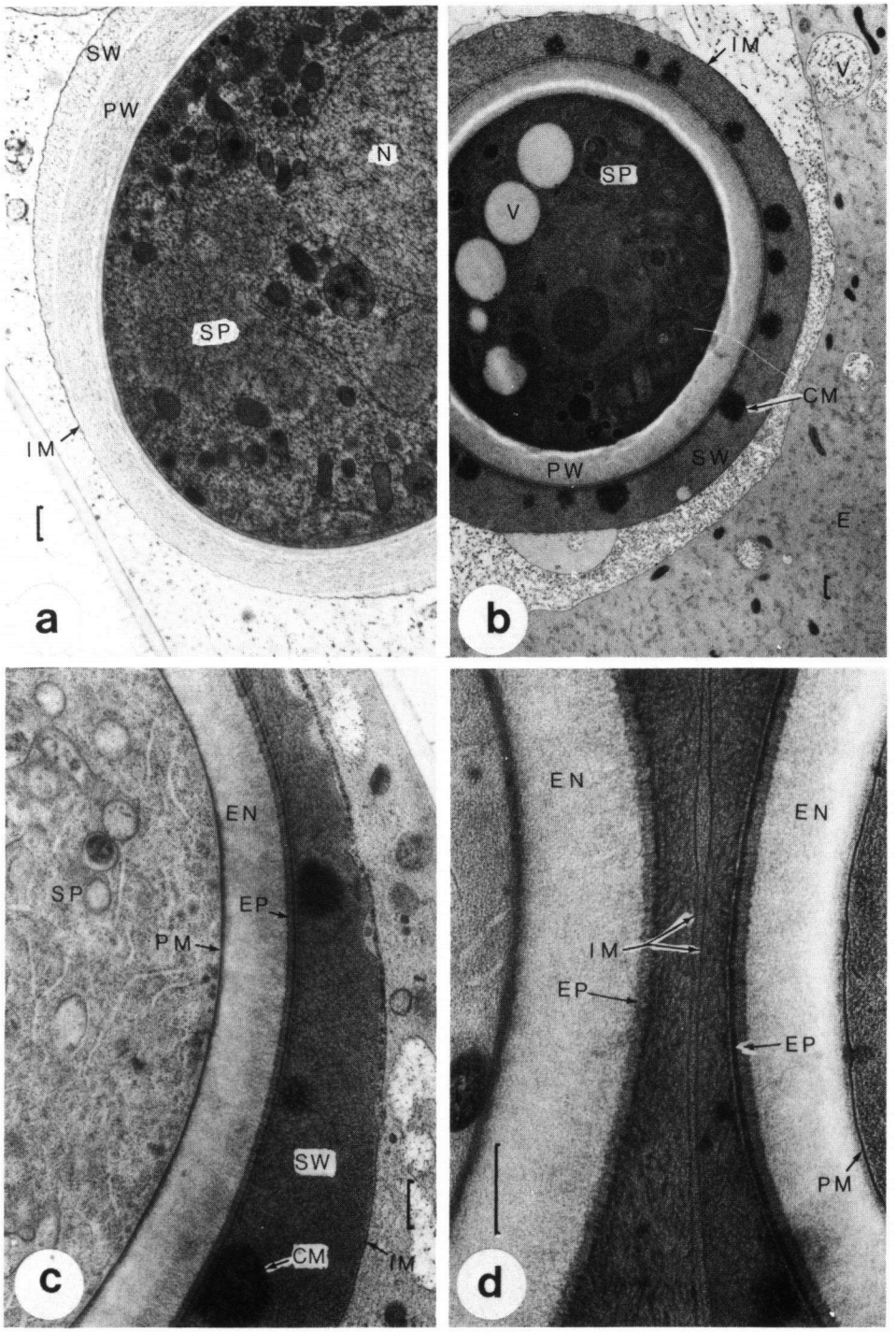

