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RECONSIDERATION OF RELATIONSHIPS WITHIN THE THELEBOLACEAE BASED ON ASCUS ULTRASTRUCTURE

J. VAN BRUMMELEN

Rijksherbarium/Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands

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.

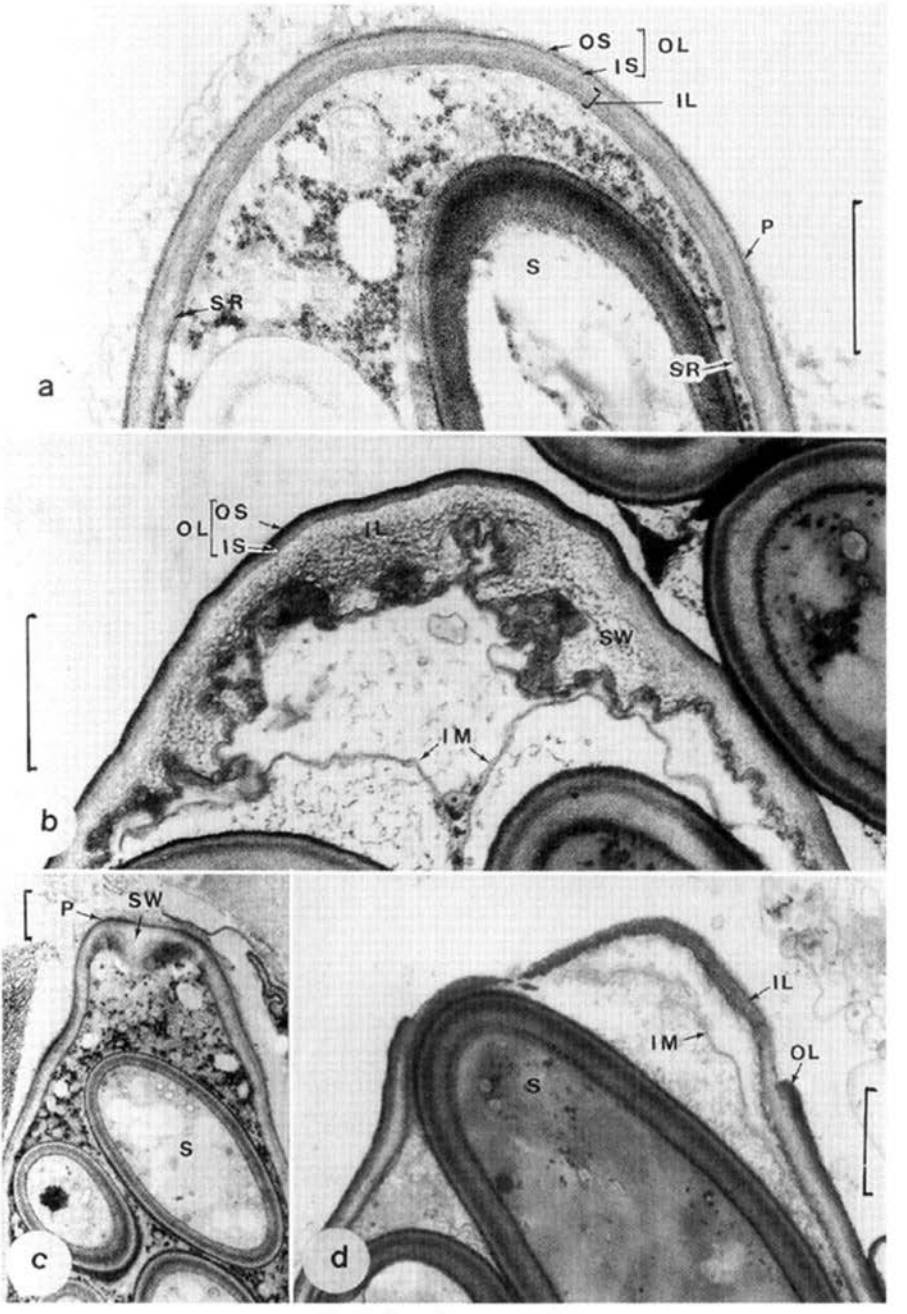
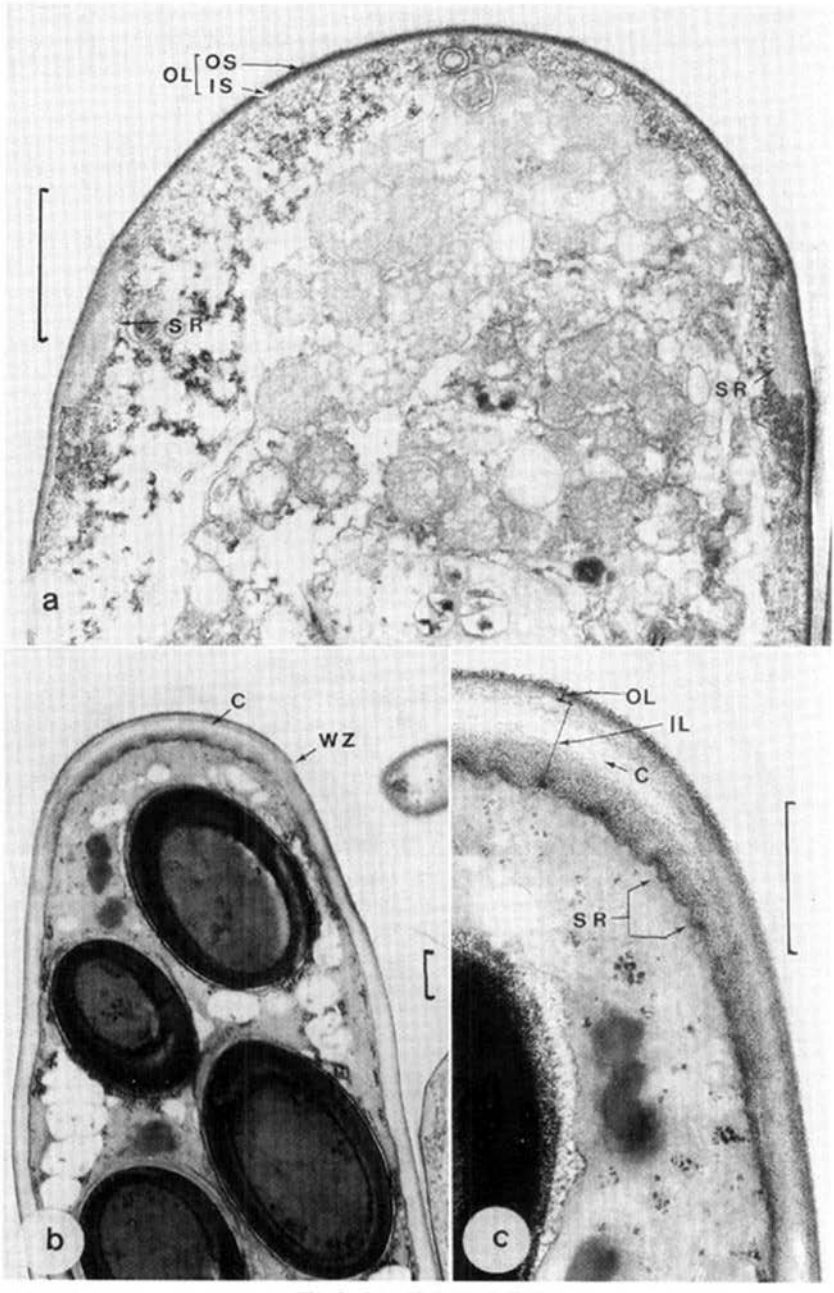


