PERSOONIA
Published by Rijksherbarium / Hortus Botanicus, Leiden
Volume 16, Part 4, pp. 425–469 (1998)

RECONSIDERATION OF RELATIONSHIPS WITHIN THE THELEBOLACEAE BASED ON ASCUS ULTRASTRUCTURE

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Genera that have been included in the family Thelebolaceae Eckblad are considered for the structure of the apical apparatuses of their asci. In the absence of such information, other characters could sometimes be used to clarify their most likely taxonomic position. The affinities of *Cleistothelebolus*, *Coprobolus*, *Coprotiella*, *Dennisiopsis*, *Lasiobolidium*, *Lasiothelebolus*, *Leptokalpion*, *Mycoarctium*, *Ochotrichobolus*, and *Zukalina* are discussed.

The ultrastructure of ascus tops has been studied in *Thelebolus microsporus*, *T. coemansii*, *T. caninus*, *T. crustaceus*, *T. polysporus*, *T. nanus*, *T. stercoreus*, *Caccobius minusculus*, *Lasiobolus pilosus*, *L. cuniculi*, *L. monascus*, *Ascozonus woolhopensis*, *A. solms-laubachii*, *Ramgea annulispora*, *Coprotus lacteus*, and *Trichobolus zukalii*. At least six different types of asci can be distinguished within the fungi studied by electron microscopy. (1) The first (typical) *Thelebolus* type, in *Thelebolus microsporus*, *T. crustaceus*, *T. stercoreus*, *Caccobius*, *Ramgea*, and *Pseudascozonus*, opening after splitting within the inner wall layer in the apex, mostly accompanied by a central apical thickening. (2) The second *Thelebolus* type, in *T. caninus* and *T. polysporus*, with a breakdown of the inner layer in the apex above the subapical ring, followed by an irregular tear in the outer layer. (3) The third *Thelebolus* type, in *T. microsporus* and *T. coemansii*, with an irregular operculum just above the subapical ring. (4) The *Ascozonus* type, restricted to *Ascozonus*, with a very prominent subapical ring and a very small operculum. (5) The *Trichobolus* type, restricted to the uni-ascal multi-spored genera *Trichobolus* and *Leptokalpion*, without any trace of a subapical ring or weakened zone, showing a very large operculum or an irregular tear, caused by a regular retraction of the outer layer from a circular apical region. (6) The asci of *Lasiobolus* and *Coprotus* agree with the earlier defined *Octospora* type. New combinations were necessary for *Thelebolus coemansii* and *Ascozonus solms-laubachii*.

Boudier (1879, 1885) used the presence of an operculum at the top of the ascus as the main character to subdivide the discomycetes. Since then several groups have been included within the Pezizales or 'operculate discomycetes' that possess asci without an operculum or with an aberrant opening mechanism (cf. van Brummelen, 1978; 1994a).

Most families of the Tuberales are now considered as hypogeous representatives of the Pezizales that have lost the ability to release their ascospores by a shooting mechanism (Trappe, 1979; van Brummelen, 1994a; 1994b).

Species of *Eleutherascus* Arx also show asci without an operculum, but their resemblance to species of the operculate genus *Ascodesmis* Tiegh. is so striking in other respects, such as the development and structure of ascospore ornamentation and the structure of ascan plugs, that both genera are united in the family Ascodesmidaceae of the Pezizales (van Brummelen, 1989a, 1989b; Kimbrough, 1994).

Another group of fungi that most authors include in the Pezizales are those showing an affinity with the genus *Thelebolus* Tode: Fr. They possess a variety of dehiscence mecha-

nisms, ranging from a simple irregular apical slit to a conventional operculum, and are placed in the tribe Theleboleae of the Pezizaceae (Kimbrough & Korf, 1967) or the Pyronemataceae (Korf, 1972), or in a special family Thelebolaceae (Eckblad, 1968). All have very small globular to disk-shaped ascomata. The development varies considerably from a type in which the ascomata remain closed until the asci are fully mature (extremely cleistohymenial) to types with a fully exposed development of the asci (eugymnohymenial, van Brummelen, 1967). The asci are wide and usually protrude far at maturity, are eight- or multi-spored, with walls not staining blue with iodine. Carotenoid pigments are absent. The habitat is exclusively coprophilous.

Polyspory occurs in most genera and is considered a special adaptation to the coprophilous habitat to produce large projectiles for a more efficient dispersal. When there is, within a genus, a range of species with different spore numbers, the eight-spored species are considered the most primitive (cf. Kimbrough, 1981; Montemartini-Corte, 1993).

The original circumscription of the Thelebolaceae (Eckblad, 1968) included nine genera: *Ascozonus* (Renny) E.C. Hansen, *Caccobius* Kimbr. in Kimbr. & Korf, *Coprobolus* Cain & Kimbr., *Coprotus* Korf ex Korf & Kimbr. in Kimbr. & Korf, *Lasiobolus* Sacc., *Leporina* Velen., *Thecotheus* Boud., *Thelebolus* Tode: Fr., and *Trichobolus* (Sacc.) Kimbr. & Cain.

The genus *Thecotheus* has an ascan wall staining blue with iodine, and should be referred to the Ascobolaceae (Aas, 1992; van Brummelen, 1994b).

Several new, very small, and extremely rare genera have since been added to the Thelebolaceae, such as: *Cleistothelebolus* Malloch & Cain, *Coprotiella* Jeng & Krug, *Dennisiopsis* Subram. & Chandrashekara, *Lasiothelebolus* Kimbrough & Luck-Allen, *Leptokalpion* Brumm., *Mycoarctium* Jain & Cain, *Ochotrichobolus* Kimbr. & Korf, *Pseudascozonus* Brumm., *Lasiobolidium* Malloch & Cain, and *Ramgea* Brumm.

Most of these new genera are known only from the description of a single collection and often insufficient attention has been paid to the structure of the ascus and its dehiscence mechanism leaving their taxonomic position uncertain. Some of these genera can, however, be excluded from the Thelebolaceae on other grounds.

GENERA CONSIDERED RELATED TO THE THELEBOLACEAE

Ascozonus (Renny) E.C. Hansen, with a range of species mainly characterized by the spore number in each ascus and the shape of excipular, hyphoid hairs, has very small, cylindrical to obconical, eugymnohymenial ascomata. The asci are cigar-shaped at maturity, 16–256-spored, with a subapical very prominent ring-shaped thickening of the wall and a small apical operculum (Vuillemin, 1887; van Brummelen, 1974). The ascospores are hyaline, fusiform, and smooth, without granules or air-bubbles. The taxonomic position of the genus is somewhat doubtful; it has been placed in the Thelebolaceae (Eckblad, 1968), in the tribe Theleboleae of the Pyronemataceae (Korf, 1973), and in the *Otidea-Aleuria* complex of the Aleuriaceae and Otideaceae ss. Kimbrough (Samuelson, 1978b).

Caccobius Kimbr. in Kimbr. & Korf, with a single species, *C. minusculus* Kimbr. in Kimbr. & Korf, has very small, discoid, presumably gymnohymenial ascomata, with an excipulum located only near the base. The asci are cylindrical to broadly clavate, 1000–1500-spored, at first thick-walled; the wall is not staining with iodine, but the outer wall

layer is uniformly red in Congo red, with an apical plug staining with Waterman's blue-black ink in young asci. The ascospores are hyline, smooth, and without guttules or air-bubbles. This genus was placed in the tribe Theleboleae of the Pezizaceae by Kimbrough & Korf (1967). But its position remains doubtful, because of the anomalous apical plug (Korf, 1972).

Cleistothelebolus Malloch & Cain, with a single species, *C. nipigonensis* Malloch & Cain, produces cleistohymenial ascomata with irregularly disposed, thin-walled, evanescent 8-spored asci. The ascospores are hyaline, smooth, and withou guttules or air-bubbles. While conidia are produced as blastospores on short peg-like conidiophores. This is not in accordance with a position close to *Thelebolus* (cf. also Benny & Kimbrough, 1980). It was initially placed in the Thelebolaceae by Malloch (1970), but soon transferred to the Eoterfeziaceae (Malloch & Cain, 1971), a family whose position and circumscription seem rather uncertain (Malloch, 1994: 398).

Coprobolus Cain & Kimbr. with a single species, *C. poculiformis* Cain & Kimbr., has 'bowl- to goblet-shaped' cleistohymenial ascomata that open at an early stage of development and are covered with a net of closely appressed bundles of reddish brown pigmented hairs. The asci are thin-walled, sometimes with a small, undefined thickening at the apex before maturity, about 250-spored, staining uniformly in Congo red, not blueing with iodine. The ascospores are hyaline, smooth, and easily producing air-bubbles in anhydrous media. They have no visible apical apparatus and open with an apical bilabiate split. The genus was placed by Cain & Kimbrough (1969) in the tribe Theleboleae of the Pezizaceae, close to the genera *Caccobius*, *Thelebolus*, and *Ascozonus*. The type material of *C. poculiformis* proved too scanty for an ultrastructural study of the asci.

Coprotiella Jeng & Krug, with a single species, *C. gongylospora* Jeng & Krug, has ascomata that are globose and remain closed at all stages. The asci are 8-spored, thin-walled, 'unitunicate', 'non-amyloid', evanescent, without croziers or apical apparatus. The ascospores are globose, hyaline, smooth, and produce air-bubbles in certain media rather easily when fully mature. The genus was placed in the tribe Theleboleae of the Pyronemataceae sensu Korf (1972) by Jeng & Krug (1977), because of 'similarity in many ways to *Coprotus*', but in the absence of ascus characters its position remains uncertain.

Coprotus Korf ex Korf & Kimbr. in Kimbr. & Korf., is a rather large genus of which the species are mainly characterized by the size and number of spores in each ascus, hymenial pigments, and the shape of excipular cells and tips of paraphyses. The ascomata are eugymnohymenial (van Brummelen, 1967), lenticular to discoid, 0.1–3.0 mm diam., hyaline, yellow, or orange, and smooth. The excipulum is of restricted development. The asci are broadly clavate, 8–256-spored, protruding above the hymenium at maturity; the wall is not staining with iodine; but the outer wall layer stains with Congo red. The ascospores are hyaline, smooth, and easily produce air-bubbles. All species are strictly coprophilous. The genus was placed in the Thelebolaceae (Kimbrough & Korf, 1967). Kish (1974) found arguments in cytological and developmental studies on *C. lacteus* to transfer *Coprotus* to the Pyronemataceae emend. Eckblad. A revision of *Coprotus* for North America (Kimbrough et al., 1972) included 18 species.

Dennisiopsis Subram. & Chandrashekara has two species, *D. octospora* Subram. & Chandrashekara, the type species, and *D. multispora* Subram. & Chandrashekara. The ascomata are eugymnohymenial with a complete absence of an excipulum. The asci are operculate with a thin-walled apex, eight- or multi-spored, and 'non-amyloid'. The ascospores are hyaline, smooth, thin-walled, and easily produce air-bubbles in anhydrous mounting media, like lactophenol. The genus is considered closely related to *Coprotus* and consequently placed in the tribe Theleboleae of the Pyronemataceae by Subramanian & Chandrashekara (1977).

Lasiobolidium Malloch & Cain has two species, *L. spirale* Malloch & Cain, the type species, and *L. orbiculoides* Malloch & Benny. The ascomata are subglobose or irregular in shape, remaining closed and covered with distinct helical appendages. The asci are 8-spored, irregularly disposed, without an apical apparatus, and becoming evanescent at maturity. The ascospores are hyaline, smooth, and without guttules and air-bubbles. The genus was at first placed in the Thelebolaceae (Malloch, 1970), because of a superficial resemblance to *Lasiobolus* Sacc., but soon transferred to the Eoterfeziaceae (Malloch & Cain, 1971). Extensive developmental studies by Janex-Favre & Locquin-Linard (1979) of *L. orbiculoides* showed little evidence for a relationship with *Lasiobolidium* with *Lasiobolus*, nor with the Thelebolaceae. In their opinion the early development of the primordium and the structure of the ascogonial apparatus strongly suggest a relationship with the operculate discomycetes, especially with the genus *Ascodesmis* Tiegh.

Lasiobolus Sacc., with a number of species mainly characterized by the size, shape, and number of ascospores and the size of the setae. The ascomata are closed at first (cleistohymenial) and open in the late-mesohymenial phase, soon covered with usually unicellular setiform excipular hairs, becoming globose to cupulate, 0.1–1.0 mm diam., hyaline, yellowish, or orange. The asci are broadly clavate, with 8 to more than 1000 spores, protruding above the hymenium at maturity; the wall is not blued with iodine; the outer wall layer stains with Congo red. The ascospores are hyaline, smooth and produce air-bubbles rather easily in anhydrous media. All species are coprophilous. The affinity of the genus is clearly with *Coprotus*. It has been included in the Thelebolaceae (Kimbrough & Korf, 1967). From the results of ontogenetic studies of *L. ciliatus* a relationship with the Pyronemataceae emend. Eckblad has been suggested (Conway, 1975). In a revision of *Lasiobolus* (Bezerra & Kimbrough, 1975) eleven species have been distinguished.

Lasiothelebolus Kimbr. & Luck-Allen (Kimbrough & Luck-Allen, 1974) is based on a mixed collection. From accompanying illustrations it was found that the type species, *L. oblongisporus*, consist of fruit-bodies of an eight-spored species of *Thelebolus* partly overgrown with a phialidic anamorph of another fungus (van Brummelen, 1984). This is now confirmed by a study of the type specimen (TRTC 45247). Since the *Thelebolus* element corresponds most nearly with the original description, that part is indicated as the lectotype. So *Lasiothelebolus* becomes a synonym of *Thelebolus* Tode: Fr. (Greuter et al., 1994: Art. 9.10).

Leptokalpion Brumm. with a single species, *L. albicans* Brumm., has paragymnohymenial ascomata developing a marginal rim, each with a single ascus. The asci are

ovoid with a dome-shaped apex, about 4000-spored, with a rather thick, bi-layered wall, not blued with iodine, and opening by an irregular bilabiate split at the top or by an irregular operculum the shape of which is defined by the opening of the covering receptacle. A subapical ring is not observed in the ascan wall. The ascospores are hyaline, smooth, and ejected all together in a single mass; their contents are without oil-guttules or air-bubbles. The genus was placed in the Thelebolaceae (van Brummelen, 1967).

Mycoarctium Jain & Cain, with a single species, *M. ciliatum* Jain & Cain, was placed in the Thelebolaceae (Jain & Cain, 1973) because of the presence of thick-walled hairs, resembling those in *Trichobolus* and *Lasiobolus*. The asci are clavate at first, without an operculum or other opening mechanism, and fully evanescent before maturity. Mature, reticulate, ascospores become exposed as a white, dry, powdery mass between the long, rigid, curved, uncinate hairs. Because of these characters, the genus *Mycoarctium* should be excluded from the Thelebolaceae and transferred to the Onygenaceae (incl. Gymnoascaceae).

Ochotrichobolus Kimbr. & Korf with a single species, *O. polysporus* Kimbr. & Korf, has discoid to scutellate ascomata that are presumably gymnohymenial, with prominent hyaline, septate, bristly, rooting hairs. The asci are operculate, about 128-spored, and stain uniformly in Congo red. The ascospores are hyaline, smooth, thin-walled, and without oil-guttules or air-bubbles. According to Kimbrough & Korf (1983) *O. polysporus* shows clear similarities not only with species of *Lasiobolus* and *Trichobolus*, but also with the other setose operculate discomycetes *Cheilymenia* and *Scutellinia*. These five genera, all with setose ascomata, were therefore included in the tribe Scutellinieae of the Pyronemataceae.

Pseudascozonus Brumm. with a single species, *P. racemosporus* Brumm., has small colourless, eugymnohymenial ascomata without an excipulum. The asci are broadly clavate with a rounded apex, 8-spored, opening either by a small round operculum or by a bilabiate split at the top. The ascospores are hyaline, smooth, thin-walled, and without oil-guttules or air-bubbles. The ultrastructure of the ascus top revealed (van Brummelen, 1987) the presence of a hemispherical body as a thickening of the inner ascus wall just below the very irregularly delimited apical operculum (c. 2 µm diam.) and the absence of a subapical ring. During ascus dehiscence wall layers split easily in the apex (Figs. 17k–n). The genus *Pseudascozonus* was considered related to the genus *Ascozonus* and to '*Ascophanus*' *coemansii* Boud., and was placed in the Thelebolaceae (van Brummelen, 1985).

Ramgea Brumm. with a single species, *R. annulispora* Brumm., has cylindrical to turbinate, paragymnohymenial ascomata. The asci are clavate with a dome-shaped apex, opening with an irregular tear at the top where wall layers are separating above a ring in the wall. The outer ascan wall stains with Congo red, except for a small apical region, while the inner wall stains blue with Waterman's blue-black ink in a small central zone in the apex. The number of ascospores is variable, but mostly four. An ornamentation of ring-shaped ridges is formed on their outer surface. *Ramgea* was placed in the Thelebolaceae, close to *Caccobius*, because of a resemblance in the opening mechanism (van Brummelen, 1992).

Thelebolus Tode: Fr., has a range of species mainly characterized by the number of spores formed in each ascus, as the spore-number in each isolate has proved to be constant (Wicklow & Malloch, 1971). The ascomata are small, subglobular, cleistohymenial, opening in the late mesohymenial or telohymenial phase (van Brummelen, 1967). The asci are cylindric-clavate to subglobose, 8- to over 3000-spored, thick-walled; the wall not staining blue with iodine, but the subapical ring and the outer wall layer below the level of the ring stain strongly with Congo red, leaving the apical dome hyaline (Kimbrough, 1966b; 1972; 1981; Samuelson & Kimbrough, 1978a). The inner layer appears to be stratified. Prior to dehiscence the wall layers of the apex become much thinner. The dehiscence is usually irregular, but occasionally, in 8-spored species, a rather regularly shaped operculum occurs.