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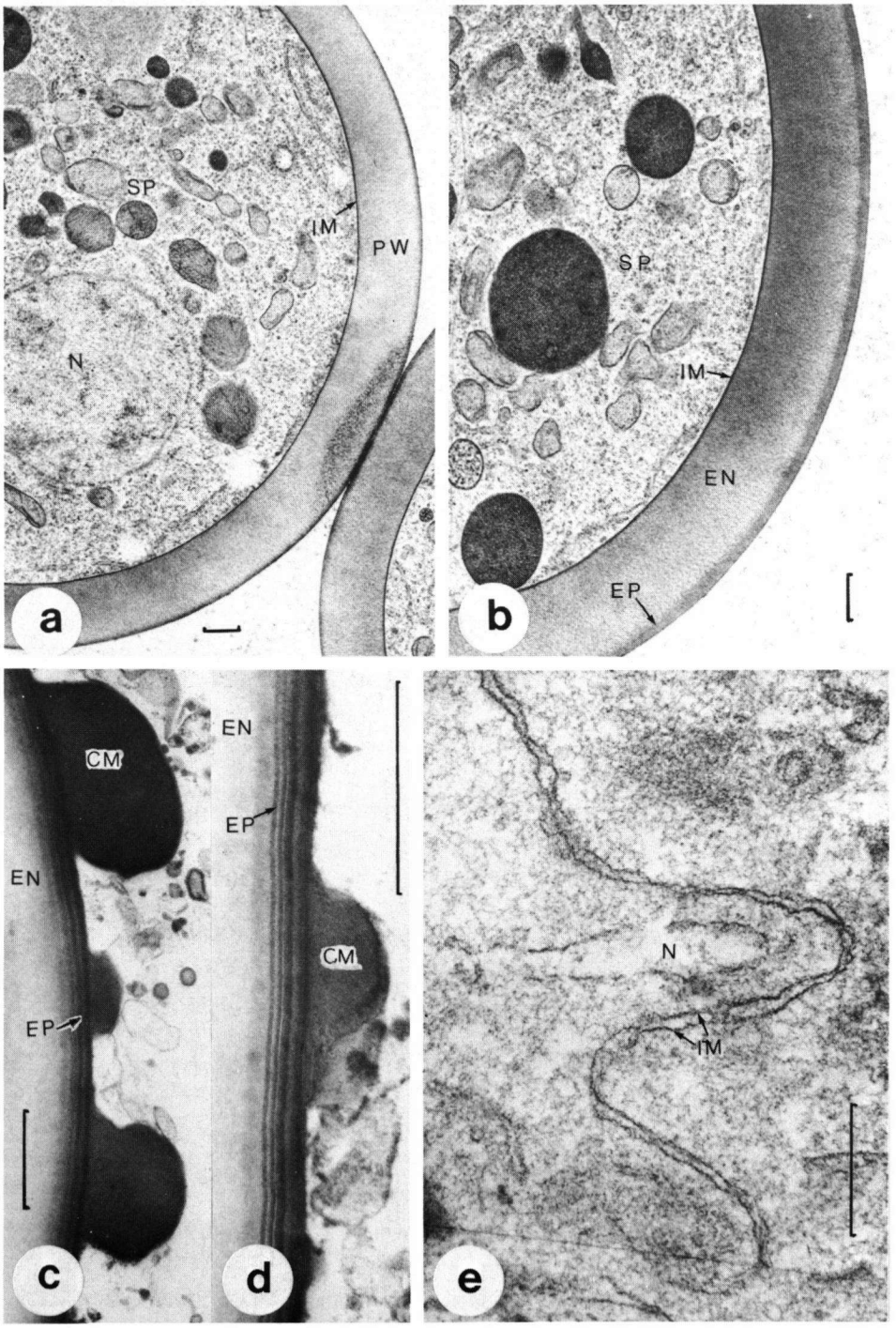