Fig. 1 (legend on page 433)



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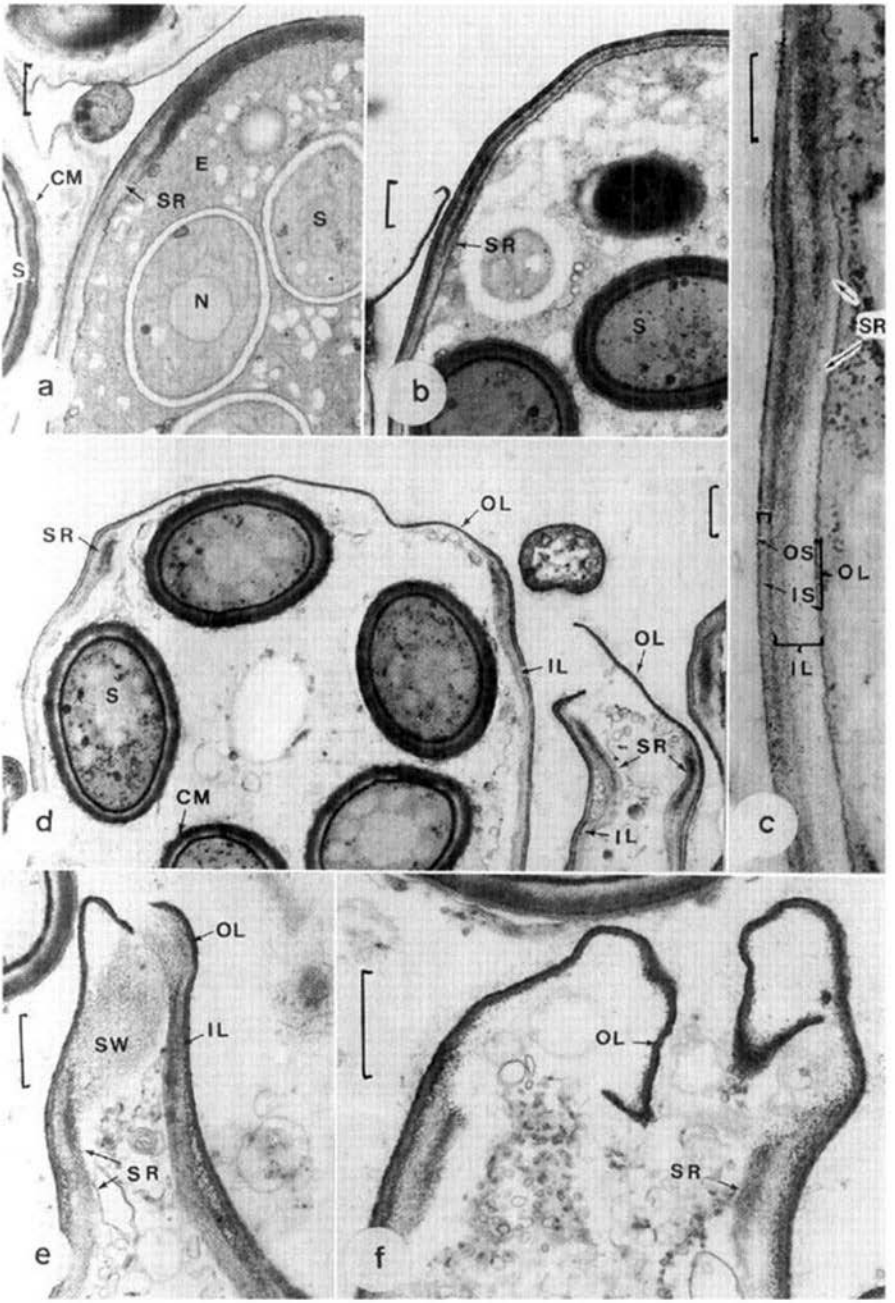

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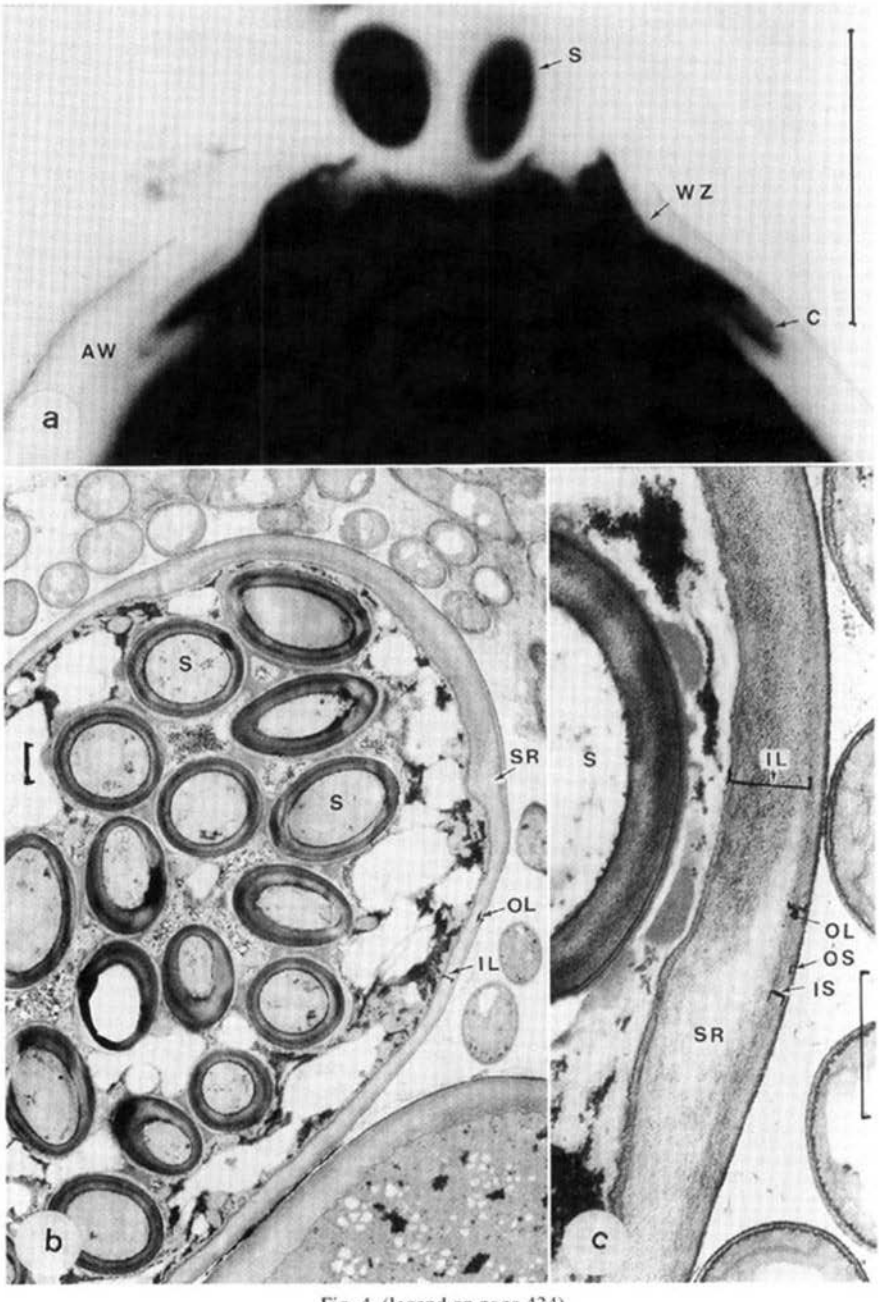