The taxonomic relationship of *Thelebolus* has been a continuous source of speculation. The genus was considered to be related to: gasteromycetous fungi (Fries, 1823), Perisporiacei of the Pyrenomycetes (Fuckel, 1869), section Ascobolei of the Pézizes (as '*Ryparobius*'; Boudier, 1869), Erysiphales (Zukal, 1886; Cooke & Barr, 1964); Ascoboleen of the Discomycetes close to *Rhyparobius* (Heimerl, 1889; Rehm, 1895; Barker, 1903), Hemiasci (Brefeld, 1891); tribe Theleboleae of the Pezizaceae (Kimbrough & Korf, 1967), subfam. Theleboloideae of the Ascobolaceae (van Brummelen, 1967), and Thelebolaceae of the Pezizales (Eckblad, 1968). The ascan wall in species of *Thelebolus* was found to differ structurally in one major aspect from that of the true operculate species: 'stacks' of microfibrils of the inner layer were arranged in a banded pattern. This structure resembled that of the 'bitunicate' ascus with a 'Jack-in-the-box' opening mechanism (Samuelson & Kimbrough, 1978a; Kimbrough, 1981). Therefore *Thelebolus* was considered to be related to the Pleosporales (Samuelson & Kimbrough, 1978a), or the Hysteriales of the Loculoascomycetes (Kimbrough, 1981), but as the authors state, its position there is still unclear.

Trichobolus (Sacc.) Kimbr. & Cain in Kimbr. & Korf. is based on *Trichobolus zukalii* (Heimerl) Kimbr. in Kimbr. & Korf, a species initially placed in a separate section of *Thelebolus* because of the setose cleistohymenial ascomata opening in the telohymenial phase (van Brummelen, 1967). Two other species, also multi-spored, *T. pilosus* (Schroet.) Kimbr. in Kimbr. & Korf and *T. sphaerosporus* Kimbr. in Kimbr. & Korf, are very closely related to *T. zukalii* (Kimbrough & Korf, 1967).

The structure and the dehiscence mechanism of the ascus in *T. zukalii* were studied by Heimerl and Zukal (Heimerl, 1889), who reported a complete absence of the ring-shaped thickening of the ascus wall, so characteristic of *Thelebolus stercoreus* Tode: Fr.

Krug (1973) enlarged the concept of the genus *Trichobolus* by adding an eight-spored species, *T. octosporus* Krug, but this has asci with an apical apparatus showing an operculum and a 'definite apical ring'. Relationship of this species with *Lasiobolus* was suggested (Samuelson & Kimbrough, 1978b). While the ascospores in *T. zukalii* and *T. pilosus* produce 'de Bary-bubbles' rather easily, such air-inclusions are not found in *T. sphaerosporus* and *T. octosporus*.

The ascus of *Trichobolus zukalii* has been the subject of studies by light microscopy (Kimbrough, 1966a, 1972) and electron microscopy (Samuelson & Kimbrough, 1978b).

Each fruit-body develops only a single spherical to shortly ovoid ascus, 350–510 × 259–425 µm with a large dome-shaped apex, no stalk and up to 7000 spores. The structure of the young ascus is not well known. In the later stages the lateral wall is rather constant in thickness and reaches 3.7–4.0 µm in the upper part. The outer layer is an almost constant 0.8–0.9 µm width in the upper part and c. 1.2 µm lower down, consisting of an outer stratum strongly staining with silver methenamine and a weaker staining inner stratum, showing a fine lamellation near its inner face. The inner layer, 2.6–3.2 µm thick stains only weakly with silver methenamine and shows a less clear, fine lamellation. In the apical region no subapical ring or other differentiation of the ascus wall or of the acroplasm is observed. In the mature ascus the apical region of the ascus wall continues to protrude more and more and becomes gradually thinner towards the top, decreasing from an initial width of about 3 µm to less than 2 µm at maturity. Ascospore release was reported to occur by a 2–4-lobed split at the top (Heimerl, 1889), by an irregular tear (Kimbrough, 1966a), or by a circumscissile rupture of the apex (Samuelson & Kimbrough, 1978). No distinct apical apparatus appears to be present.

Trichobolus was placed in the tribe Theleboleae of the Pezizaceae (Kimbrough & Korf, 1967), in the Thelebolaceae (Krug, 1973), or close to the species of the '*Otidea-Aleuria*-complex' (Samuelson, 1978d).

Zukalina O. Kuntze was introduced by Zukal (1887) as *Gymnodiscus* Zukal with a single species, *Zukalina* (*Gymnodiscus*) *neglecta* (Zukal) O. Kuntze. This remarkable fungus was observed only once in 1885 on horse dung in Vienna. The ascomata are gymnohymenial up to 250 µm across with excipular tissue only at the base of the asci. The asci are multi-spored, about 86 × 21 µm, straight or somewhat curved, and after throwing off the top cap become 'ear-trumpet-shaped'. The ascospores are hyaline, fusiform, c. 10.5 × 3 µm, surrounded by a broad layer of mucus.

Although this fungus was well described by Zukal (1887) and Rehm (1896) it shows a combination of characters, which make it difficult to recognize in the absence of authentic material. *Zukalina* was placed in the Theleboloideae as a genus of uncertain position by van Brummelen (1967). Korf (1973) and Aas (1992) consider *Zukalina* as a possible synonym of *Thecotheus* Boud. But since the dimensions of ascomata, asci, and ascospores in that genus are at least twice as large as in *Zukalina*, a relationship is unlikely. An affinity of *Zukalina* with *Ascozonus*, as suggested by Velenovský (1934) is more likely. Empty asci of *Thelebolus* and *Ascozonus* may show the truncate shape as described and depicted by Zukal (1887), especially when, after spore release, the torn remains of the apex above the subapical ring turn inwards into the ascus below the level of the ring (cf. e.g. Zukal, 1887, 1889; Vuillemin, 1887). However, the presence of hymeneal mucus, ascospores with mucus only at the sides (Zukal, 1887, Taf. 1, fig. 1c), and the absence of a strong subapical ring in the ascus do not agree with such an interpretation. The identity of *Zukalina* remains uncertain.

MATERIALS AND METHODS

As far as possible fresh material, either from cultures or collected in the field, has been studied. Minor fragments or isolated bundles of asci were fixed and embedded in Epon,

ultrathin sections were cut using a diamond knife. In most cases selected sections were treated with the periodic acid-thiocarbohydrazide-silver proteinate procedure (PA-TCH-SP), a slightly modified Thiéry (1967) technique, as described by Verkley (1992). If not stated otherwise the material described and illustrated was handled according to these methods. In addition, part of the material was fixed by using the ultra-rapid freeze fixation method followed by freeze substitution as described elsewhere (van Brummelen, 1993). Asci of *Caccobius minusculus* and *Ramgea annulispora* were only available from dried material. Here a few asci were rehydrated in water for 24 hours and then further treated as fresh material.

Photomicrographs of ascus apices were also made with light microscopy with a Leitz microscope using a Plan Apo 100 × objective. The stains used were 1% Congo red in 10% ammonia and 0.02% methyl blue in lactophenol.

The following list gives details of the origin of the material referred to in this paper.

Ascozonus woolhopensis (Berk. & Br. in Renny) Boud. — Ekeren, near Antwerp, Belgium, on dung of rat (comm. Vervliet), 12.II.1972, *J. van Brummelen* (culture, L).

Ascozonus solms-laubachii (Rabenh.) Brumm., *comb. nov.* — Basionym: *Ascobolus solms-laubachii* Rabenh. in Fungi europ. exc., Cent V, No. 420. 1862; Rabenhorst, Bot. Ztg. 20: 198. 1862; not *Ascobolus solms-laubachii* sensu Fuckel, Hedwigia 5 (1866) 2; Jb. nassau. Ver. Naturk. 23–24 (1870) 288; not *Ascobolus solms-laubachii* sensu Schröter in Kryptog.-Fl. Schles. (ed. Cohn) 3 (2) (1893) 53. — *Rhyparobius solms-laubachii* (Rabenh.) Rehm, Rabenh. Kryptog.-Fl., Pilze 3 (1895) 1101. — Lectotype: Rabenhorst, Fungi europ. exs. No. 420 (Herb Rehm, S). — Overveen, The Netherlands, on horse dung, 13.IX.1973, *J. van Brummelen* (culture, L).

Caccobius minusculus Kimbr. — S. of Whitney, Nipissing Distr., Ontario, Canada, on dung of rabbit, 26.IX.1956, *R.F. Cain* (TRTC 32390; holotype of *C. minusculus*).

Coprobolus poculiformis Cain & Kimbr. — S.W. of Palgrave, Peel Co., Ontario, Canada, on rabbit dung, 7.X.1962, *R.F. Cain* (TRTC 38822; holotype of *C. poculiformis*).

Coprotus lacteus (Cooke & Phill. in Cooke) Kimbr. et al. — Elspeet, The Netherlands, on dung of sheep, 19.XII.1972, *J. van Brummelen* (L).

Lasiobolus cuniculi Velen. — Leiden, The Netherlands, on dung of goat, 27.VIII.1993, *J. van Brummelen 8249* (L).

Lasiobolus monascus Kimbr. — Fontaines Chauds, near Epau, S. of Le Mans, La Sarthe, France, on dung of rabbit, 2.III.1984, *J. van Brummelen 7167* (L).

Lasiobolus pilosus (Fr.: Fr.) Sacc. [*L. ciliatus* (J.C. Schmidt: Fr.) Boud. sensu auct., non sensu Schmidt, Fries, or Boudier; *L. equinus* (O.F. Müller) P. Karst., not sanctioned by Fries; *L. papillatus* (Pers.: Fr.) Sacc. sensu auct., non sensu Persoon or Boudier (cf. Boudier, 1869; van Brummelen, 1967)]. — Overveen, The Netherlands, on dung of rabbit, 15.V.1973, *J. van Brummelen* (L).

Lasiothelebolus oblongisporus Kimbr. & Luck-Allen — S of Paul Smith College, Saranac Lake, New York, USA, on deer dung, 12.IX.1965, *E.R. Luck-Allen C1594* (TRTC 45247; holotype of *L. oblongisporus*).

Leptokalpion albicans Brumm. — Khao Luang, Prov. Nakhon Si Thammarat, Thailand, on roe deer dung (comm. Dr. H.O. Sleumer), VI.1968, *J. van Brummelen 2490* (L; holotype of *L. albicans*).

Pseudascozonus racemosporus Brumm. — Tourbière de Frasne, dép. Doubs, France, on dung of deer, 20.III.1985, *J. van Brummelen 7398* (L; holotype of *P. racemosporus*).

Ramgea annulispora Brumm. — Stiphoutse Bossen, Helmond, The Netherlands, on dung of pheasant, 11.III.1990, *L. Raaijmakers* (L; holotype of *R. annulispora*).

Thelebolus caninus (Auersw.) Jeng & Krug — S. of Dorset, Haliburton, Ontario, Canada, on dung of deer, 18.IX.1967, *D. Malloch* (culture TRTC 45563, CBS 708.69).

Thelebolus coemansii (Boud.) Brumm., *comb. nov.* — Basionym: *Ascophanus coemansii* Boud., Annls Sci. nat. (Bot.) V 10 (1869) 244; *Thelebolus coemansii* (Boud.) Kuyper in E. Arnolds et al., Overz. Paddestoelen Nederl. (1995) 732 (not validly published). — Type specimen not preserved; type represented by: Boudier, Annls Sci. nat. (Bot.) V 10 (1869) 244, pl. 10 f. 30. — Overveen, The Netherlands, on horse dung, 21.VI.1973, *J. van Brummelen* (L).

Thelebolus crustaceus (Fuckel) Kimbr. in Kobayasi et al. — Mt. Speke, Ruwenzoni Mts., Uganda, on dung of carnivore, 23.VII.1969, *R.F. Cain et al.* (culture TRTC 45566, CBS 715.69).

Thelebolus microsporus (Berk. & Br.) Kimbr. — Gondwana Pond, near German Base, Antarctica, on mud polluted with skua dung, *A. Montemartini-Corte* 'Gondw. S1' (culture); Edmonson Point, Camp 56, Antarctica, on mud polluted with skua dung, *A. Montemartini-Corte 56/1* (monosporic isolate; cf. Montemartini et al., 1993).

Thelebolus nanus Heimerl — Robbenoord Bos, Flevoland, The Netherlands, on dung of deer, 1.X.1982, *J. van Brummelen 6687* (L).

Thelebolus polysporus (P. Karst.) Otani & Kanzawa — Warren Lake, Cape Breton, Highlands National Park, Nova Scotia, Canada, on dung of carnivore, 9.VI.1967, *D. Malloch* (culture TRTC 45548, CBS 711.69).

Thelebolus stercoreus Tode: Fr. — S. of Coldwater, Simcoe Co., Ontario, Canada, on dung of deer, 13.V.1968, *D. Malloch* (culture TRTC 45546, CBS 717.69).

Trichobolus octosporus Krug — Bosler, Albany Co., Wyoming, USA, isolated from deer dung, 1.IX.1964, R.F. Cain (TRTC 43801; holotype of *T. octosporus*)

Trichobolus zukalii (Heimerl) Kimbr. in Kimbr. & Korf — Mt. Tamalpair, near San Francisco, USA, on dung of deer (comm. Dr. H.O. Sleumer), 1.IV.1962, *J. van Brummelen 1447* (L).

Legends to Figures 1–12 (on pages 435–446)

Abbreviations used in figures: A, ascus; AS, ascostome; AW, ascus wall; C, cleavage or splitting of ascus wall; CM, condensed material; CT, central thickening; E, epiplasm; F, line or zone of fracturing; FI, fibrillar elements; IL, inner layer; IM, investing membrane; IS, inner stratum; N, nucleus; O, operculum; OL, outer layer; OS, outer stratum; P, periascus (extra-ascan layer); PM, plasma membrane or plasmalemma; S, ascospore; SL, sublayering of the ascus wall (usually indicated by dotted lines); SR, subapical ring; SW, strongly swollen wall material; WZ, weakness zone. — The scale markers in electron micrographs without further indication equal approximately 1.0 μm, in photomicrographs 10 μm. Unless otherwise stated, the illustrated material was fixed in 1% glutaraldehyde and contrasted with the Thiéry technique.

Fig. 1. *Thelebolus microsporus*, electron micrographs of longitudinal median sections of ascus apices. a, c. Tops of immature asci, fixed by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique. b. Mature ascus shortly before opening at the top. d. Top of just opening ascus.

Fig. 2. *Thelebolus coemansii*, electron micrographs of longitudinal median sections of ascus apices. a. Very young ascus, with beginning of wall differentiation and formation of subapical ring. b. Ripening ascus. c. Id. detail of apical and subapical wall regions.

Fig. 3. *Thelebolus caninus*, electron micrographs of longitudinal median sections of ascus apices. a. Detail of young ascus. b. Ripening ascus. c. Detail of ascus wall near subapical ring. d. At left mature ascus with break down of inner wall layer; at right the top of a dehisced ascus. e, f. Tops of dehisced asci with swelling of inner wall layer.

Fig. 4. *Thelebolus crustaceus*, ascus development. a. Photomicrograph of ascus apex at the beginning of opening, stained with methyl blue. b. Electron micrograph of longitudinal median section of immature ascus top. c. Id. detail of ascus wall near subapical ring.

Figs. 5a, b. *Thelebolus polysporus*, electron micrographs of longitudinal median sections. a. Part of top of immature ascus. b. Detail of ascus near subapical ring. — Figs. 5c, d. *Thelebolus crustaceus*, photomicrographs of ascus apices. c. Ripening ascus stained with Congo red. d. Mature ascus stained with methyl blue.

Fig. 6. *Thelebolus stercoreus*, ascus development. a. Photomicrograph of living mature ascus in closed ascoma. b. Photomicrograph of isolated living mature ascus, showing bulging out of inner wall layer at the top. c–e. Electron micrographs of asci fixed in 1% glutaraldehyde and contrasted with Thiéry technique. c. Longitudinal median section of mature ascus, with thinner wall above the subapical ring. d. Detail of ascus wall near subapical ring. e. Id. near base of ascus, showing fibrils after extreme swelling; between an inner and outer region with fibrils more or less parallel to the ascus surface a less dense region with oblique or irregularly disposed fibrils occurs.

Fig. 7. *Caccobius minusculus*, electron micrographs of longitudinal median sections of ascus apices. a. Young ascus before ascosporogenesis. b. Immature ascus. c. Top of mature ascus, shortly before spore release. d. Top of dehisced ascus.

Fig. 8. *Lasiobolus pilosus*, electron micrographs of longitudinal median sections of ascus apices. a. Top of mature ascus. b. Id. detail of transition between apical and subapical regions. c. Top of dehisced ascus with operculum. d. Id. detail of ascostome and opercular margin.

Fig. 9. *Lasiobolus cuniculi*, electron micrographs of longitudinal median sections of ascus apices, after fixation by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique. a. Detail of mature ascus. b. Detail of transition between apical and subapical regions. c. Detail of operculum of dehisced ascus.

Fig. 10. *Ascozonus*, electron micrographs of longitudinal median sections of ascus apices. Figs. 10a, b. *Ascozonus woolhopensis*. a. Detail of subapical ring in immature ascus. b. Detail of operculum of dehisced ascus. Figs. 10c–e. *Ascozonus solms-laubachii*. c. Detail of weakened places in the top of mature ascus. d. Top of mature ascus with spore release beginning. e. Detail of operculum of dehisced ascus.

Fig. 11. *Ramgea annulispora*, electron micrographs of longitudinal median sections of ascus apices. a, b. Ripening asci. c. Mature ascus with ornamented ascospores. d. Ascus at the beginning of spore release.

Fig. 12. *Coprotus lacteus*, electron micrographs of longitudinal median sections of ascus apices. a. Apex of almost mature ascus. b, c. Details of transitional zone between subapical and apical regions in mature ascus, showing beginning of fracturing of inner layer. d, e. Details of dehisced asci near base of operculum.