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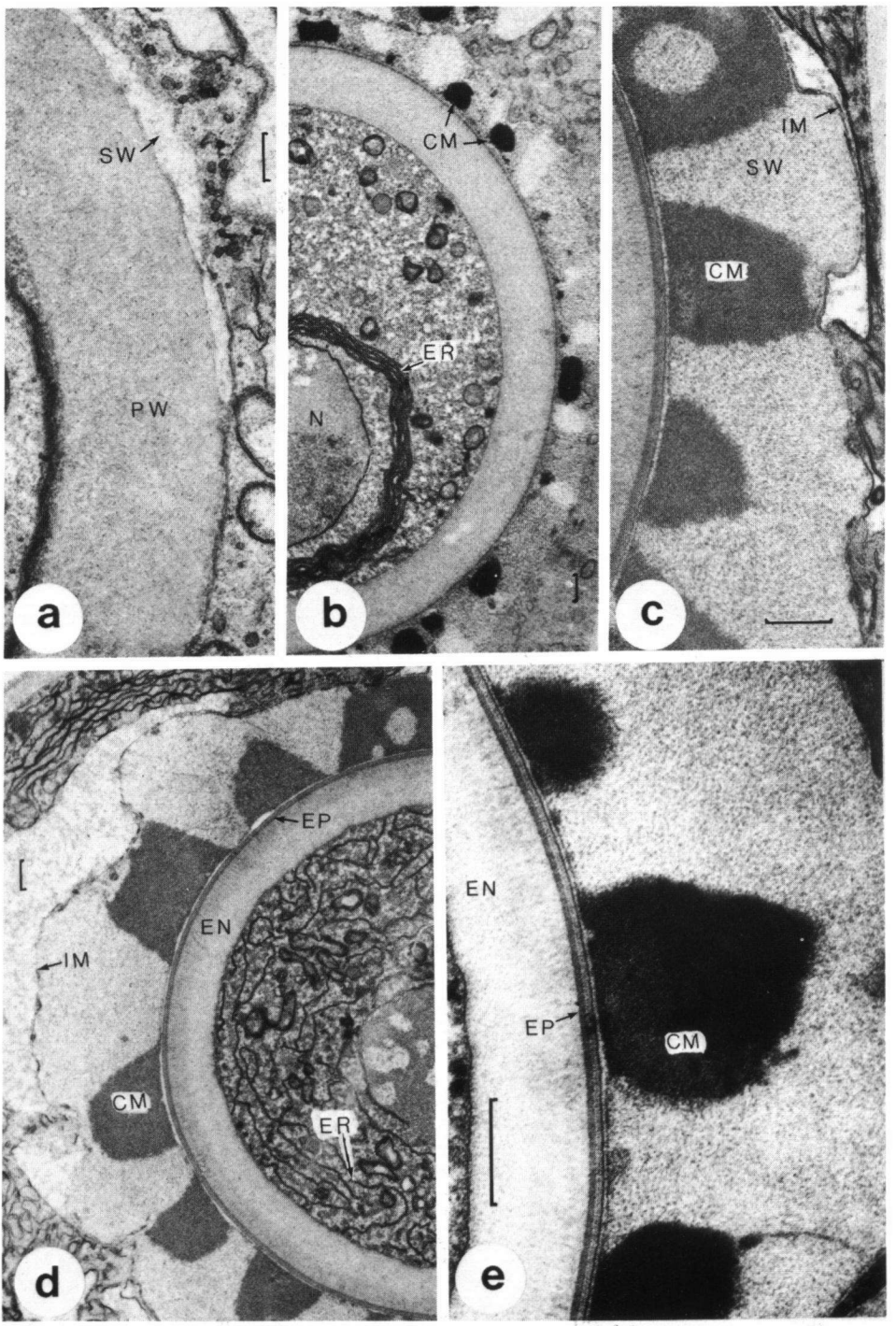