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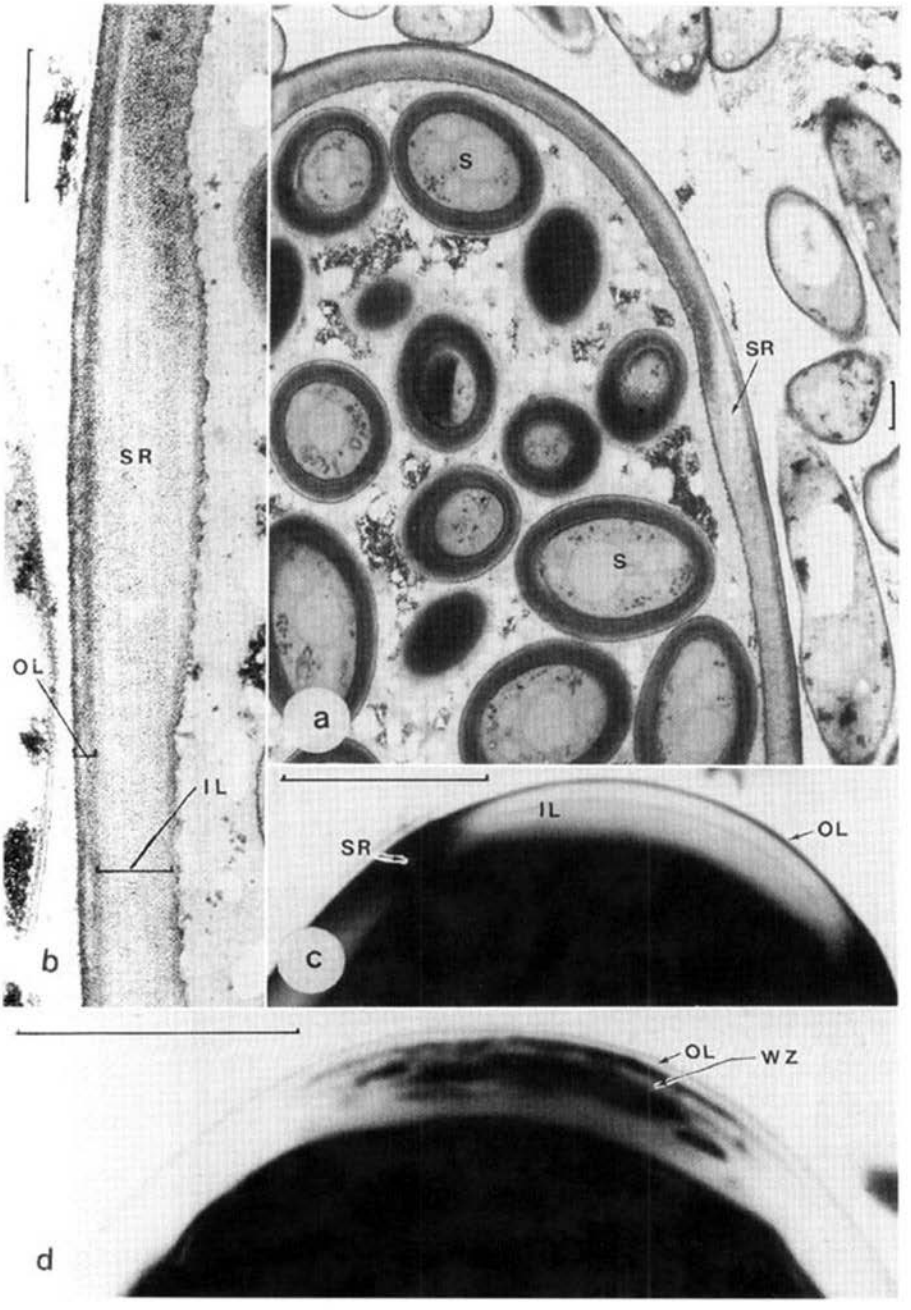