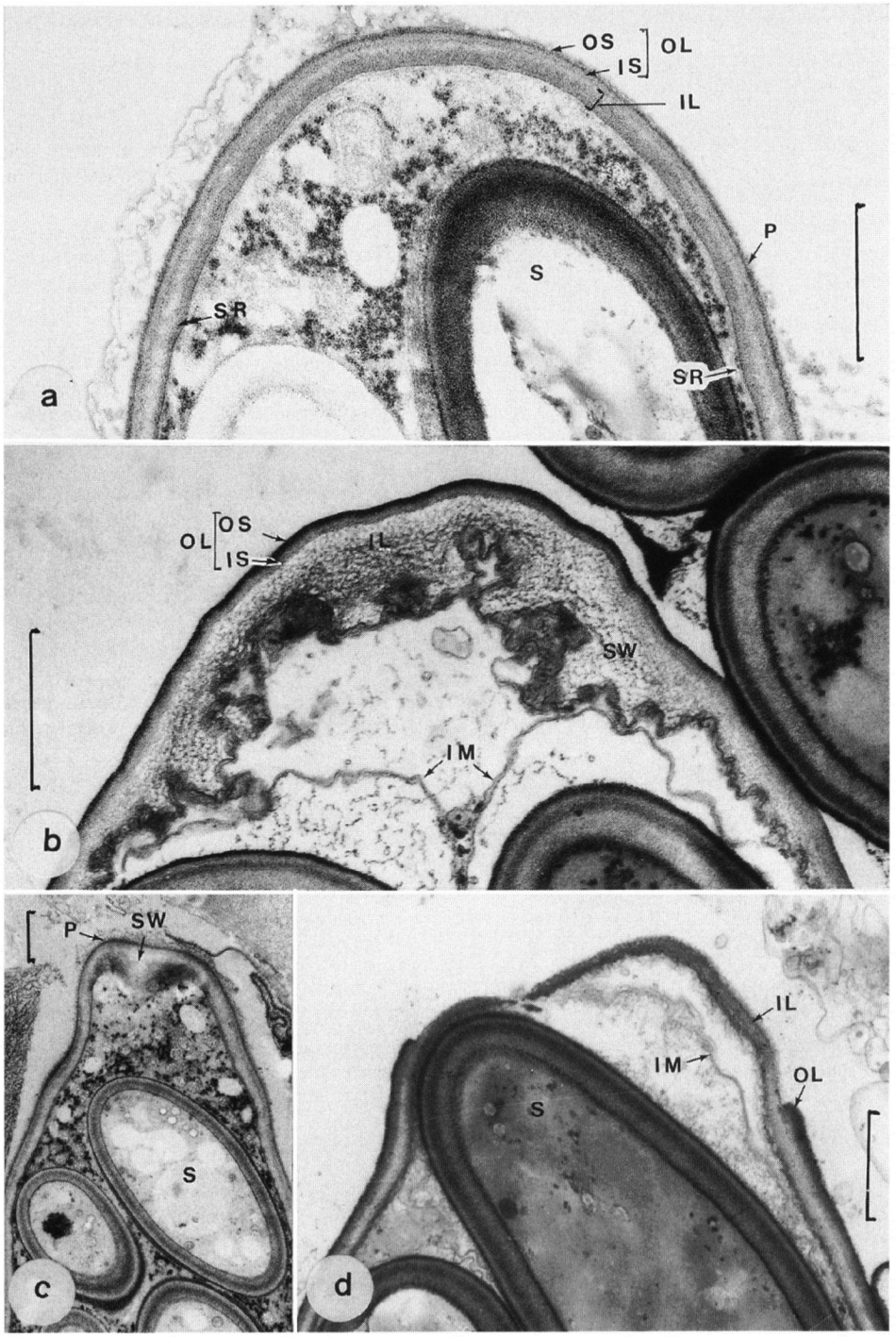
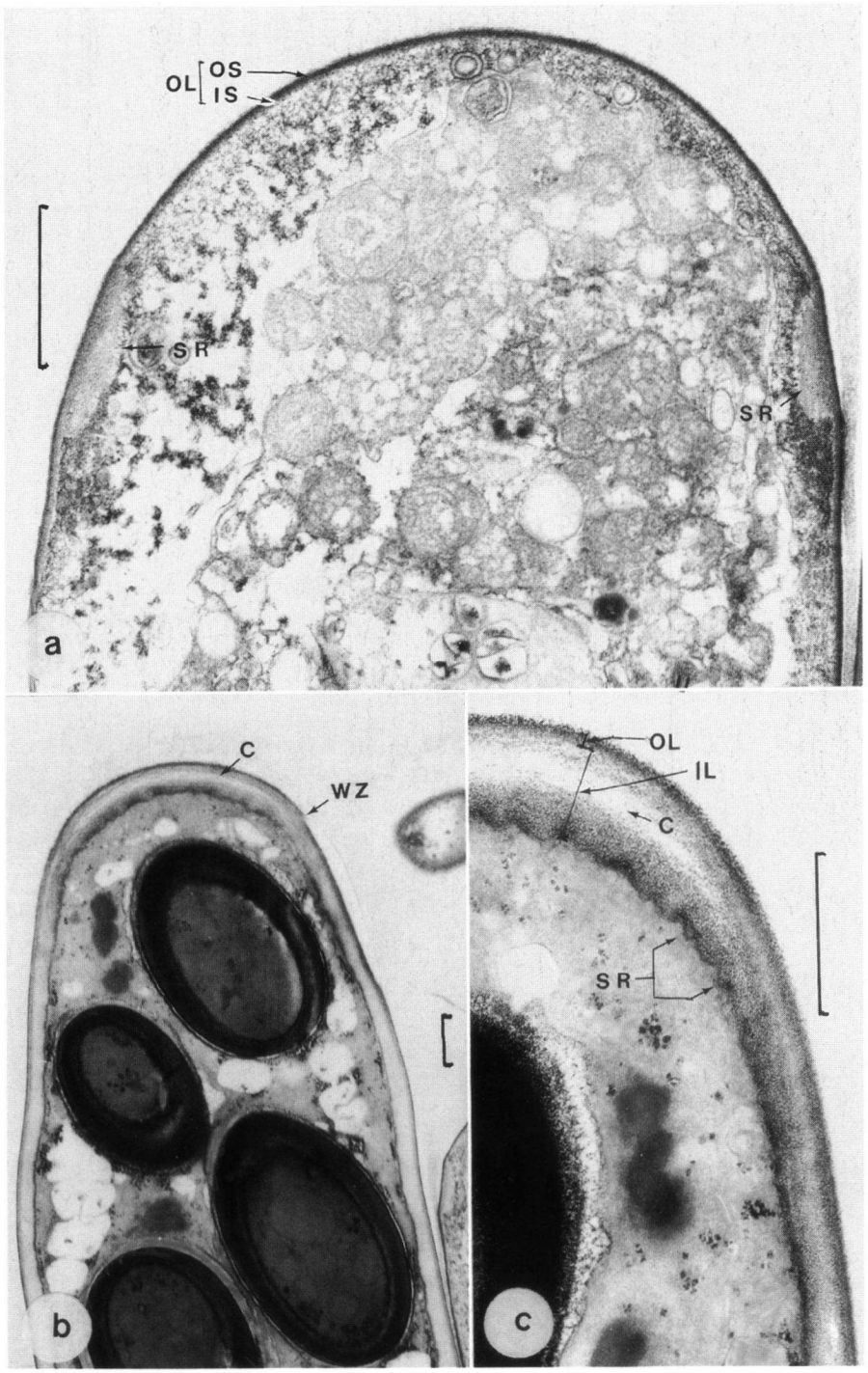


Fig. 1 (legend on page 433)



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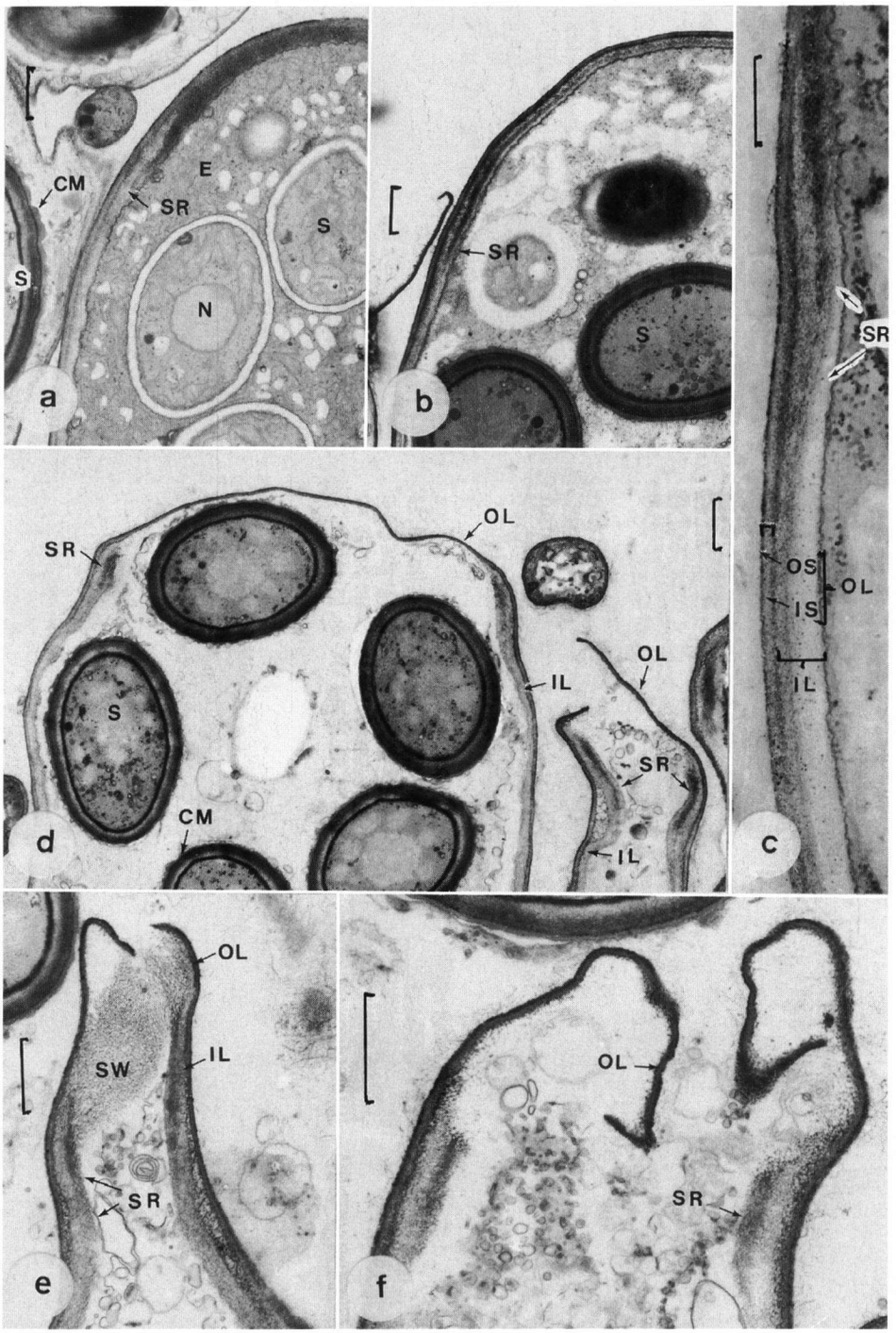

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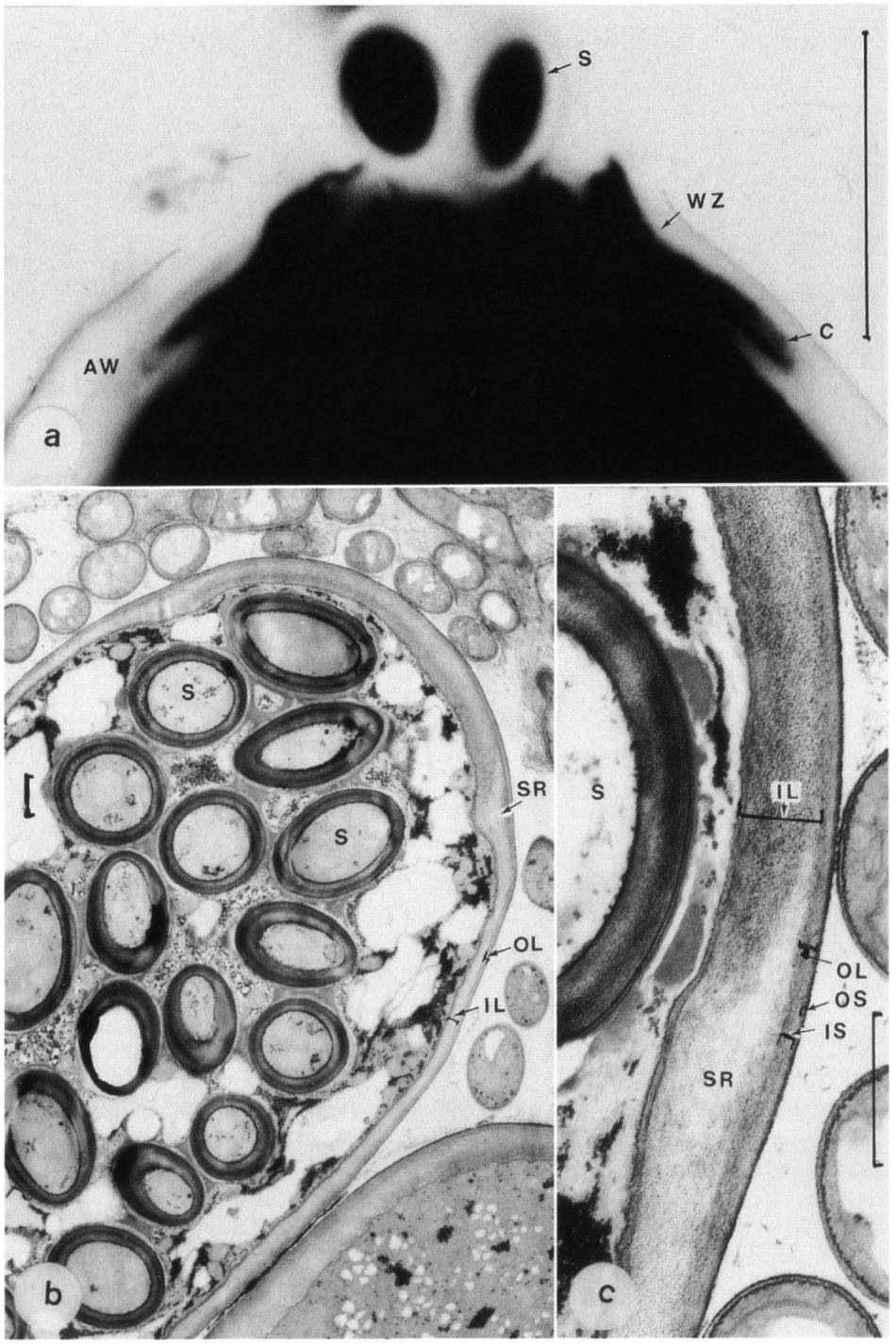

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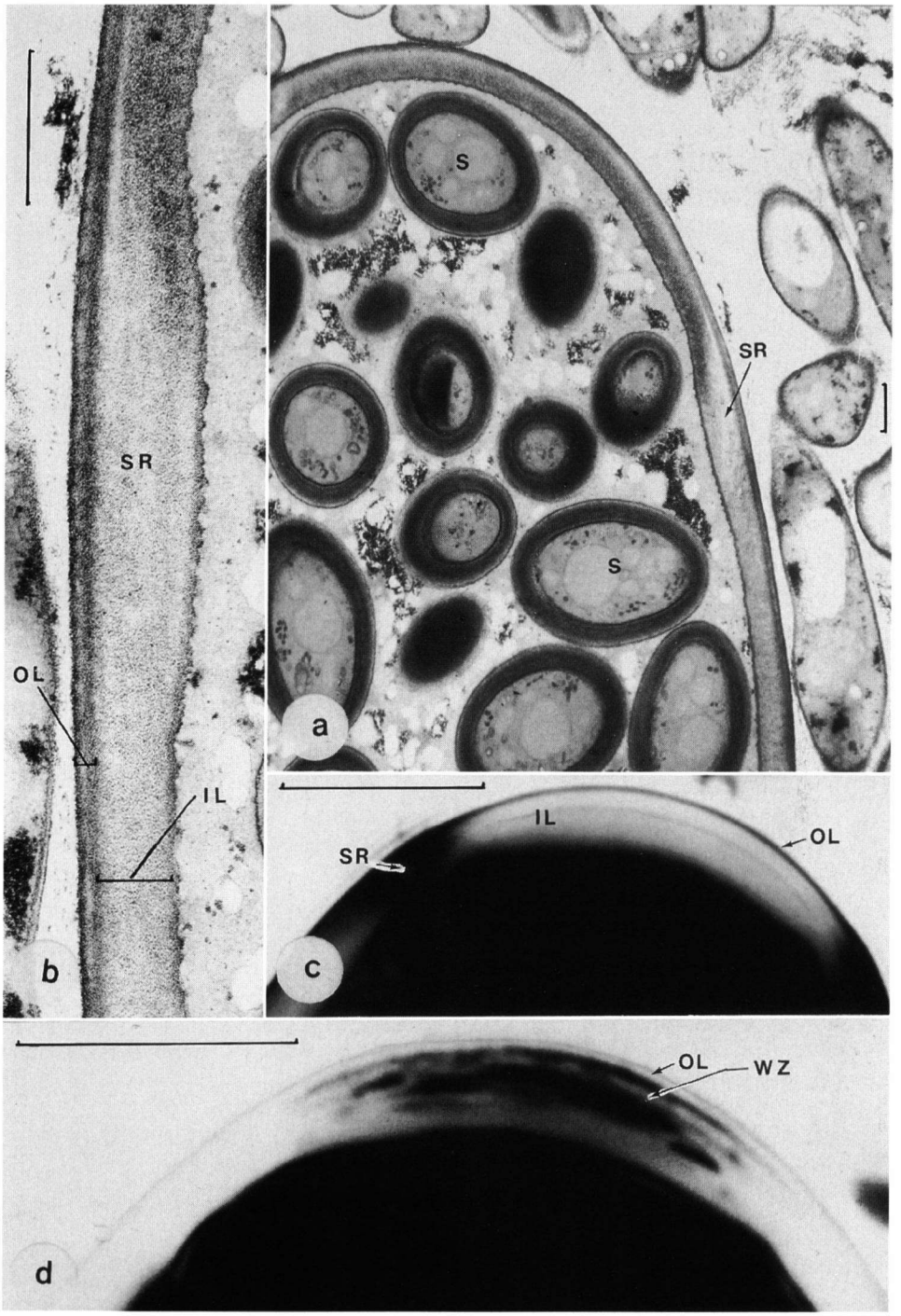