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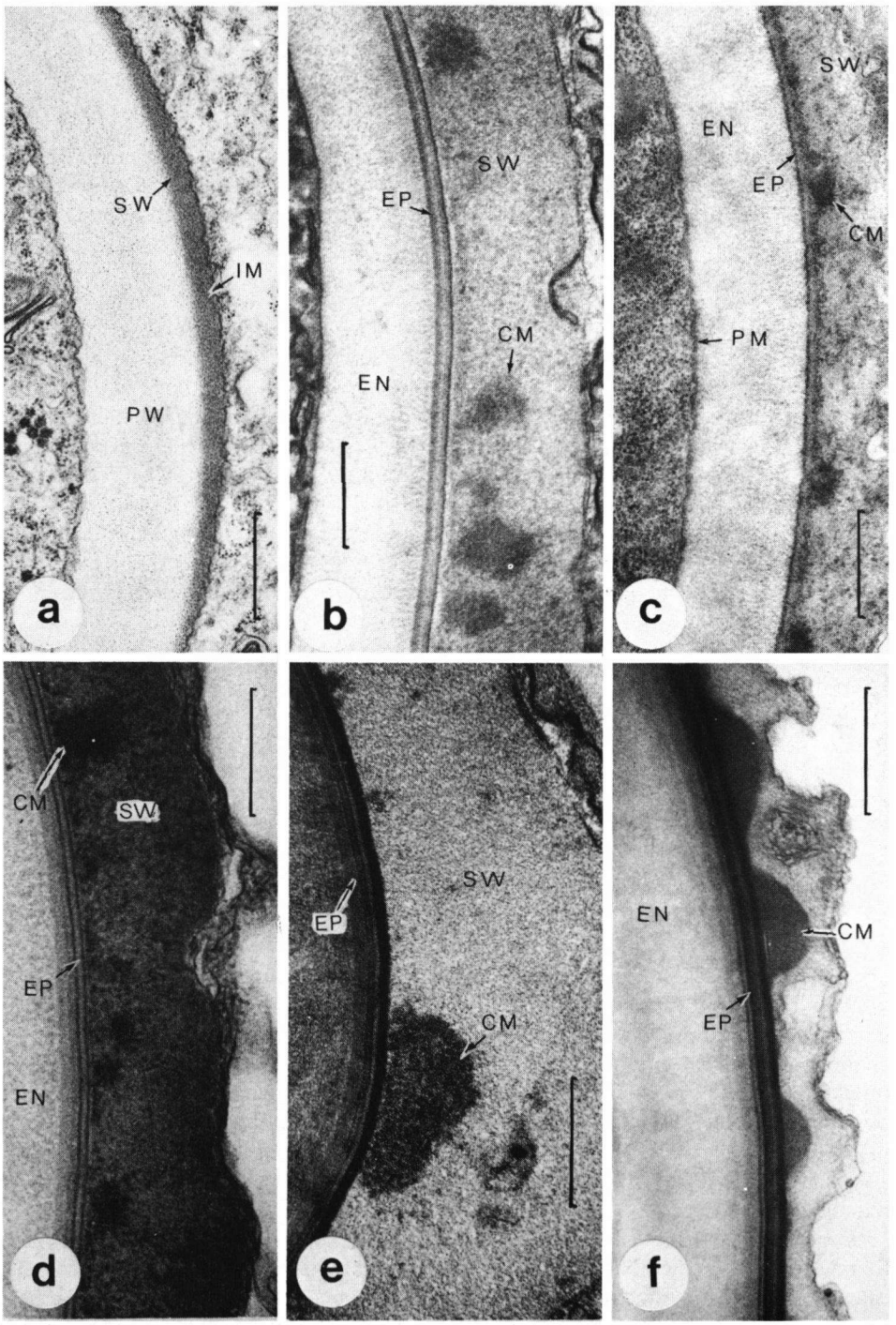

Fig. 10 (legend on page 131)

OBSERVATIONS

The ascus top

For a comparison of ascus apices among representatives of the Pezizales to be of value it is important to note the development and possible changes in the structure of the ascus wall and the apical ascoplasm. The regions of main importance in the wall of the ascus top are the operculum, the zone of dehiscence, and the subapical region.

Scutellinia pseudotrechispora — Figs. 1, 2

Mature asci are cylindrical with a rounded tip, 200–260 × 15–20 µm.

In the young ascus, before and during early ascospore formation, the ascan wall appears to be still undifferentiated, thicker throughout the lateral face, 540–630 nm, and thinner at the tip, 300–380 nm (Figs. 1a–d). From the beginning the outer surface of the whole ascus is covered with a rather electron dense extra-ascan layer or periascus, which increases in thickness from 80–110 nm at the lateral face to 180–250 nm at the top.

At the inner side of the lateral wall, about 5 µm under the tip, a ring-shaped band 800–1000 nm broad and 100–120 nm thick is formed (Figs. 1a–d). The initiation of this ring is often signalled by the presence of an adjacent band of lomasome-like structures in the ascoplasm (Figs. 1b, d). During further ripening at the inner face of the ascus wall an inner layer is produced. This layer can be distinguished in the opercular region and near the subapical ring (Fig. 1e) in $KMnO_4$-OsO_4-fixed material.

With the Thiéry technique a thin reactive stratum 15–20 nm thick just at the outside of the inner layer can be demonstrated in the top of almost mature asci (Fig. 2d). This outer stratum of the inner layer is more reactive in the region of the operculum and near the subapical ring.