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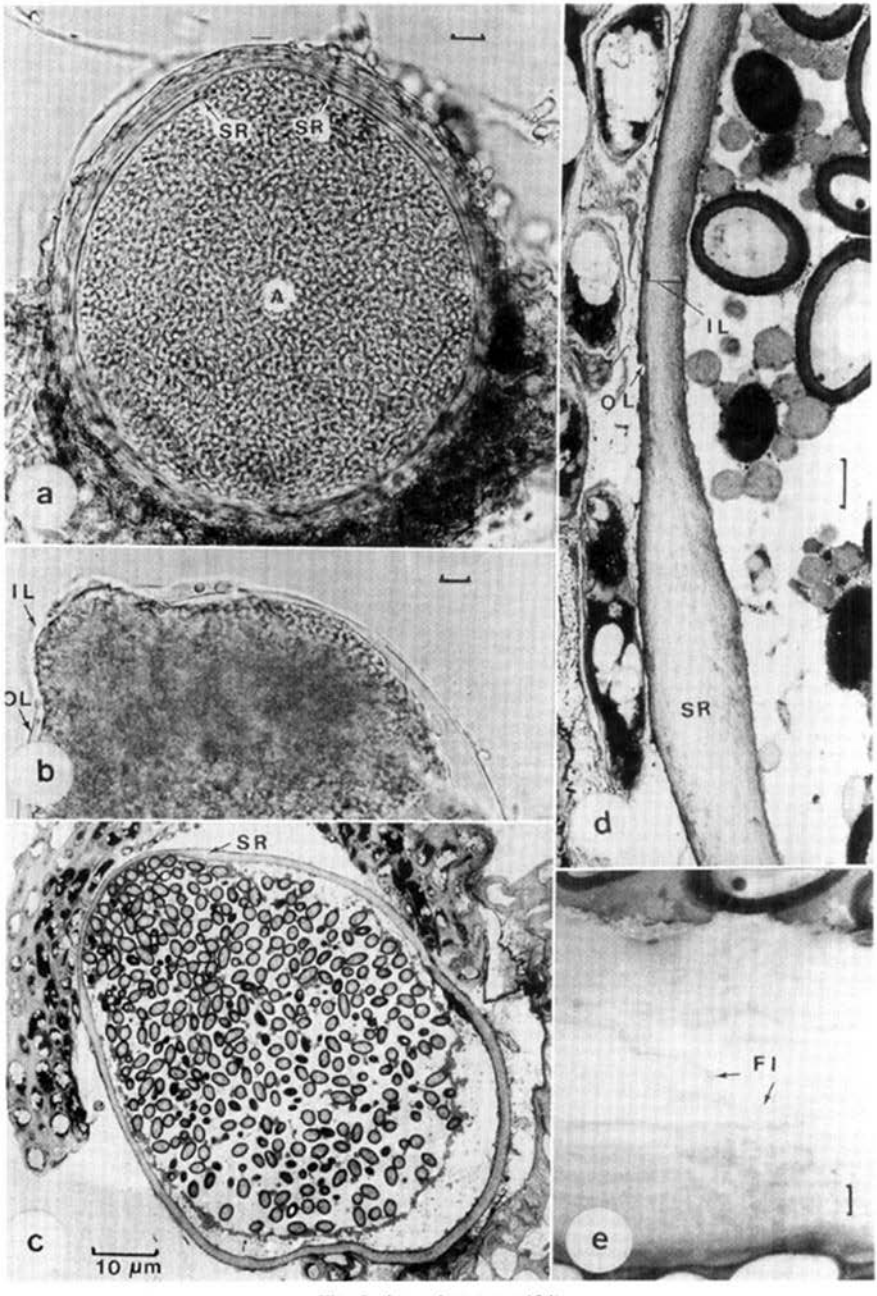