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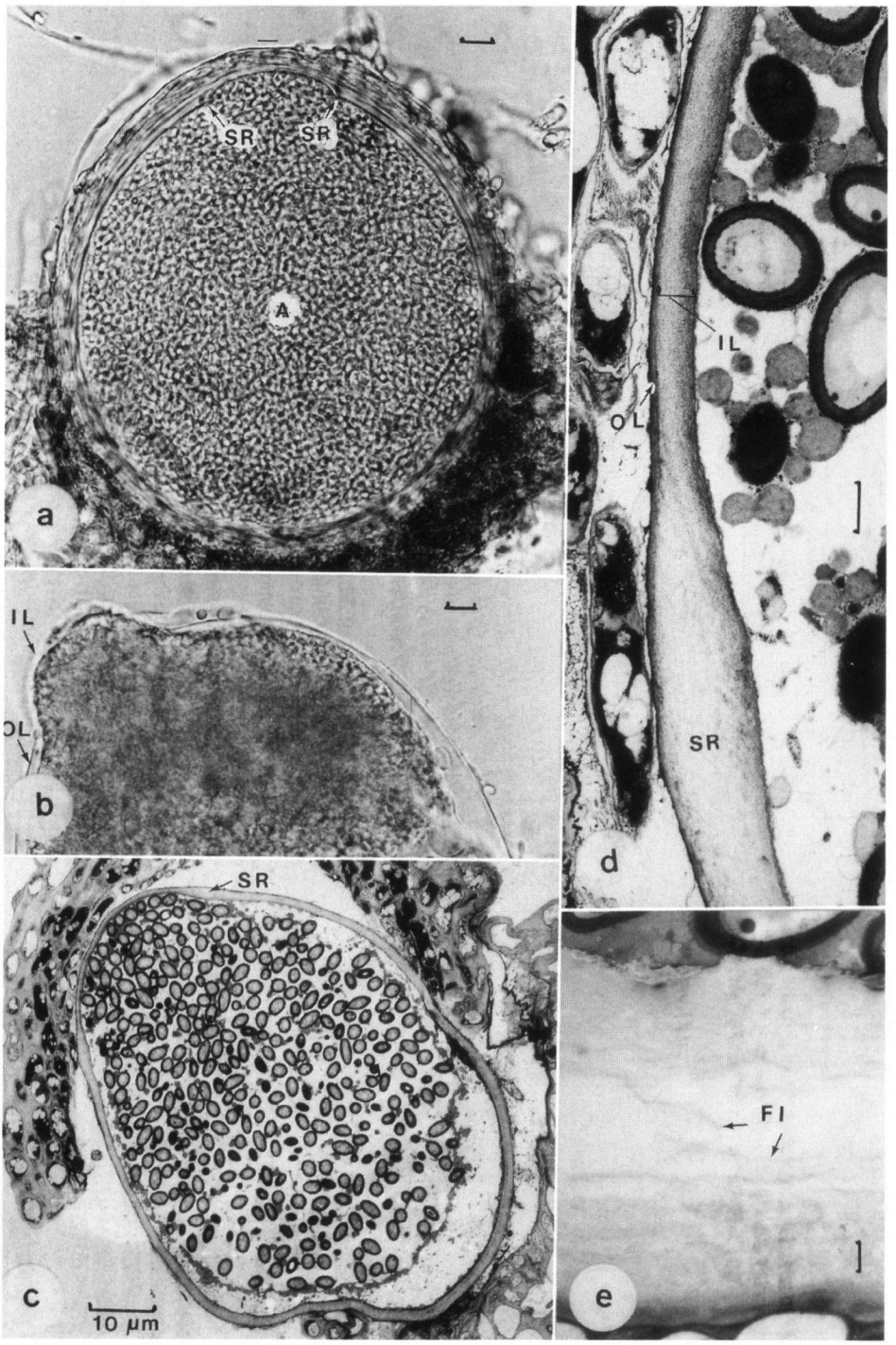

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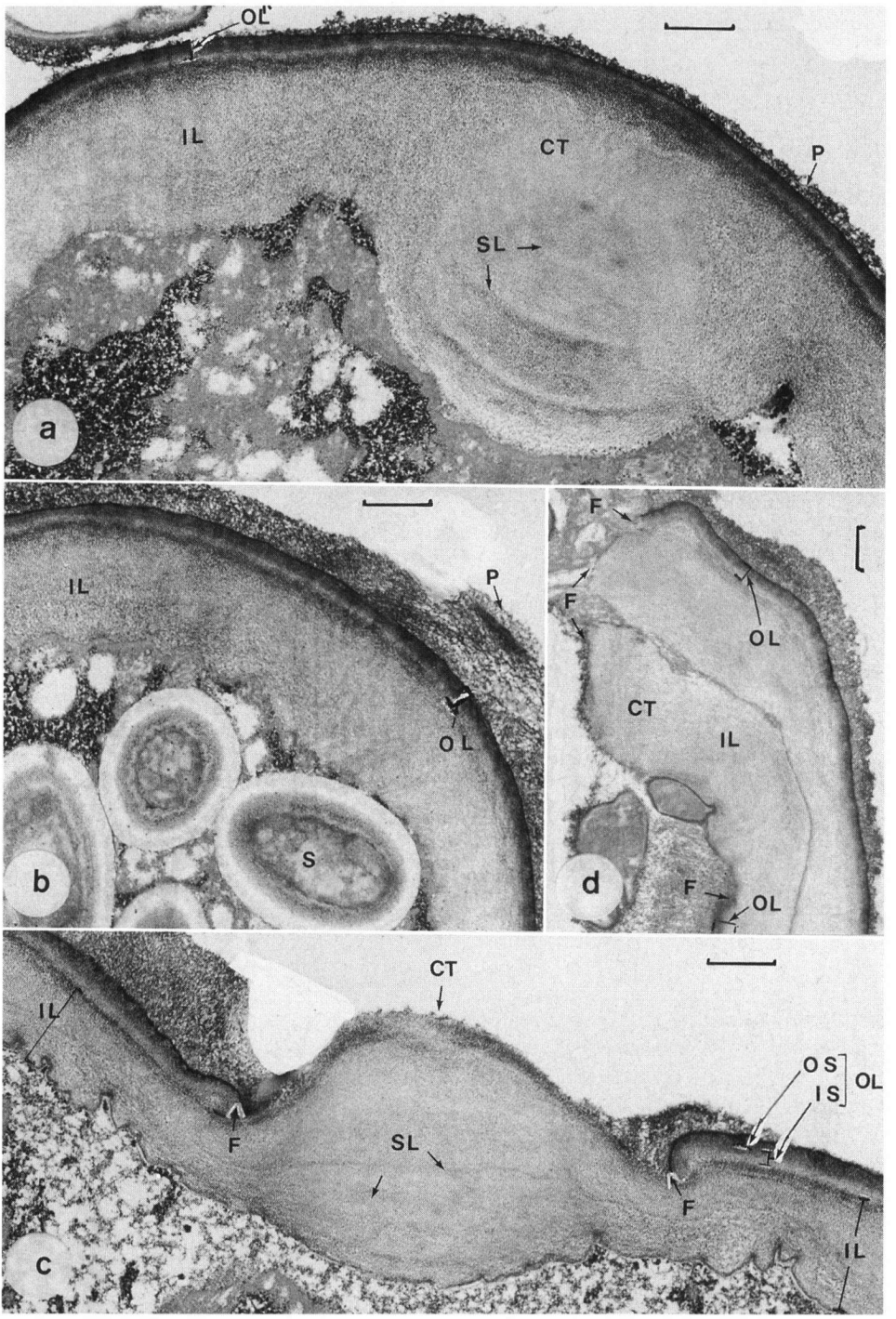

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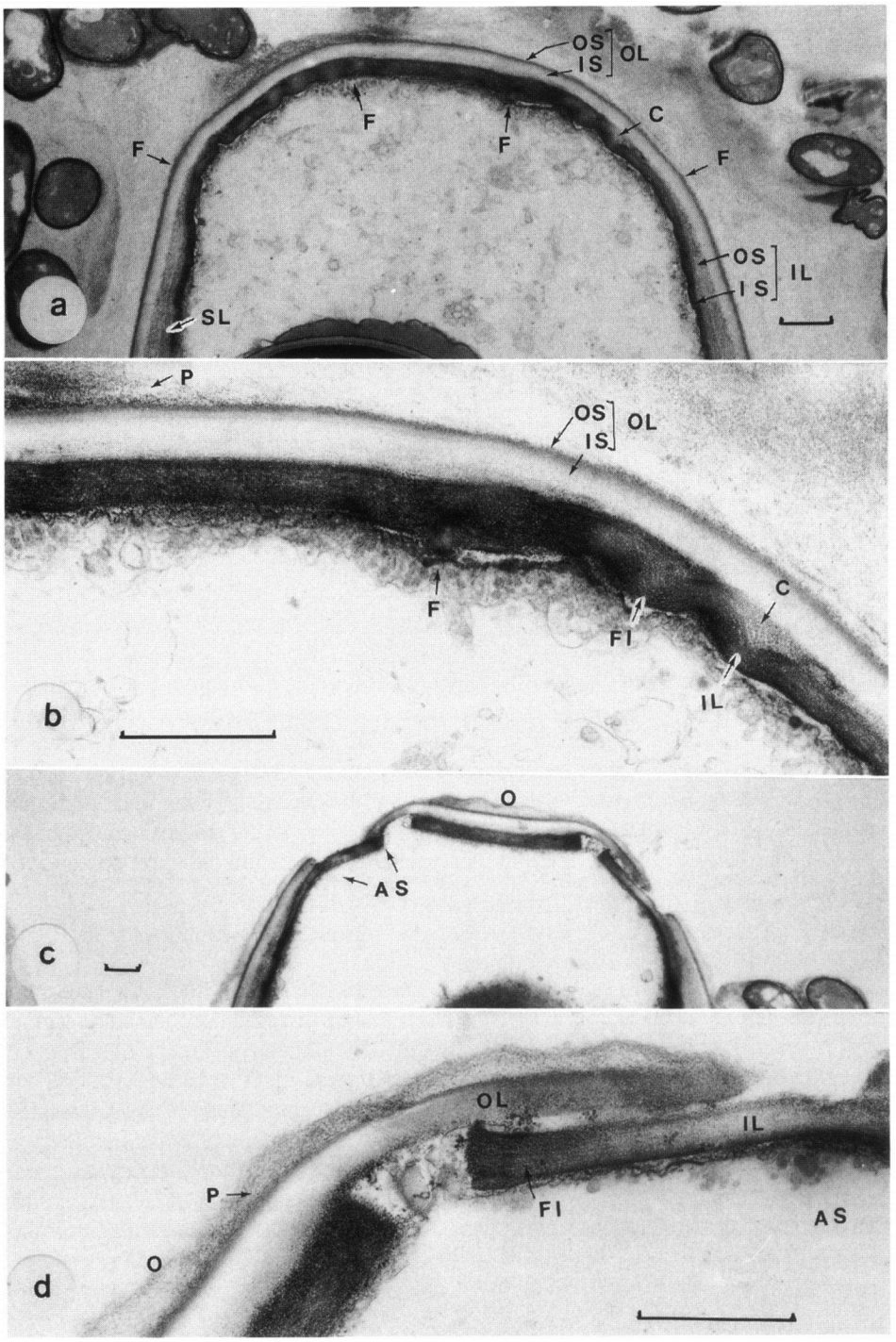

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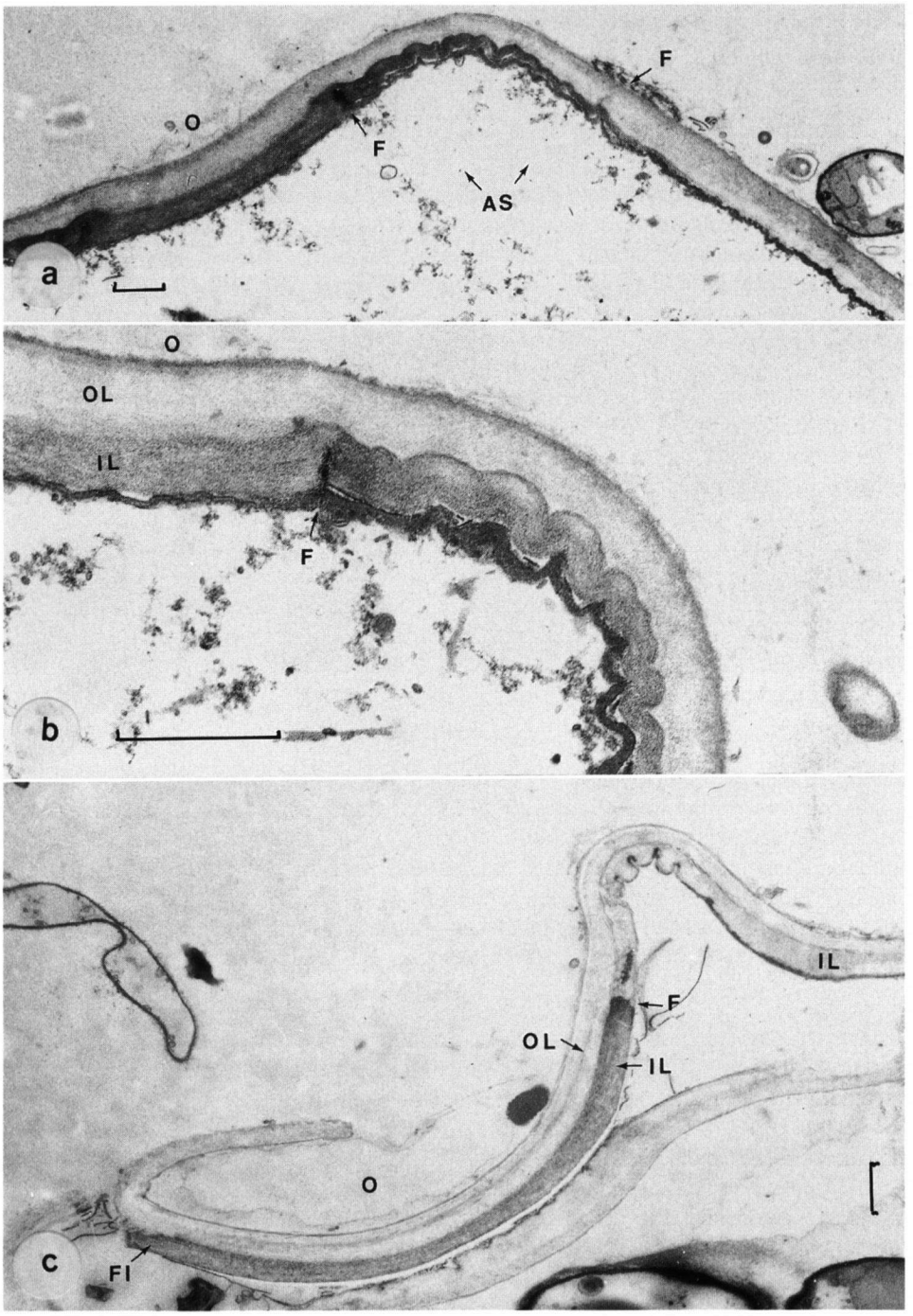