In the mature ascus the inner layer increases in thickness from 30–45 nm in the lateral wall and 45–60 nm at the level of the subapical ring to 120–150 nm in the opercular region. Over the same distance the outer wall layer decreases from 500–540 nm in the lateral wall and 200–260 nm near the subapical ring to 180–210 nm in the opercular region.

The apical ascoplasm or 'acroplasm' is rich in endoplasmatic reticulum, the elements of which rearrange to form a more or less central bundle (Figs. 1c, 2c). This tract or funiculus can be followed from the inner face of the future operculum, where it is forming an apical funnel, down to the lateral side of the uppermost ascospore (Figs. 2a–d). The tubular elements of the funnel are usually found closely attached to the future operculum by a plate or sheet, 80–120 nm thick, of two or three layers of closely adpressed and interwoven tubules. These tubules reach a diameter of about 30 nm at the outside and 15 nm at the inside, which closely corresponds with the other elements of endoplasmatic reticulum in the ascus.

Even at full maturity no structural evidence for the rupturing of the operculum can be found in the ascus wall. No indentation or other preformed superficial structures demarcate the place of the future operculum (Fig. 2a). The place of the formation of a zone of dehiscence is just indicated by a region of wrinkling of the inner wall layer and the margin of the attachment of the funnel (Fig. 2d).

The lines of fracturing in the inner and outer layers do not exactly correspond, while the fracture will occur within a relatively broad zone. This results in rough, more or less torn, margins of the operculum and the ascostome. The operculum reaches a diameter of about 8 µm.

Scutellinia umbrorum — Figs. 3, 4f

Mature asci are cylindrical with a rounded or slightly flattened tip, 220–300 × 14–20 µm.

In the young ascus the wall is still undifferentiated, slightly thicker at the sides than at the tip. When examining material fixed by ultra-rapid freezing, followed by freeze substitution and contrasted for polysaccharides with the Thiéry technique the wall layers can be distinguished (Figs. 3a, c, d). Rather early a reactive (electron dense) subapical ring is formed at the inner face of the lateral wall about 6 µm behind the tip. At first the subapical ring is about 600–700 nm broad, but it may reach 1600 nm with maturity of the ascus.

At the later stages no increased activity of the endoplasmatic reticulum at the level of this ring could be seen. An electron dense inner layer, 130–180 nm thick in the lateral wall, reaches a thickness of 300 nm in the opercular region. An electron transparent outer layer is rather constant with a thickness of 310–350 nm. The reactive periascus is present from the beginning with a thickness of 300–350 nm over the whole ascan surface. At the top of the ascus it is often difficult to distinguish the delimitation of the periascus from the outer ascan layer, because a thin, also reactive, outer stratum has been differentiated in the latter (Figs. 3a, c, d).

The apical ascoplasm in the ripening ascus is rich in endoplasmatic reticulum, small vacuoles, and mitochondria (Fig. 3c). Elements of the endoplasmatic reticulum unite to form a more or less central tract (Fig. 3b), which connects the lateral side of the uppermost ascospore with the opercular region (Fig. 3b). At the top, the tubules of the tract form an obconical figure or funnel, usually with a central apical globule inside (Fig. 4f). A sheet of two or more layers of interwoven tubules of endoplasmatic reticulum above the funnel covers the inner face of the future operculum. The dehiscence mechanism shows the same pattern as in *S. patagonica.*

Scutellinia patagonica — Figs. 4a–e

Mature asci are cylindrical to subcylindrical with a rounded tip, 240–300 × 22–25 µm.

The young ascus shows an undifferentiated wall and an electron dense strongly mucilaginous periascus of varying thickness, 150–500 nm.

In material fixed by ultra-rapid freezing followed by freeze substitution and contrasted with the Thiéry technique the wall layers can be clearly distinguished. At the inner face of the lateral wall about 6.5 µm behind the tip a ring-shaped reactive band about 600 nm broad is formed. During further development this subapical ring is found again somewhat deeper as a reactive band at the outer face of the outer stratum of the inner wall layer (Figs. 4c, e), and after ascus dehiscence at the lower end of a strongly swollen ring-shaped zone of the inner wall (Fig. 4a). A rather strongly reactive inner layer is shown only about 50–60 nm thick in the lateral wall and in the subapical ring, increasing in thickness to 150–200 nm in the opercular region.