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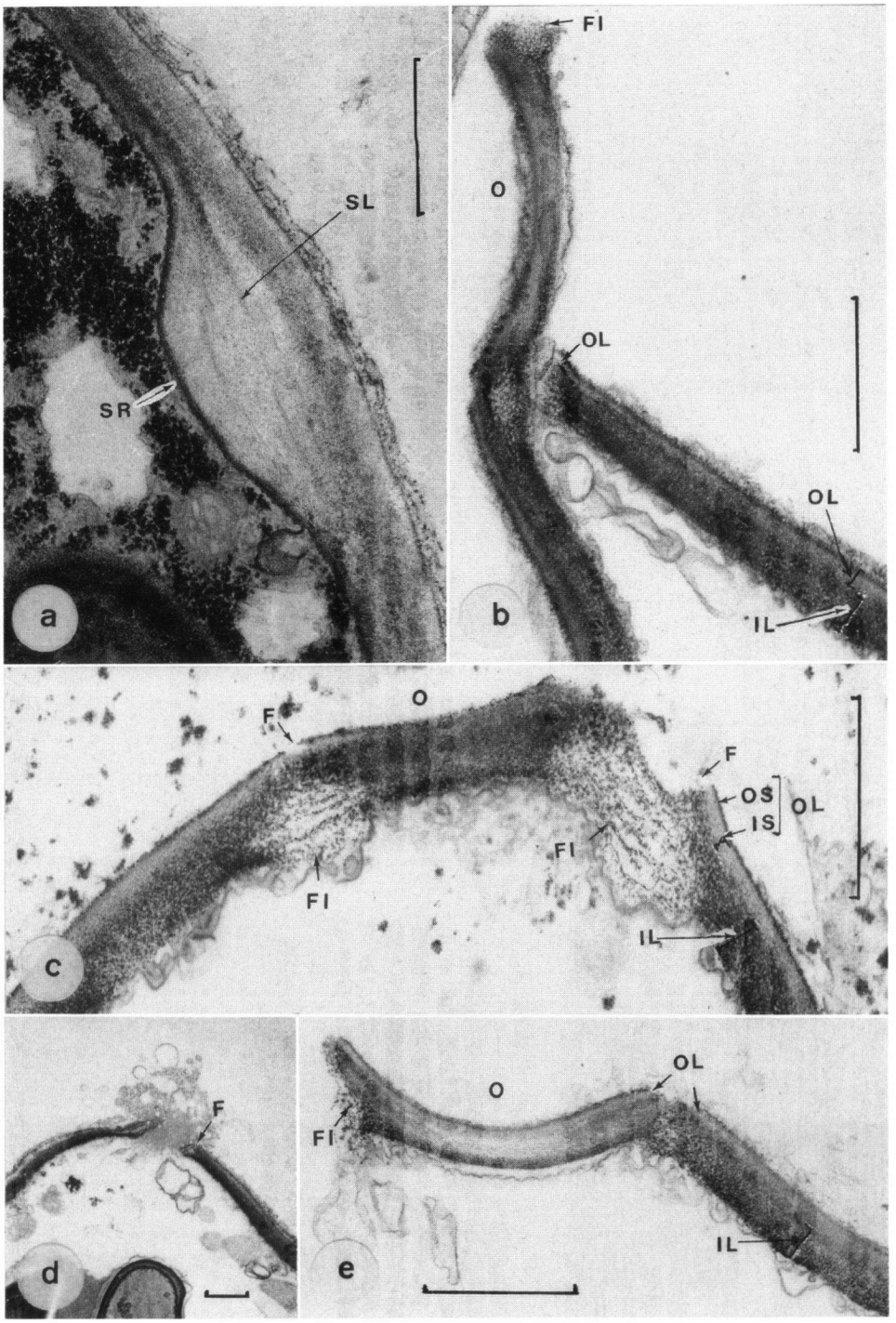

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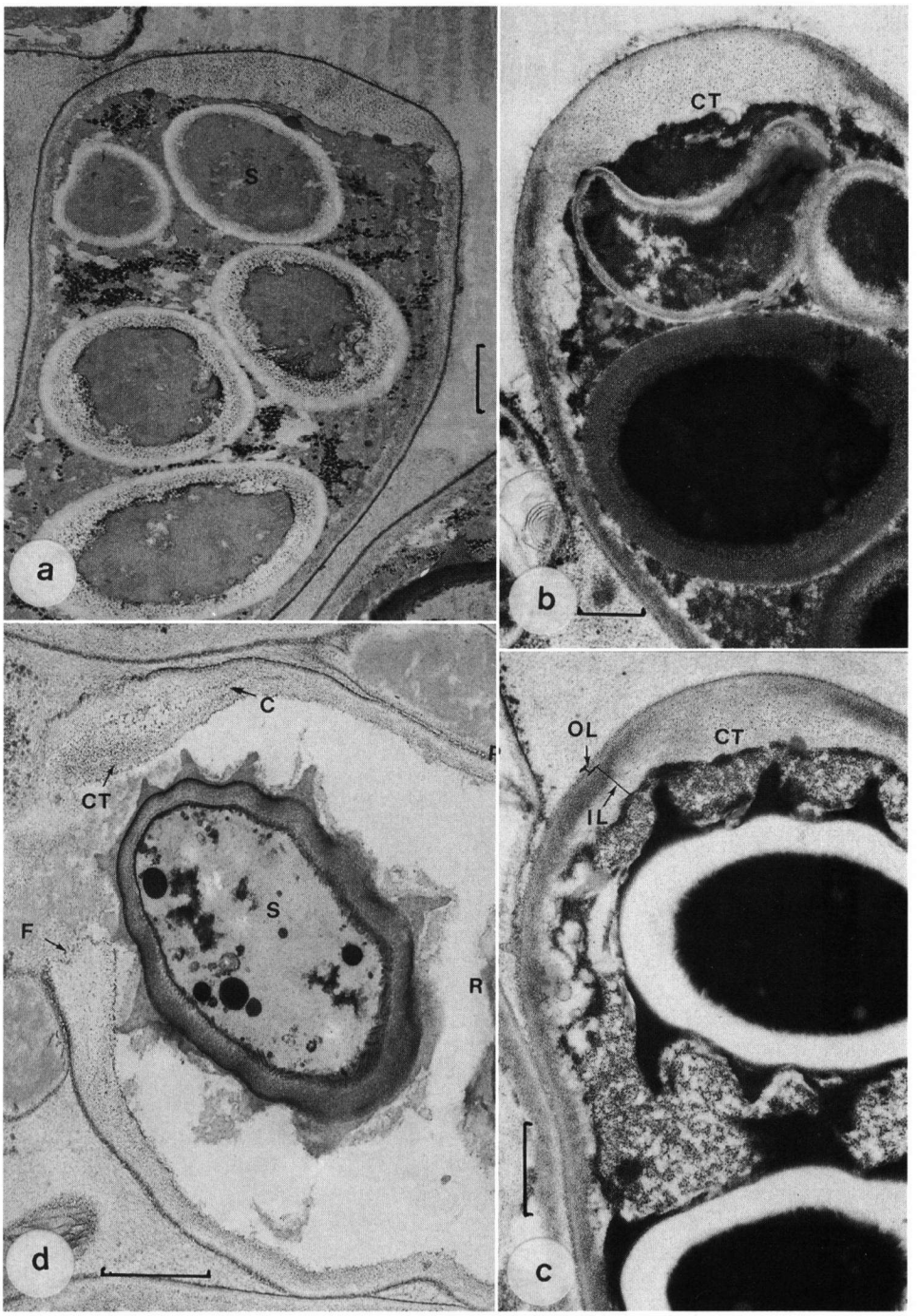

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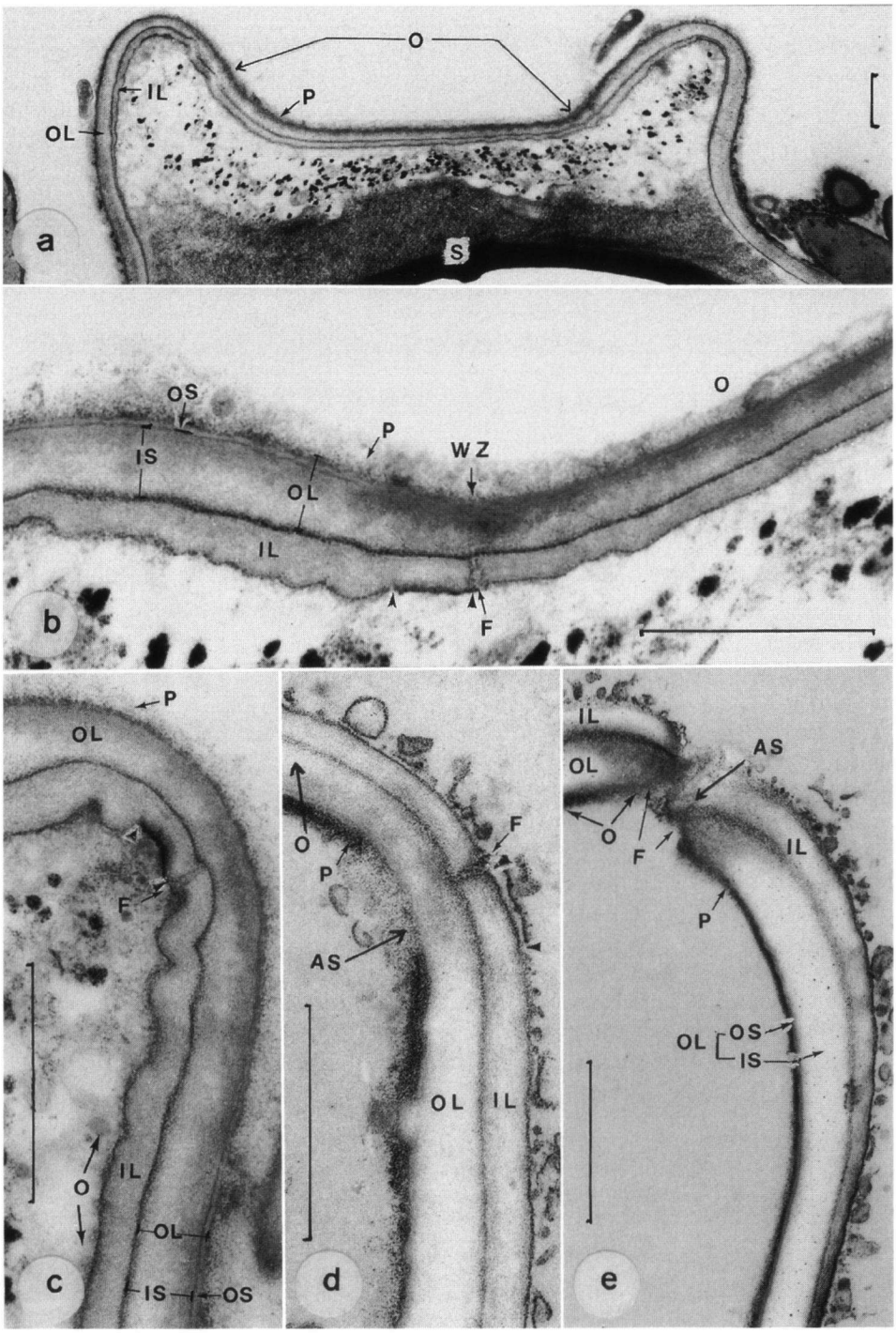


Fig. 12 (legend on page 434)

OBSERVATIONS ON THE ASCUS APEX

Observations on the structure and differentiation of the lateral and apical ascus wall in this study are mainly based on electron micrographs of material treated with the Thiéry technique, since this proved to be the most reliable test for carbohydrates.

In the past many results by light microscopy were obtained after staining with Congo red. This is both an indicator and a stain. As an indicator the dye acid is blue and its (alkaline) sodium salt red. The red colour of the salt changes easily with a small quantity of weak acid into blue.

Since Klebs (1886) Congo red has been extensively used as a reagent for cellulose. Especially in alkaline solutions cellulose stains intensely red. But the staining is not very specific, since it was also found to stain plant mucus, pectines, callose, and hemicellulose. The staining with Congo red is based, neither on ionogenic nor on chemical bindings, but depends on induced physical dipole forces, where the complexes of Congo red planar molecules neatly fit into the cavities between ± parallel oriented fibrils of structural polysaccharides or other fibrillar wall polymers (Frey-Wyssling, 1959; Harms, 1965; Drawert, 1968). By the polarized character of the Congo red staining, oriented structures that bind this stain become strongly dichroic. In polarized light such structures show red with light polarized in one direction, but green with light polarized perpendicularly (cf. Pearse, 1968). Such dichroic staining with Congo red does not characterize a certain chemical substance, but indicates the presence of micellar structures with orientated internal surfaces.

The results of more recent investigations of wall components of fungi (van der Valk et al., 1977; Vermeulen & Wessels, 1986; Wessels, 1990; Ruiz-Herrera, 1992) indicate that, although Congo red is not very specific, it binds best with not-yet crystallized native β-glucans, such as in cellulose and nascent chitin (with unbranched chains of acetylglucosamine). Since fungal walls are generally rich in chitin in non-crystalline form, these readily stain with Congo red.

In electron microscopy different polysaccharides are localized with the Thiéry technique. This gives much finer results in the deposition of metallic silver at the site of action than are obtained with the silver methenamine technique (Martino & Zamboni, 1967). Structures in fungal walls, that react negatively with the Thiéry-technique are rich in chitin and β-1–3 glucans, while those that react positively are rich in other glucans and mannans (Ruiz-Herrera, 1992). Images obtained with the Thiéry technique are therefore not comparable with those obtained with Congo red, since this also stains e.g. precursors of chitin.

Thelebolus microsporus — Figs. 1a–d, 14a–e

Fixed with rapid cryo fixation and chemical fixation, both followed by treatment with the Thiéry technique.

General — Asci numerous, broadly cylindric-clavate with a short broad stalk and a rounded apex, reaching 80–125 × 20–26 µm, with 8 smooth spores; the wall not staining with iodine.

Young ascus — The wall is undifferentiated and of constant thickness, 55–70 nm, in both lateral and apical parts. Special activity at the site of the future subapical ring was not observed. There is an outer stratum of strong reactivity 27–32 nm thick and an inner stratum of lower reactivity 32–42 nm thick. A thin reactive periascus is observed from the beginning and at later stages.

Lateral wall — The outer layer, about 90 nm thick, becomes more clearly delimited, remains rather constant, and consists of a strongly reactive outer stratum 42–46 nm thick and a moderately reactive inner stratum 37–46 nm thick. After cryo fixation the inner layer shows little variation in thickness, laterally the thickness is 93–116 nm, in the apex about 185 nm, while it reaches 230–275 nm in the subapical ring.

Ripening and mature ascus — While the outer layer remains unchanged, the inner layer differentiates into two strata, an outer stratum of low reactivity and an inner of strong reactivity. The latter is up to 162 nm thick in the apex, becomes gradually thinner in the subapical region and is scarcely distinguishable lower down the lateral wall. At maturity a great accumulation of wall material is observed in the apex.

In cryo-fixed material the inner layer increases up to 700 nm and its inner boundary remains regular and smooth, although vague in the apex; while in chemically fixed material it reaches 740 nm and shows a strongly undulated or folded inner stratum.

Dehiscence mechanism — Shortly before dehiscence the ascus wall stretches strongly. Regular dehiscence occurs in asci with freely exposed ascus tops. Here the more rigid outer layer breaks with an irregular tear above the level of the subapical ring, which gives space for the more flexible compressed inner layer to extend through this opening. Immediately, or very soon afterwards, the inner layer also breaks irregularly and the ascospores are forcibly released. Structurally the actual splitting occurs within the outer stratum of the inner layer ('couche c' of Bellemère, 1977). In asci that are not freely exposed this process of regular dehiscence is hampered.

Thelebolus coemansii — Figs. 2a–c, 14f, g

General — Asci numerous, broadly cylindric-clavate with a short stalk and a rounded apex, reaching 85–110 × 20–25 µm, with 8 smooth spores embedded in a reticulum; the wall not staining blue with iodine.

Young ascus — The wall is rather uniform with a constant thickness of 60–70 nm in the lateral and apical parts; consisting of a strongly reactive outer stratum and a less reactive inner stratum. An outer floccose, reactive periascus is present. Exactly at the place where the future subapical ring will be formed a rather conspicuous annulus 930–1200 nm wide and 210–230 nm thick of moderately reactive wall material is deposited on the inner face of the single layered young wall. In the neighbouring ascoplasm increased activity is observed.

Lateral wall — The outer layer, 70–80 nm thick, consists of a strongly reactive outer stratum 18–23 nm thick and a less reactive inner stratum 46–56 nm thick. The inner layer shows a uniform thickness of about 100 nm in the flanks, but reaches 265 nm in the subapical region.

Ripening and mature ascus — The outer layer remains constant in thickness and reactivity. The inner layer is deposited on the inner face of the outer layer as a secondary wall. The inner stratum of this layer is more reactive than the outer stratum and forms the main part of the apical wall with a thickness up to 510 nm. It is clearly fibrillose and often shows internal regions free of reactive wall material, orientated parallel to the surface of the apex. Often rather large cavities are formed in the apex, which can be easily demonstrated with light microscopic stains. On ripening the inner surface of the inner stratum becomes undulated or folded in the entire apex above the subapical ring. At these stages

the subapical ring is not easily detected, but it can be seen as the narrow region in the wall where both strata of the inner layer change rather abruptly in thickness. Just above the subapical ring a weakened zone in the outer wall layer is often observed.

Dehiscence mechanism — Not observed in detail with TEM.

Thelebolus caninus — Figs. 3a–f, 15a–e

General — Asci numerous, broadly clavate with a short broad stalk and a rounded or slightly flattened apex, reaching 105–120 × 30–35 µm, with 32 finely verrucose spores; the wall not blued with iodine.

Young ascus — Shortly after early ascosporogenesis a secondary wall can already be distinguished at the inner face of the outer layer (primary wall). The outer layer is uniform in contrast and thickness 112–140 nm thick, both apically and laterally.

Lateral wall — The outer layer decreases in thickness to 80–100 nm when it becomes strongly extended during further development and remains constant in all parts. It consists of a strongly reactive outer stratum 26–33 nm thick and a less reactive inner stratum about 65 nm thick. The inner layer is clearly delimited from the outer layer by a strongly reactive boundary line and shows a thickness of 165–195 nm at the sides and reaches 400 nm in the subapical ring. In this layer usually a moderately or weakly reactive outer stratum, 200–230 nm thick, and a very thin strongly reactive inner stratum can be distinguished.

Ripening and mature ascus — While the outer layer remains constant, all further differentiation is observed in the inner layer. Especially in the apical and subapical regions this layer becomes more stratified. An initially moderately reactive inner stratum occupying the major part of the inner layer differentiates into a complex of three to four laminae of alternating degrees of reactivity. An intermediate lamina of strong reactivity becomes prominent in the apex of mature asci, but narrows down and ends in the upper part of the subapical ring.

Dehiscence mechanism — In mature asci sometimes the outer layer becomes eroded, but protrusion of the inner layer at this site is not observed (Fig. 15b). On the contrary in many mature asci a gradual disintegration and disappearance of major parts of the inner layer takes place, at first in the apex above the subapical ring, but then also in the lateral wall, leaving the subapical ring more or less intact. Finally the inner stratum of the outer layer is also weakened by disintegration. Then the thin outer layer tears open at the apex with a bilabiate split or an irregular lid, to discharge the ascus contents. The subapical ring can still be recognized in the discharged asci by the termination of the strongly reactive lamina of the inner stratum of the inner layer. After discharge disproportional swelling of parts of the inner layer is a common phenomenon.

Thelebolus crustaceus — Figs. 4a–c, 5c, d, 14h

General — Asci numerous, broadly clavate with a broad short stalk and a rounded apex, 115–140 × 40–52 µm, with 64 smooth spores; the wall not blued with iodine.

Young ascus — The primary wall is uniform and of constant thickness up to 160 nm in all parts, consisting of a strongly reactive outer stratum and a less reactive inner stratum. A periascus is not observed. On further development the primary wall becomes the outer layer of the ascus wall when a secondary wall is deposited as an inner layer against its inner face.

Lateral wall — While the ascus wall increases in thickness in the apex to 950–1125 nm, and in the subapical ring to 1125–1350 nm, it reaches only 500–675 nm laterally. The outer layer remains almost constant in thickness, 135–160 nm, with an outer stratum 14–23 nm thick and an inner stratum 110–125 nm thick. In the inner layer, 290–450 nm thick, an outer stratum of low reactivity, 325–370 nm thick, and a strongly reactive inner stratum, 65–80 nm thick, can be distinguished.

Ripening and mature ascus — Further differentiation of the ascus wall is observed in the inner layer, while the outer layer remains almost unchanged. In the apical and subapical region the inner layer becomes more stratified. An initially moderately reactive inner stratum is differentiated into three lamellae with different reactivity. The ending of an intermediate lamina of strong reactivity is clearly visible in the upper part of the subapical ring. Also a well delimited strongly reactive lamina about 75 nm thick can be distinguished in the innermost part of the inner layer. At the level of the ring increased reactivity is also observed in a narrow region of the outer stratum of the inner layer close to the outer layer. In apical and subapical regions the boundary between the inner and outer strata of the inner layer is vague.

Dehiscence mechanism — Not observed in detail with TEM.

Thelebolus polysporus — Figs. 5a, b, 15f

General — Asci 2–5 in each fruit-body, at first broadly clavate, then subellipsoid to ovoid with a large dome-shaped apex and without a distinct stalk, 80–160 × 60–90 µm, with 256 smooth spores; the wall not blued with iodine.

Young ascus — The primary wall shows a rather simple fine structure consisting of a strongly reactive outer stratum and a weakly reactive inner stratum throughout the whole ascus. During early ascosporogenesis an inner layer of weak reactivity is formed at the inner face of the primary wall. A regular periascus is not observed.

Lateral wall — During further development the primary wall becomes the outer layer and remains rather constant in thickness 140–160 nm in all parts and consists of a strongly reactive outer stratum 45–60 nm thick and an only moderately reactive inner stratum 90–115 nm thick. The inner layer with a thickness of 480–680 nm is of low reactivity at first, but later at its inner side a strongly reactive lamina, 55–70 nm thick, becomes distinct.

Ripening and mature ascus — Further differentiation is mainly restricted to the inner layer which becomes more stratified in the apical and subapical regions. A rather strongly reactive internal lamina in the apex ends in the upper part of the subapical ring. The rest of the ring is always of low reactivity and sometimes a vague lamellation is observed. While the lateral and apical wall are about 770 nm in thickness, in the ring it reaches 910 nm.

Dehiscence mechanism — Not observed in detail with TEM, but light microscopic observations show a protrusion of the inner layer trough an irregular rupture of the outer layer at the apex, and finally a rupture of the inner layer releasing the ascospores (Fig. 4a).

Thelebolus nanus

This species with a single ascus containing 1000 to 1500 ascospores was studied with methods of light microscopy and found to be very similar in structure to *Thelebolus stercoreus*. It differed mainly in the lower spore number and the sizes being smaller in all parts.

Thelebolus stercoreus — Figs. 6a–e, 15g–i

General — In each fruit-body there is usually only a single shortly ellipsoid to ovoid ascus with a large dome-shaped apex and without a stalk, 165–230 × 120–175 µm, with 2000 to over 3000 smooth spores; the wall is not blued with iodine.

Young ascus — Shortly after early mitotic divisions prior to ascosporogenesis the ascus expands and the thickness of the ascan wall increases markedly. An internal weakly reactive layer develops at the inner face of the primary wall which becomes the outer layer consisting of a strongly reactive outer stratum and a moderately reactive inner stratum. A perispore is not clearly observed.

Lateral wall — On further development the outer layer remains constant in appearance and shows a thickness of about 210 nm in the lateral and subapical region. A strongly reactive outer stratum of about 50 nm thick and a less reactive inner stratum 140–170 nm thick can be distinguished at all stages. The inner layer is still less reactive, except for a 55–70 nm thick inner stratum, and is very variable in thickness, because of extreme swelling of its outer stratum. This stratum shows a fibrillar fine structure with elements parallel to the ascus surface, from 1.2–2.5 µm thick in ripening asci reaching sometimes more than 9 µm in fully mature ones.

Ripening and mature ascus — Most differentiation is restricted to changes in the inner layer which shows changes in the thickness and also variation in stratification. Because of this the wall is only 910–980 nm in the apex and increases to more than twice this size in the subapical ring. In the apex and the upper part of the apical ring the presence of a strongly reactive inner stratum is accentuated. Both in the apical dome and laterally below the ring a separation of the outer stratum of the inner layer is observed shortly before maturity. Because of the low reactivity the fibrillar elements can be distinguished only with difficulty. In the outer and inner part of this stratum the elements are rather densely packed parallel to the wall surface. In an intervening zone of increasing thickness between these parts the elements are irregularly arranged, as if torn apart. If almost mature asci are forcibly crashed a rupture of the outer layer under the level of the subapical ring is followed by separation of the ascan wall along this zone.

Dehiscence mechanism — Shortly before full maturity the ascus increases strongly in size and the fruit-body forcibly opens, usually exposing the top of the ascus. Within the apex above the subapical ring a slightly protruding central apical dome about 45 µm across stretches. When the outer layer in the apex opens with an irregular tear the inner layer extends through an opening of about 50 µm wide. Immediately, or shortly afterwards, the inner layer also ruptures, releasing the mature ascospores. During dehiscence all parts of the inner layer may swell enormously.

Caccobius minusculus — Figs. 7a–d, 16a–f

General — Asci 10–20 in each fruit-body, subcylindrical, rather sharply tapering downwards into a short stalk, with a rounded or somewhat conical top, with 500–1000 smooth spores. The wall not staining in iodine; before maturity a central thickened apical part of the inner layer stains with Waterman's blue-black ink.

Young ascus — The wall is already thick from the earliest stages studied. Before ascosporogenesis the thickness is rather uniform, 1.6–2.1 µm in the lateral and subapical regions, but increases in the apex up to 3.0 µm. The outer layer, 220–240 nm thick, is at

first rather vaguely delimited, but becomes more distinct later, especially in the apex, by increased reactivity. The inner layer, 2.3–2.8 µm thick, shows low reactivity, except for a narrow zone in the apex at about 160 nm below the outer layer. At this early stage a conspicuous thickening develops in the central part of the apex in the inner layer. This central thickening may reach 3.6–6.0 µm in width and is sharply delimited by folds in the subapical region. At first it shows little or no differentiation, but during early ascosporogenesis it becomes clearly stratified by the presence of three to four strata of alternating reactivity. On further ripening the stratification becomes less evident, but can still be seen as fine horizontal lines. A perispore is not observed at very young stages, but from early ascosporogenesis onwards a more or less continuous or fragmented perispore, 200–1000 nm thick, of reticulate structure and moderate reactivity is present.

Lateral wall — The outer layer, 210–240 nm thick, consists of a strongly reactive outer stratum 35–50 nm thick and a moderately reactive inner stratum 160–185 nm thick. The inner layer, at first 2.3–2.9 µm thick, decreases in size after stretching of the ascus to 1.6–1.9 µm, and is of low reactivity with a subtle but clear stratification as a continuation of strata in the apical and subapical regions. In the subapical region no evidence of a subapical ring could be found.

Ripening and mature ascus — Differentiation is mainly due to changes in the inner layer. At maturity this layer becomes folded in the subapical region. When the ascus stretches the fibrillar structure of the inner layer becomes evident.

Dehiscence mechanism — At maturity the outer layer of the ascus together with the outer non-reactive stratum of the inner layer split and open usually at the centrum of the apex. The central thickening of the inner layer bulges out through this opening, exposing its thin reactive stratum. The opening remains rather restricted, 5–7 µm, just wide enough to expose the central thickening. Later the inner layer breaks along the deep folds bordering the thickening and the ascus contents are released.

Here it was observed that the structural and functional inner and outer layer of the ascus wall do not fully correspond with each other. The separation takes place within the inner layer close to the boundary plane of its outer and middle stratum.

Lasiobolus pilosus — Figs. 8a–d, 16g, h

General — Asci numerous, cylindric-clavate with a short stalk and a rounded or slightly flattened apex, 159–300 × 15–35 µm, with 8 smooth ascospores; the wall not staining blue with iodine.

Young ascus — The wall is rather undifferentiated and of constant thickness (140–170 nm) in both lateral and apical parts; consisting of a reactive outer stratum, 55–70 nm thick, and an inner stratum of low reactivity, 84–100 nm thick.

Lateral wall — On further development the bilayered nature of the ascus wall becomes obvious. An outer layer (140–180 nm) and an inner layer (420–490 nm) can be distinguished, each with an outer and an inner stratum. In the subapical region the inner wall increases in thickness towards the apex up to 700 nm.

Ripening and mature ascus — The outer layer remains constant in thickness and reactivity, and a strongly reactive periascus can be distinguished on its outer surface. S condary wall material appears to have been deposited on the inner face of the existing ascus wall. This is especially obvious in the apical and subapical regions with the presence of an

often strongly reactive, fibrillose inner stratum of the inner layer, reaching a thickness of 420 nm in the apex, but gradually diminishing in thickness in the subapical region, down to 40 nm. In the transitional zone between the subapical region and the apex this inner stratum becomes clearly undulated or folded, except for an apical, central flat region, the future operculum. On the other hand the outer stratum of the inner layer, which is moderately reactive, reaches its maximal thickness in the subapical region. This annular thickening corresponds both in position and structure with the subapical ring in other species. In this laminated outer stratum three to five strata may be distinguished after chemical fixation and Thiéry reaction.

Dehiscence mechanism — The opening is by an operculum at the top. The parting of the operculum from the ascostome is not a single clear fracturing line. In the inner part the fracture is at the sharp demarcation between the flat and the undulating regions of the inner stratum of the inner layer. In the outer part the fracture is at the line where the outer stratum of the inner layer abruptly diminishes in thickness. In the rather wide zone of overlap (1.4–1.7 µm) between both parts of the operculum separation of the wall layers can already be observed before the opening. This separation is located at the external side of the inner stratum of the inner layer.

Lasiobolus cuniculi — Figs. 9a–c, 16i, j

Fixed with rapid cryo fixation, followed by freeze substitution and treatment with the Thiéry technique.

General — Asci numerous, broadly clavate with a broad base and a short stalk and rounded above, finally often slightly flattened, 120–210 × 20–32 µm, with 8 smooth ascospores; the wall not staining blue with iodine.

Young ascus — Not observed with TEM.

Lateral wall — The outer layer, 106–115 nm thick, consists of a strongly reactive outer stratum (46–55 nm) and a weakly reactive
inner stratum (55–64 nm). The inner layer is 46–55 nm thick in the lower part. With this method of fixation the stratification of the inner layer is less evident than after chemical fixation. In the subapical region the outer stratum of the inner layer increases considerably in thickness, up to 830 nm, at the level of the subapical ring.

Ripening and mature ascus — The outer layer remains constant in thickness and in contrast; a reactive periascus is present on its outer surface. An inner layer, consisting of two strata, appears to have been deposited on the internal face of the ascus wall. Its outer stratum is moderately reactive and becomes weakly stratified in the apex and in the upper part of the apical wall. Its inner stratum is thickest in the apex, up to 420 nm, and becomes considerably thinner in the lateral wall towards the base (less than 42 nm), where it becomes almost indistinguishable. In the transitional zone between the subapical region and the apex it becomes strongly undulated or even folded.

Dehiscence mechanism — The opening is by an apical operculum. The sharp fracturing line in the inner stratum of the inner layer is at the well marked transition between the flat and the undulated regions in the apex. While the fracture in the outer part is just above the subapical ring at the level of the outer delimitation of the undulate region of the inner stratum. In the operculum the upper part (diam. c. 24 µm) may exceed the lower part (diam. c. 12.5 µm) considerably on all sides (about 6 µm).

Lasiobolus monascus — Fig. 16k

This description is based on earlier observations (van Brummelen, 1984) with glutaraldehyde/OsO_4 fixation and contrasting with uranyl and lead and on illustrations published by Kimbrough & Benny (1978) with silver methenamine contrasting.

General — In each fruit-body only a single ovoid to ellipsoid ascus without a stalk and with a rounded or somewhat flattened apex, 270–400 × 170–250 µm, with over 1000 smooth ascospores; the wall not staining blue with iodine.

Young ascus — The wall is almost undifferentiated and uniform, 1080–1190 nm thick in the lateral part and only 750–860 nm in the apical part, consisting of a reactive outer stratum (c. 215 nm thick) and an inner stratum of low reactivity, 540–860 nm thick.

Lateral wall — The outer layer remains almost constant in thickness and reactivity. A periascus is not observed. On further differentiation of the ascus wall an inner layer is formed at the inner face of the already existing outer layer. Practically all further changes in the ascus wall are due to changes in thickness and differentiation in the inner layer. In this layer a strongly reactive inner and an only moderately reactive outer stratum can be distinguished. Within this outer stratum often a finer stratification can often be observed and, especially in the subapical region, a considerable increase in thickness to more than 2500 nm forms a subapical ring of low reactivity.

Ripening and mature ascus — At the inner side of the inner layer an inner stratum of strongly reactive, parallel fibrillar elements reaches a thickness of 1550 nm in the apex, but gradually decreases downwards till it becomes almost indistinguishable in the lower lateral wall. In the transitional zone between the suboperculate region and the apex this stratum becomes strongly undulate. Shortly before dehiscence the future line of fracturing can be observed at the delimitation of the flat and the undulated region of this inner stratum (cf. Kimbrough & Benny, 1978: fig. 34).

Dehiscence mechanism — Not observed in detail with TEM.

Ascozonus woolhopensis — Figs. 10a, b, 17a, b

General — Asci numerous, at first broadly clavate, then more slenderly clavate with a flattened top, finally clavate with a conical top ('cigar-shaped') gradually tapering downwards into a rather broad base, 100–150 × 20–30 µm, with 64 fusiform smooth spores; the wall not staining with iodine.

Young ascus — Before early ascosporogenesis the wall is almost undifferentiated and of constant thickness, 172–280 nm in the lateral and apical parts, consisting of a strongly reactive outer stratum 45–65 nm thick and an only weakly reactive inner stratum 120–220 nm thick. A strongly reactive periascus 170–260 nm thick is observed from the beginning, later it becomes thinner, c. 70 nm, and may become partly loosened from the ascus wall.

Lateral wall — Shortly after the beginning of meiosis a secondary wall can be distinguished at the inner face of the outer layer and a conspicuous ring-shaped thickening is formed subapically. The outer layer 190–210 nm thick, consisting of a strongly reactive outer stratum 45–65 nm thick and a less reactive inner stratum. The inner layer is moderately reactive and not clearly stratified below the level of the ring, except for a 45–65 nm wide uniform, strongly reactive inner stratum. In the subapical region the inner layer strongly increases in thickness to 600–925 nm to form a ring that may reach a width of 1.4 µm and a height of 3.1–4.4 µm. The reactivity in the inner layer varies from low to

moderate and four strata of different reactivity may be distinguished in the ring and subapically; in the extreme apex only two strata are seen.

Ripening and mature ascus — After karyogamy the apical wall increases strongly in thickness and may reach 1000 nm, but on further ripening it decreases to a thickness of about 490 nm. At maturity the top above the ring becomes more or less conical in shape with a small flattened central region. In the apex the outer stratum of the outer layer remains constant at 45–60 nm, while the inner stratum decreases in thickness towards the apex from 138 nm near the ring to 45 nm in the central flattened part.

Dehiscence mechanism — Shortly before dehiscence the ascus stretches strongly and the wall in the apex becomes thinner. Weakening of the inner layer is observed in the central part by breaking down of wall material. An apical disk or operculum 2.5–3.0 µm in diameter is differentiated by decreased thickness of the outer layer and a more dense and granular structure of the inner layer as opposed to a fibrillar structure of the bordering wall. At dehiscence the operculum tears loose along a circular line and initiates from there downwards a bilabiate split of the apex above the thick resistant ring and the ascus contents are forcibly ejected.

Ascozonus solms-laubachii (see page 432) — Figs. 10c–e, 17c, d

General — Asci numerous, at first broadly clavate with a somewhat flattened top, finally more slenderly clavate with a conical top ('cigar-shaped') gradually tapering downwards into a rather broad base, 80–120 × 22–25 µm, with 32 fusiform smooth spores, 12–13 × 3–4 µm; the wall not blued with iodine.

The ultrastructure and the dehiscence of the ascus are found to be exactly the same as described above for *Ascozonus woolhopensis*, but certain stages, just at the beginning of the opening of the operculum, were only observed in this species (Fig. 10c, d).

This species, as *Streptotheca boudieri* Vuill., was also studied by Vuillemin (1887) He described the structure and dehiscence mechanism of the ascus with the characteristic annular thickening and the small operculum at the apex.

Ramgea annulispora — Figs. 11a–d, 17h–j

General — Asci numerous, broadly clavate with a short broad stalk and a dome-shaped apex, 27–35 × 6.7–9.0 µm, with 8 ornamented ascospores; the wall not staining blue with iodine.