The less reactive outer layer is most conspicuous with a thickness of 260–350 nm in the lateral wall, decreasing to 180–220 nm in the tip.

Under optimal conditions a contrasting sublayering of both the inner as well as the outer layer can be observed. In the opercular region within the inner layer an inner stratum of about 110 nm thick of strong reactivity can be followed in close contact with a slightly less reactive outer stratum of 120–150 nm thick (Fig. 4c). At the margin of the future operculum the outer stratum decreases rather abruptly in thickness, while its delimitation becomes less distinct in the zone of dehiscence and again more evident around the subapical ring. In the outer layer a thin moderately reactive outer stratum of 40–60 nm can be followed in places where it sufficiently contrasts from the almost equally reactive periascus (Figs. 4c–e).

At maturity the site of the margin of the future operculum is not marked by an indentation or a preformed weakness zone. Dehiscence takes place in a zone between the subapical electron transparent zone and the area of abrupt decrease in the thickness of the outer stratum of the inner layer in the top.

In the apical ascoplasm of the ripening and mature ascus a funnel and a tract can be found (Fig. 4c). After discharge of the ascospores, the operculum, with a diameter of 9–13 µm, remains attached to the ascostome by a narrow hinge.

In the emptied ascus layers may swell considerably by imbibition. Especially the inner ascan layer near the subapical ring swells disproportionally (Fig. 4a). Cleavage of the wall of the operculum and of the ascostome occurs along the face between inner and outer layers or the 'thimble-shaped lamina' (van Brummelen, 1978).

Scutellinia trechispora — Figs. 5a–c

Mature asci are cylindrical with a rounded tip, 240–300 × 20–24 µm.

The development of the ascus top is very much the same as decribed here for *S. pseudotrechispora*. A short, more functional description of it was given earlier (van Brummelen, 1978; as *S. armatospora*).

The different layers of the apical wall are easily visible in the mature ascus just before dehiscence (Fig. 5b) and after spore discharge (Fig. 5a, c), when examining $KMnO_4$-OsO_4-fixed material.

Scutellinia scutellata

Mature asci are cylindrical with a rounded tip, 220–300 × 18–22 µm.

The development of the ascus top of this species also closely agrees with that described here for *S. pseudotrechispora* (see below). It has also been the subject of a study by Samuelson (1978).

The ascospore wall

The ultrastructure of the early development of ascospores in these species of *Scutellinia* closely accords with the general process, in which after three nuclear divisions eight nuclei are formed. Each nucleus becomes enclosed in a double unit membrane (Fig. 8e). The primary wall is formed between these membranes; the inner one becomes the sporoplasmalemma and the outer one the investing membrane of the ascospore. The substance of the primary wall is electron transparent in permanganate and glutaraldehyde fixed material.

In the species studied the young undifferentiated primary wall may reach 700–1000 nm in thickness. In mature spores this will be reduced to 500–750 nm.

The development of the primary wall continues during further ripening. In the outer zone of the primary wall a series of up to five closely spaced electron dense bands becomes evident, and form the epispore with a thickness of 90–110 nm (Figs. 6c, d; 8c, d; 9e; 10d–f). It is this layer which develops, at full maturity of the spores, a resistence against the chemicals of fixation and embedding. The primary wall is usually rather uniform in appearance and forms the most constant part of the ascospore wall.

Between the outer surface of the primary wall and the spore delimiting membrane the secondary spore wall is formed as an extra layer. The substance of this layer is rather homogeneous at first and increases in electron density. The development of the secondary wall is of great importance for the formation of the ornamentation on the ascospores and differs slightly in each of the species under investigation.

Scutellinia pseudotrechispora — Figs. 5d–f, 6

In material fixed in glutaraldehyde-OsO_4 or in permanganate-OsO_4 the secondary wall shows a moderate electron density and further increases in density without forming centres of condensation (Figs. 5d, e). Secondary wall material is deposited in very large quantities. The investing membrane becomes locally strongly elevated up to 2 µm or more high (Figs. 5f; 6a–d). The internal differentiation of the secondary wall becomes rather complex with large subglobose areas of somewhat lower electron density at the periphery and caps on the epispore, showing a tubular or laminose substructure (Figs. 6a, c, d). The ornamentation of mature ascospores consists of a wide network of crests up to 3.5–6.0 µm high.