Young ascus — Not studied with TEM.

Lateral wall — The outer layer, 78–90 nm thick, consists of a thin strongly reactive outer stratum and a less reactive inner stratum. The inner layer, 210–235 nm thick, shows no clear stratification and is moderately reactive.

Subapical region — While the outer layer remains almost constant in thickness over the whole ascus, the inner layer increases rather abruptly in thickness, up to 670–750 nm, in the subapical and apical regions.

Immature ascus — The inner layer occupies the major part of the apex and shows little internal differentiation. The subapical annulus, which was observed by light microscopy and interference contrast (van Brummelen, 1992, figs. 2 c, d) could not be verified with electron microscopy.

Mature ascus — Without significant changes. Only in the transitional region between the lateral and the apical part of the wall the inner layer is often undulated or folded.

Dehiscence mechanism — The central region of the apex is weakened and opens either subcentrally or with an irregular tear. Within the inner layer a separation of strata is observed. The outer layer is partly eroded. The protrusion of the expanding inner layer through the outer layer is restricted to a zone 1.4–1.9 µm wide. At this stage a central part of the inner stratum in the inner layer shows increased reactivity.

By light microscopy it was observed (van Brummelen, 1992), that the outer layer stains with Congo red and that in the inner layer a small central zone at the apex stains with Watermann's blue-black ink.

Coprotus lacteus — Figs. 12a–e, 17e–g

General — Asci numerous, cylindric-clavate with a short stalk and a rounded or somewhat flattened apex, 70–110 × 15–20 µm, with eight smooth ascospores; the wall not staining blue with iodine.

Young ascus — Not observed.

Lateral wall — At the later stages the wall is rather constant in thickness 420–480 nm in the lower part and reaching 580 nm in the subapical region. It consists of an outer layer of low reactivity 400–520 nm thick and an inner more reactive layer 45–50 nm thick below and 90–210 nm subapically. The inner layer is clearly separated by a reactive boundary line from the outer layer, but internally is not stratified.

Ripening and mature ascus — The outer layer remains constant in thickness c. 469 nm and low reactivity, only in the apical and subapical regions a thin outer stratum, 29–35 nm thick, of moderate reactivity can be distinguished. A strongly reactive floccose periascus, 85–175 nm thick, covers its outer surface, especially in the upper part of the ascus. The inner layer which is not clearly differentiated in an inner and outer stratum, is most evident in the apex 230–275 nm thick and in the subapical region where it gradually diminishes in thickness to about 45 nm. In the transitional zone between the subapical region and the apex the inner layer becomes clearly undulated or folded, except for an apical, central flat region, the future operculum. Only a slight annular thickening can be observed in the subapical wall region of the mature ascus. No differentiation of the wall is found at the level where in species of Pyronemataceae a subapical ring is formed.

Dehiscence mechanism — The dehiscence is by an apical operculum regularly shaped and about 10 µm wide. The fracture starts at the inner face of the inner layer at a rather sharp demarcation between the flat and undulated regions. Often in mature asci a fissure can be observed in the inner layer at the apical side of a less swollen region c. 300–500 nm wide with a more reactive inner face (Fig. 12b–e, between arrow-heads). The weakened zone for fracturing in the outer layer is close to the fissure in the inner layer. The fracturing lines in both layers are close together, thus producing relatively smooth margins at the operculum and the ascostome. Although the folding of the inner layer in the subapical region is considerable, a separation of both wall layers in that region is not observed. After dehiscence the ascus wall near the ascostome swells considerably (Fig. 12e).

Trichobolus zukalii — Figs. 13a–e

Our observations on this species agree for the main part with those of Heimerl (1889), Kimbrough (1966a), and Samuelson & Kimbrough (1978b). But the dehiscence of the ascus in this species can only be observed with great difficulty (cf. Heimerl, 1889).

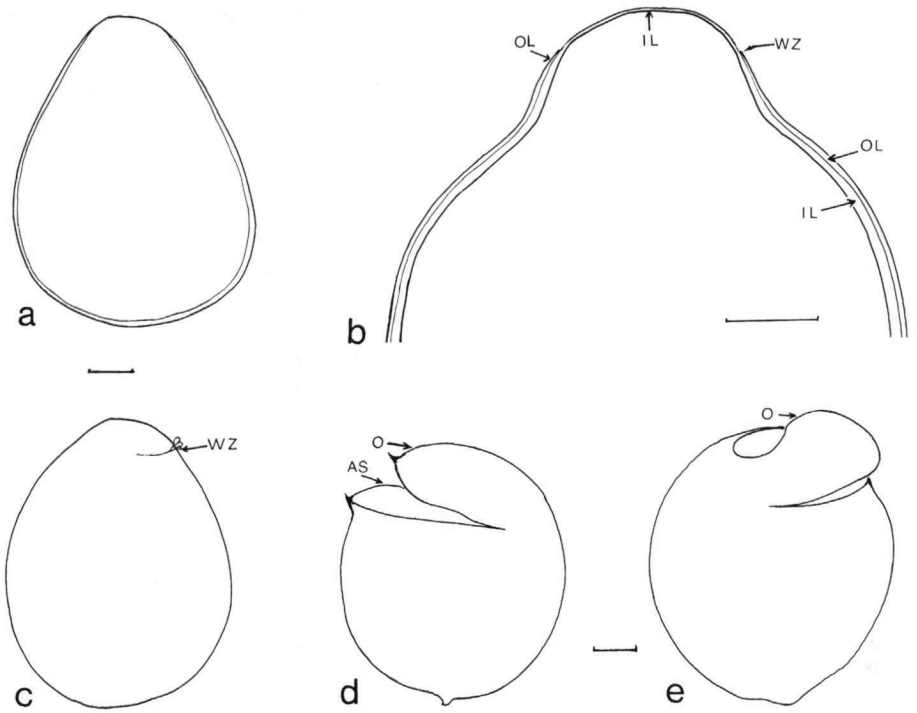


Fig. 13. *Trichobolus zukalii*, ascus development viewed by light microscopy. a. Mature ascus. b. Idem, detail, showing discontinuity of outer wall layer in the top. c. Beginning of opening along circular line. d, e. dehisced asci showing a large operculum. All scale markers represent 50 μm.

Apparently fully mature asci remain intact for many hours or stop their development without opening at all, while on the other hand, probably dependant on external conditions, asci suddenly release all spores. The empty ascus immediately collapses and contracts within the fruit-body, leaving no opportunity for adequate observations. Methods of fixation used for electron microscopy also cause fully mature asci to collapse. The illustrations of ascus wall ultrastructure presented by Samuelson & Kimbrough (1978b: figs. 8 –18) do not show fully mature stages, since much epiplasm is still present in the asci.

By using light microscopy combined with interference contrast or staining with Congo red the ripening and dehiscence of the ascus could be studied in a great number of asci.

In asci that could ripen and open undisturbed, without pressure from outside, all opened with a very regular and sharply delimited operculum of more than 100 μm diameter (Figs. 13d, e; cf. Kimbrough, 1966a: fig. 1h; Samuelson & Kimbrough, 1978b: fig. 7). In the last phase of ripening the protruding top of the ascus gradually diminishes in thickness. The decrease in thickness is observed both in the inner and the outer layer, but is most extreme in the outer one. At a certain moment during stretching of the wall in the top the outer layer no longer follows the inner layer and remains behind, leaving the central part free. A very sharp and regular circular border of the thin outer layer is observed. On

further increase of the ascus turgor the opening of the top starts exactly along this circular line. A movement of the inner and outer layer relative to each other is not clearly observed. Near the line of dehiscence no indentation of the wall, nor any indication of a subapical ring is present. In mature ascospores no oil-drops or air-bubbles were found.

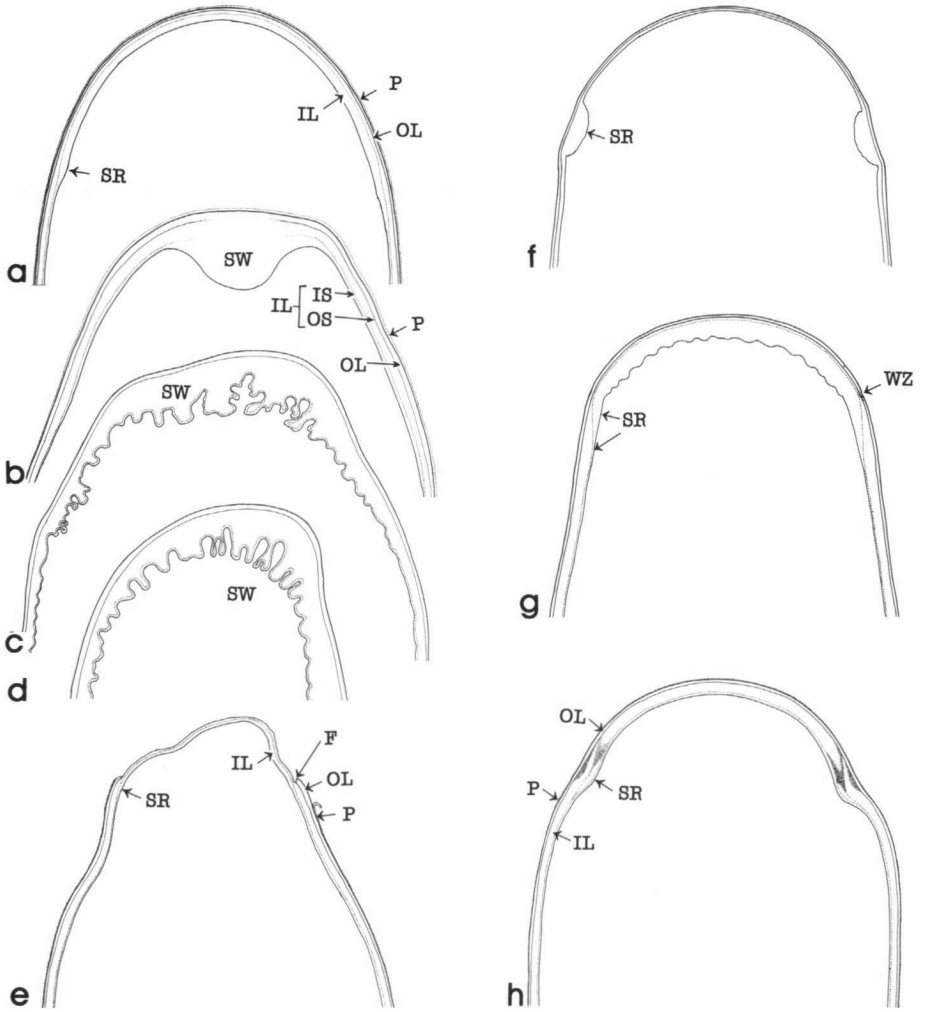


Fig. 14. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Figs. a–e. *Thelebolus microsporus* (a, b, fixed by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique; c–e, idem, but fixed in 1% glutaraldehyde). a. Almost mature ascus. b. Ripening ascus with strongly thickened inner layer. c, d. Mature asci with strongly swollen and undulating inner layer. e. Dehiscing ascus with ruptured outer layer and protruding inner layer at the top. — Figs. f, g. *Thelebolus coemansii*. f. Very young ascus, before karyogamy, showing deposition of wall material at the level of the future subapical ring. g. Mature ascus, with weakening of the wall beginning. — Fig. h. *Thelebolus crustaceus*, almost mature ascus.

DISCUSSION

The results obtained with different methods of fixation and contrasting were compared and not found to be contradictory. The combination of rapid freeze fixation and freeze substitution followed by the test for polysaccharides with the Thiéry technique proved to be especially valuable for the study of thin median sections of asci. The images obtained with this method were not, or scarcely, affected by local and sometimes disproportional swelling of wall material as sometimes occurs with methods of chemical fixation.

The seven species of *Thelebolus* included in this study show a very similar structure of the ascus top.

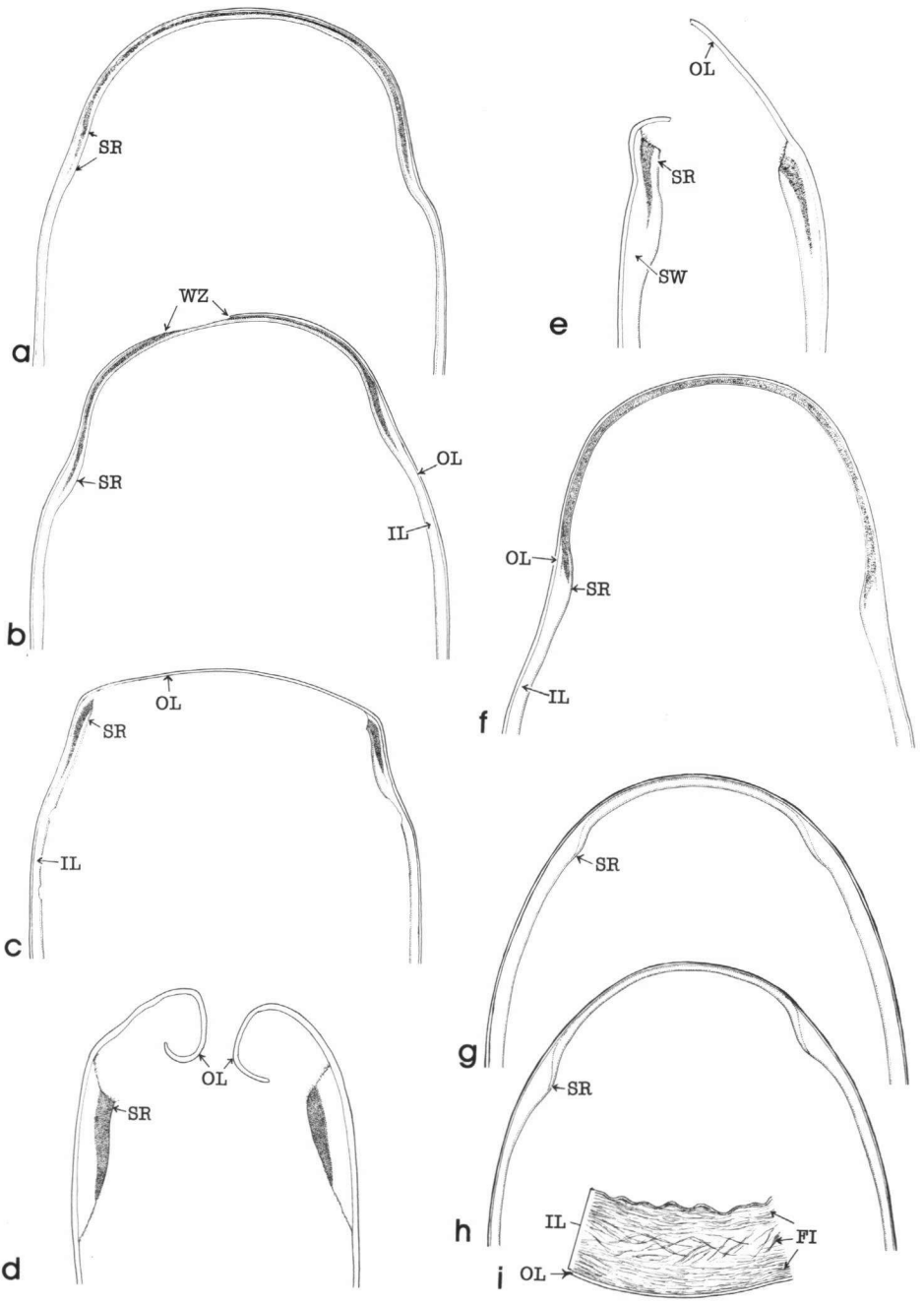
At the earliest stages the ascus shows only a single thin wall layer, which is already differentiated into an inner and an outer stratum. It is then that wall material is deposited at the inner face of the wall at the level of the future subapical ring (Fig. 2a), with a process very similar to that observed for *Pyronema* (Reeves, 1967) and many other representatives of the Pyronemataceae s.l. (van Brummelen, 1978; Samuelson, 1978b). Soon afterwards the activity at the subapical ring becomes far less evident and an inner (secondary) layer is deposited at the inner face of the primary outer layer. This two-layered wall with a subapical ring is common to most members of the Pezizales so far studied and had already been described by Boedijn (1933) and Chadefaud (1942). The localized thickening, observed at the ascus top of *Thelebolus crustaceus* by Czymmek & Klomparens (1992: fig. 18) represents a rather advanced state in the development of the apical apparatus.

The outer layer remains thin and constant in appearance. When viewed by light microscopy the outer layer in *Thelebolus* stains in all regions with Congo red (Fig. 5c), this contradicts observations by Kimbrough (1966b) and Kimbrough & Korf (1967), who described hyaline ascus apices after this staining above the subapical ring and used this as a distinguishing character for the genus *Thelebolus*. Ultrastructural observations after application of the chemically more specific Thiéry technique demonstrates the outer stratum of the outer layer with strong reactivity, while the inner stratum reacts only moderately.

As a rule the outer stratum of the inner layer shows a low reactivity and the inner stratum a moderate to strong reactivity. These differences in reactivity allow the different layers and strata to be distinguished and followed, especially when cut exactly perpendicular to the boundary planes.

A distinction should be made between the coherence of layers and strata above and below the subapical ring.