Scutellinia umbrorum — Fig. 7

The material fixed by rapid freezing and freeze substitution with OsO_4 and contrasted with uranyl acetate and lead citrate shows an electron transparent initial secondary wall (Fig. 7a) gradually increasing in density and then developing centres of condensed material on the surface of the epispore (Figs. 7b, c). Between two developing spores an area of mutual contact between their investing membranes can often be observed (Fig. 7d).

The condensed material increases to form a pattern of isolated rounded warts of unequal size 0.6–1.5 µm high. With maturity the rest of the secondary wall disappears.

Scutellinia patagonica — Figs. 8a–d

The freeze fixation gives the young spores a very natural look (Figs. 8a, b). The sporoplasm shows a globular nucleus, endoplasmatic reticulum, many vesicles with different contents, mitochondria, and a smooth plasmalemma.

The differentiation of the secondary wall is very similar to the process described for *S. umbrorum*. Here also a pattern of isolated rounded warts, 0.5–1.0 µm high, develops.

Scutellinia trechispora — Figs. 8e, 9

The permanganate-OsO_4-fixed material has been given extra contrast with barium permanganate. This shows the development of the secondary wall clearly. The initial secondary wall is rather electron transparent (Fig. 9a). In the somewhat homogeneous substance of the secondary wall electron dense condensed material forms centres on the

surface of the epispore (Fig. 9b). With increase in thickness of the secondary wall these centres grow to form homogeneous electron dense conical warts on the outer surface of the globular spores.

The development of some of these warts is restricted by the contact of the spore investing membrane, which prevents free growth at the tip (Figs. 9c–e). At maturity the ornamentation consists of isolated conical and truncate warts 1.0–2.2 μm high.

Scutellinia scutellata — Fig. 10

In material fixed in permanganate-OsO_4 and in glutaraldehyde-OsO_4 the electron transparent primary wall develops on its outside a more electron dense secondary wall. This is homogeneous at first (Fig. 10a), but soon increases in thickness and develops centres of condensed material in the vicinity of the epispore (Figs. 10b–d).

The appearance of the secondary wall becomes more granular. On the epispore warts are formed that may fuse laterally (Figs. 10e, f). With maturity the rest of the secondary wall material disappears. The mature spores are ornamented with a rather irregular pattern of short lines and isolated warts 0.3–0.7 μm high.

DISCUSSION

The species of *Scutellinia* studied show a very similar structure of the ascus top. The results obtained with different methods of fixation and contrasting have given additional information. The combination of rapid freeze fixation and contrasting for polysaccharides with the Thiéry technique proved to be especially valuable. The results when compared with other methods were not found to be contradictory.

The structure of the ascus top is summarized in a diagrammatic scheme (Fig. 11). The ascus top in *Scutellinia* is characterized by the almost complete absence of structural indications of the demarcation of the operculum.

In the operculum an outer stratum of the inner layer be recognized which decreases rather abruptly in thickness at the margin of the operculum region. Somewhat at the outside of this region the wall becomes electron transparent with all methods used. Only after use of the Thiéry technique the outer stratum of the inner layer can be vaguely followed. This stratum becomes again more evident in the subapical region. It corresponds exactly with the earlier described 'interrupted thimble-shaped lamina' (van Brummelen, 1978).

The development of the subapical ring begins in the young ascus. Electron dense material is deposited as a band on the inner face of the wall rather far behind the tip of the ascus. Later the material of the inner layer is deposited over the whole inner face of the ascus and the band of the subapical ring becomes located at the limit of the inner and the outer wall layers.

In the living ascus the subapical ring is scarcely visible as a thickening of the wall. But in non-living material, when the turgor of the ascus has strongly decreased or the wall has collapsed, there is a strong swelling at this place in the inner layer. This swelling corresponds with the structure described as 'bourrelet sousapical' by Chadefaud (1942).