In the fungi of this study all changes in the ascus apex take part in the inner layer. This layer is flexible and usually much thicker than the more rigid outer layer. Often in the thicker parts sublayering can be observed, where slight differences in density or reactivity or differences in the main direction of the microfibrils occur. It is important to realise that very considerable differences in wall thickness may occur due to swelling of the inner layer. An example is the ascus wall thickness in *T. stercoreus*, which normally is 1.5–2.0 µm below and about 900 nm above the subapical ring (Fig. 6c, d), but may reach 12 µm below and 8 µm above this ring after swelling (Fig. 6e; Samuelson & Kimbrough, 1978a: fig. 45). In such swollen walls microfibrils can be observed with low to moderate reactivity.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In the multi-spored species, *T. polysporus* and *T. stercoreus*, the inner layer below the subapical ring may show an inner and outer region with densely placed fibrils more or less parallel to the surface separated by a less dense region with obliquely or irregularly arranged fibrils (Figs. 6e, 15i). When mature asci are pressed or crushed in light microscopic preparations, the outer layer may break at a place below the level of the subapical ring causing the inner layer to separate along this less dense region. When this is followed by a considerable extension of the elastic and often somewhat folded inner region of the inner layer this resembles the 'Jack in the box' opening mechanism of asci, generally considered to be typical of ascomycetes belonging to the 'Ascoloculares'. This phenomenon is most common in multi-spored species of *Thelebolus* (cf. van Brummelen, 1978, fig. 36) but has also been observed, to a less extent, in crushed asci of e.g. *Scutellinia.*

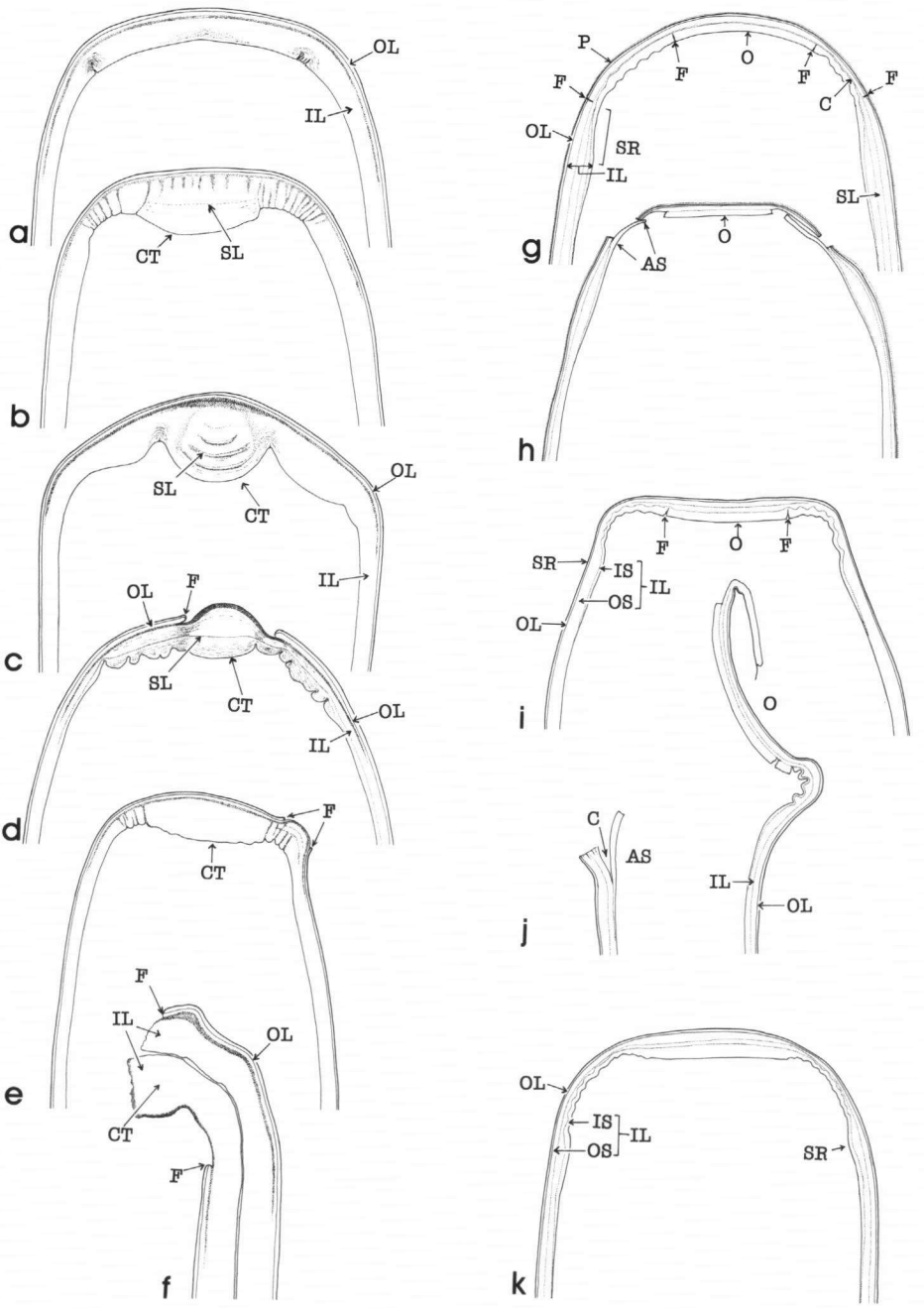
The banded appearance of the internal region of the inner layer in *T. stercoreus* was considered of great importance for the taxonomy of that genus by Samuelson & Kimbrough (1978a), since this was considered an important criterium for the 'bitunicate ascus' (Reynolds, 1971; 1989). When, however, the structure of the 'bitunicate ascus' in the Pyrenomycetes was more clearly presented (Parguey-Leduc & Janex-Favre, 1982), more characters appeared to be involved. The fibrils of the inner layer in this type of ascus are at first undulated, but soon afterwards they fold into an often rather sharp-edged zig-zag pattern. The superficial banded pattern in *T. stercoreus*, observed by Samuelson & Kimbrough (1978a), is quite different, but corresponds well with the strongly reactive fibrils often observed at the undulated or folded inner surface of the inner layer of the subapical ascus wall in species of *Thelebolus*, *Lasiobolus*, and *Ascozonus.*

Although the structure of the ascus in species of *Thelebolus* is very similar, different mechanisms of ascus dehiscence occur, sometimes even in the same species. There are three main types: (1) Proliferation of the inner layer through an opening in the outer layer is observed in *T. microsporus*, *T. crustaceus*, and *T. stercoreus*. This mechanism is considered the most typical for the genus *Thelebolus*. (2) Weakening and breakdown of the inner layer in the apex, except for the subapical ring, is found in *T. caninus* and *T. polysporus*. (3) Opening at the top by an irregularly shaped operculum just above the subapical ring may occur in the 8-spored species, *T. coemansii* and *T. microsporus*. But a flat and rigid differentiation of the inner layer, as present in typical operculates, is not present in these asci.

This supports earlier views (van Brummelen, 1978; 1994a; Bellemère, 1994) that ascus structure is more important for the taxonomy than the actual dehiscence mechanism.

The typical *Thelebolus* type shows: a subapical ring which is very pronounced in multi-spored species and much less so in 8-spored ones; a splitting of the inner layer above the subapical ring, beginning from the top downwards; a thick inner layer which tends to imbibe water and form a central thickening or accumulation of folded or undulated wall material in the whole top region. A tract or a funiculus are absent. The periascus is thin and rarely preserved.

Fig. 15. Diagrammatic sections of ascus tops, as seen with electron microscopy. Figs. a–e. *Thelebolus caninus*. a. Ripening ascus. b. Mature ascus with eroded outer layer. c. Fully mature ascus with break down of inner layer of ascus wall. d, e. Dehisced ascus showing persisting inner layer at subapical ring. — Fig. f. *Thelebolus polysporus*, mature ascus. — Figs. g–i. *Thelebolus stercoreus*. g. Immature ascus. h. Mature ascus. i. Detail of ascus wall near base, showing sublayering of inner layer.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The asci of *Caccobius*, *Ramgea*, and *Pseudascozonus* also belong to the same type, since central thickenings of different shapes are present in the inner layer at the top and a weakening occurs by splitting within the inner layer parallel to the wall surface, from the top downwards. In all cases the outer layer erodes and opens more or less irregularly allowing the swollen inner layer to extend and break just beside the central thickening.

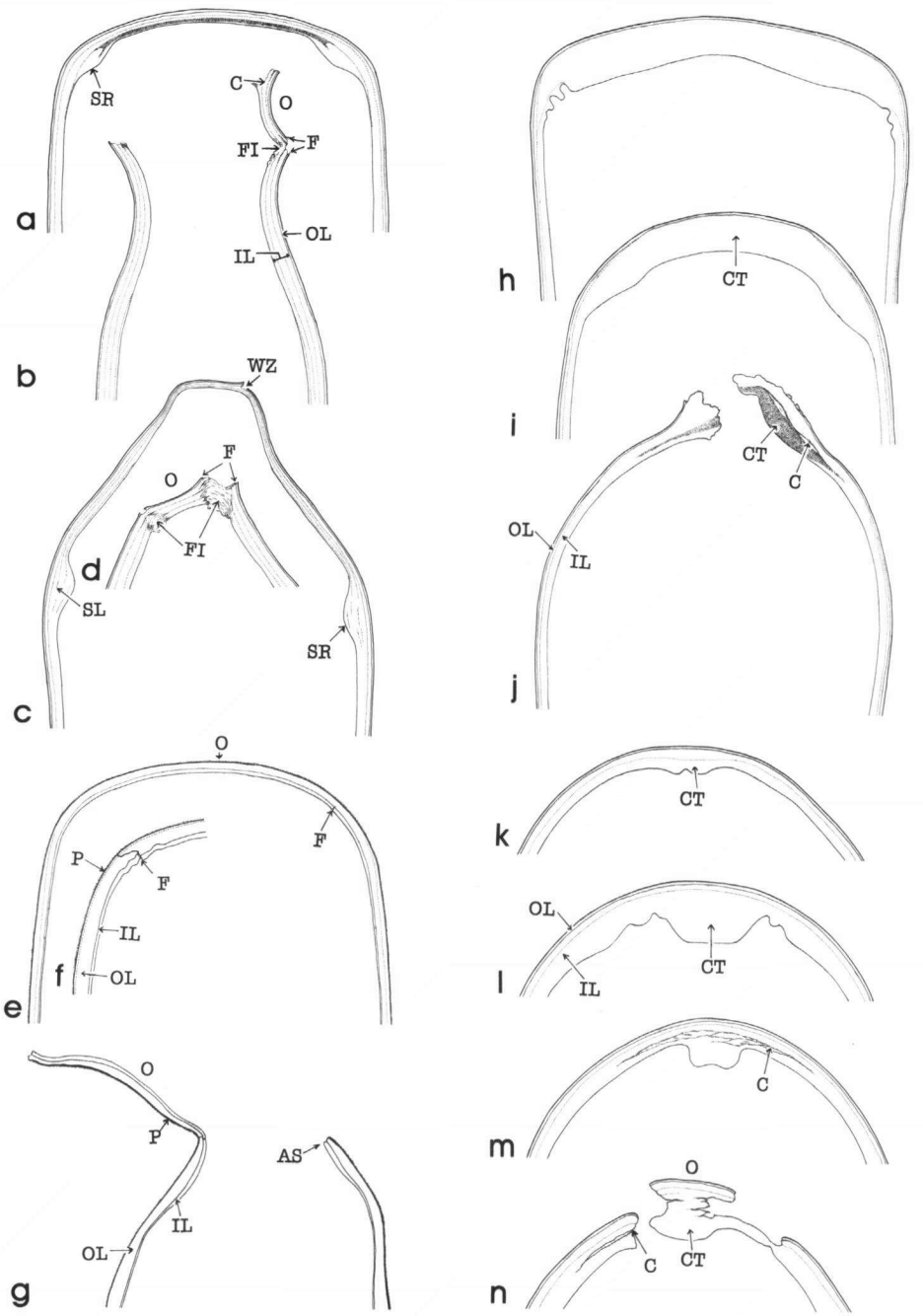
Sublayering of the inner layer is observed in the central thickening over a varying period. When treated with Waterman's blue-black ink during this period fine lines and cavities are stained blue. The staining with this dye, reported for the study of asci as a 'chitinoid' stain (Kimbrough & Korf, 1967; Chadefaud, 1973) is little documented, and chemically not very specific.

Although the dehisced ascus in *Lasiobolus monascus* was not studied with the same methods as in *L. pilosus* and *L. cuniculi*, the illustrations available strongly indicate that the structure and the morphology are the same. Characteristic of all three species of *Lasiobolus* is the wide zone of overlap between the lines of dehiscence in the inner and outer part of the operculum. The lines of fracturing in both parts are exactly predictable from the structure. The perpendicular plane of fracturing in the inner part seems to be preformed. The wrinkled or undulated region in the inner layer clearly underlines the partly independent bi-layered nature of the ascus in *Lasiobolus*. The plane of the splitting is not exactly between the structurally distinguished inner and outer layers, but within the outer stratum of the inner layer, which corresponds with Bellemère's (1977) 'couche c'. The inner stratum of the inner layer can easily be distinguished by its strong reactivity.

The structure of the ascus in *Coprotus lacteus* strongly resembles that of *Lasiobolus* and agrees with earlier results by Samuelson (1978c), except that in *Coprotus* the fracturing lines in the inner and outer part of the operculum are nearer together than in *Lasiobolus*. The rather roughly delimited strengthened operculum, without indentation or a prominent subapical ring, and the insensitivity of the wall to iodine, both indicate the *Octospora* type of ascus, which is characteristic of the large family Pyronemataceae. This also agrees with Samuelson's conclusion that according to ascal structure *Pyronema* and *Coprotus* are most closely related to the Otideaceae and Aleuriaceae sensu Kimbrough (1970).

The two species of *Ascozonus* studied show a very constant and remarkable structure and dehiscence mechanism. The ascus opens by a very small, usually somewhat concave operculum or apical disk (cf. van Brummelen, 1974). Since the opening is too narrow to allow the passage of the spore mass in the mature ascus top, a bilabiate split from the margin of the opercular opening down to a very thick and rigid subapical ring occurs during dehiscence. The inner layer is markedly fibrillar especially near the margin of the operculum. The inner layer in the central part of the operculum is less reactive and more rigid then surrounding parts (Figs. 10b, e).

Fig. 16. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Figs. a–f. *Caccobius minusculus*. a. Very young ascus, before karyogamy. b, c. Young stages, before ascosporogenesis, showing development of an apical thickening in the inner layer. d. Mature ascus with apical rupture of outer layer. e. Mature ascus with subapical opening of outer layer. f. Top of dehisced ascus. — Figs. g, h. *Lasiobolus pilosus*. g. Mature ascus. h. Dehisced ascus. — Figs. i, j. *Lasiobolus cuniculi*. i. Mature ascus. j. Dehisced ascus. — Fig. k. *Lasiobolus monascus*, mature ascus, reconstructed from Kimbrough & Benny (1978: figs. 23–35).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A distinct *Ascozonus* type of ascus top structure is defined by a very pronounced, sublayered, rigid subapical ring and a conical apex ending in a small, flat, or usually somewhat concave rigid operculum with a fibrillar margin. A considerably swollen inner wall is sometimes present (van Brummelen, 1974). And a rather thin periascus not staining blue with iodine. A funiculus is absent and mature ascospores are typically fusoid.

This type differs from the *Octospora* type (above and van Brummelen, 1978) by the obvious, permanent subapical ring, the less well defined operculum, the absence of separation in the layers at the margins of operculum and ascostome and the absence of a tract and funiculus. From the *Thelebolus* type, with which it was previously linked (van Brummelen, 1978), it essentially differs in the absence of a process of separation of wall layers from the centre of the apex downwards.

Trichobolus zukalii is a very remarkable fungus with the largest known operculate ascus containing up to 7000 spores. The ascus is unique in structure and dehiscence and as it lacks the fundamental elements of an apical apparatus it is difficult to compare with the other types of asci already described for the Pezizales. The *Trichobolus* type is characterised by the absence of a subapical ring, annular indentations, preformed weakness zones, a periascus and a funiculus. Shortly before maturity the inner layer and outer layer both decrease in thickness at the apex. At full maturity in the free top of the ascus the outer layer remains behind during stretching, leaving a circular central part free. The inner layer may slightly protrude. Then, at a certain moment, the inner layer starts to tear open along the sharp circular border of the outer layer.

The eight-spored species, *T. octosporus* (Krug, 1973), should possess unicellular hyaline excipular hairs and operculate asci with a definite apical ring, but a study of the type specimen (TRTC 43801) failed to reveal these characters. So the relationship of this species could not be cleared, but is certainly not with *Trichobolus* or *Lasiobolus* as suggested by Samuelson & Kimbrough (1978b).

A process of opening similar to that in *Trichobolus zukalii* occurs also in *Leptokalpion albicans* with a single large ovoid ascus containing about 4000 spores (van Brummelen, 1977). Here the circular shape of the opening at the top of the paragymnohymenial ascomata strongly restricts the place and shape of the broadly attached operculum, while bilabiate splits can also occur.

Fig. 17. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Figs. a, b. *Ascozonus woolhopensis*. a. Young ascus, before ascosporogenesis. b. Detail of extreme top of dehisced ascus with operculum. — Figs. c, d. *Ascozonus solms-laubachii*. c. Top of mature ascus, showing beginning of rupture of outer layer. b. Detail of extreme ascus top during dehiscence. — Figs. e–g. *Coprotus lacteus*. e. Mature ascus. f. Detail of transition between subapical and apical regions, showing beginning of fracturing. g. Dehisced ascus. — Figs. h–j. *Ramgea annulispora*. h. Ripening ascus. i. Mature ascus. j. Dehisced ascus. — Figs. k–n. *Pseudascozonus racemosporus* (fixed in 1% glutaraldehyde and 1% OsO_4 and stained with lead citrate and uranyl acetate). k. Young ascus. l. Ripening ascus. m. Mature ascus. n. Dehisced ascus.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. W. Star for his most skilful technical assistance throughout this study. Gratitude is due to the directors of the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands and the Cryptogamic Herbarium, Department of Botany, University of Toronto (TRTC), Canada for disposal of culture collections and the loan of herbarium specimens. A great debt of gratitude is due to Mrs. Dr. S.M. Francis for reading the manuscript and improvement of the English text.

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