Both after physical and chemical fixation in the apical ascoplasm a tract, an apical funnel, and an apical globule are found, more or less shaped as described by Chaudefaud. The attachment of the tubules of the funnel to the wall of the opercular region strongly

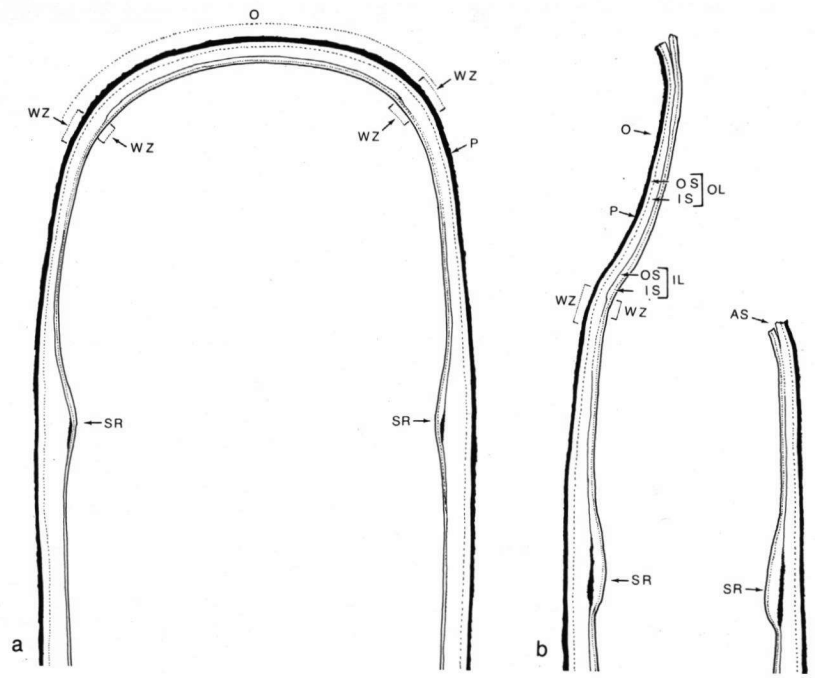

Fig. 11. Diagrammatic sections of ascus tops of *Scutellinia*, as seen with electron microscopy. a. Almost mature ascus. b. Ascus after spore discharge.

suggests a function in the opening mechanism of the ascus top. Samuelson (1978) was not able to find these plasmatic structures in the species of his '*Otidea-Aleuria* complex'.

The type of ascus top found in *Scutellinia* is closely related to that in genera such as *Anthracobia, Aleuria, Coprobia, Cheilymenia, Pyronema,* and *Octospora* and is considered to be characteristic of the family Pyronemataceae.

The development of the primary spore wall and its differentiation into an epispore and an endospore fully agrees with the general process in the Pezizales. But differences are found in the process of secondary wall formation.

In *Scutellinia umbrorum, S. patagonica, S. trechispora,* and *S. scutellata* a homogeneous, moderately electron transparent wall is formed. Within the substance of this wall local areas of condensed material are formed which concentrate on the epispore and develop into the elements of the spore ornamentation. In all stages of development the structure of these elements is homogeneous and electron dense. This is in contrast with the development in *S. pseudotrechispora,* where the secondary spore wall is only homogeneous at the beginning. But soon a rather complex structure develops with large subglobose areas of lower electron density and caps on the epispore with a tubular or laminose substructure.

This type of secondary wall development with a lasting complex structure is unusual among the Pyronemataceae (cf. Merkus, 1976).

The taxonomic position of *S. pseudotrechispora* within the genus *Scutellinia* is therefore probably more isolated than suggested in a classification mainly based on patterns of spore ornamentation and hair structures (cf. Schumacher, 1990).

ACKNOWLEDGEMENTS

The author wishes to thank Mr. W. Star for his enthusiasm and most skilful technical assistance throughout this study. He is also much indebted to Drs. A. Bellemère, N.C. Janex, and H. Clémençon for their detailed information concerning the application of the Thiéry technique to fungi. Gratitude is expressed to the director of the Botanical Laboratory (Leiden) for the use of the electron microscope. Thanks are due to Mr. H. Huyser who provided us with living material of *Scutellinia umbrorum* and *S. patagonica* and to Mrs. Dr. S.M. Francis for the correction of the English text. Financial support was given by the Netherlands Organization for Scientific Research (NWO) for collecting material in Scandinavia. This organization also placed the equipment for ultra-rapid freezing and freeze substitution at our disposal.

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