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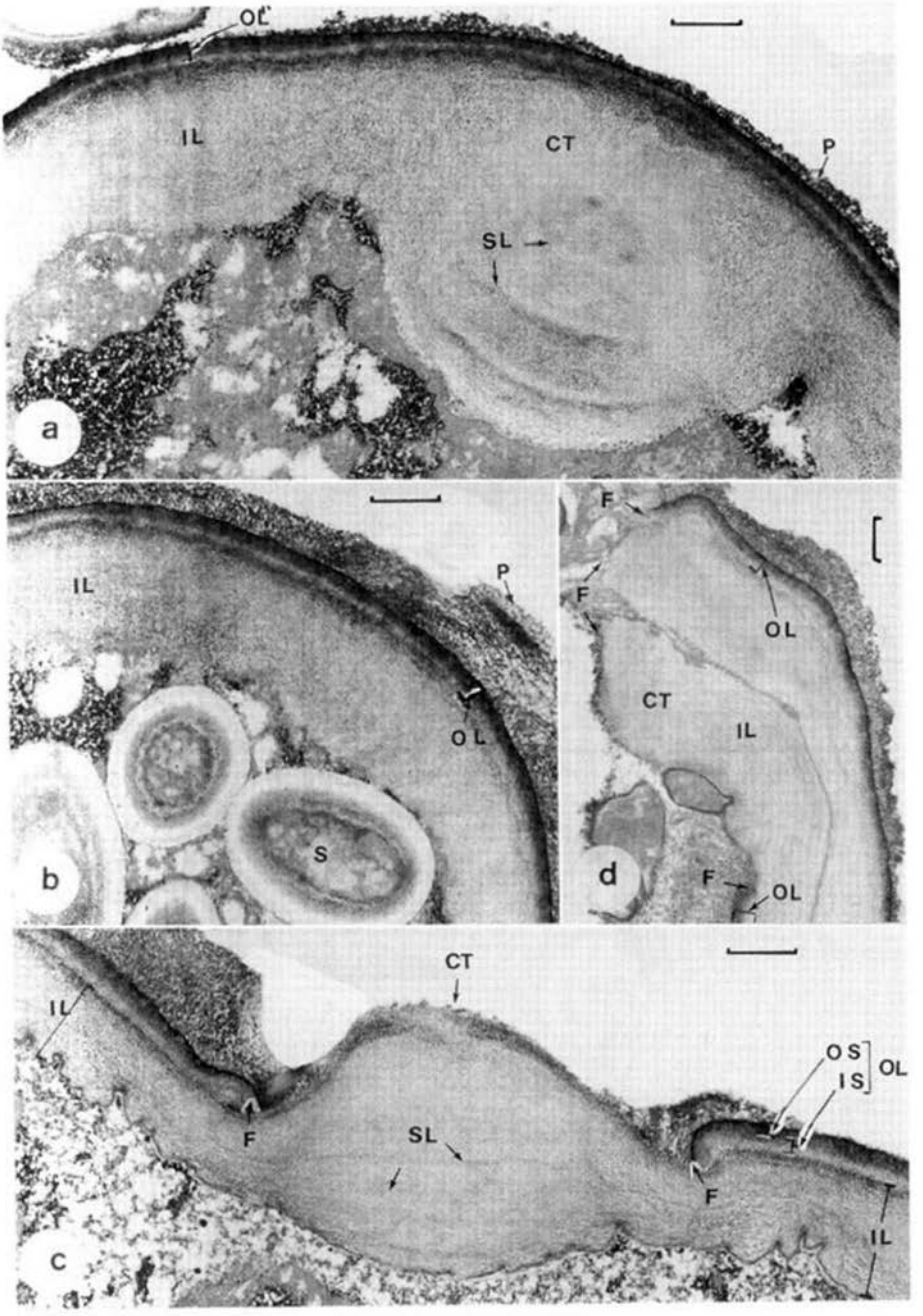

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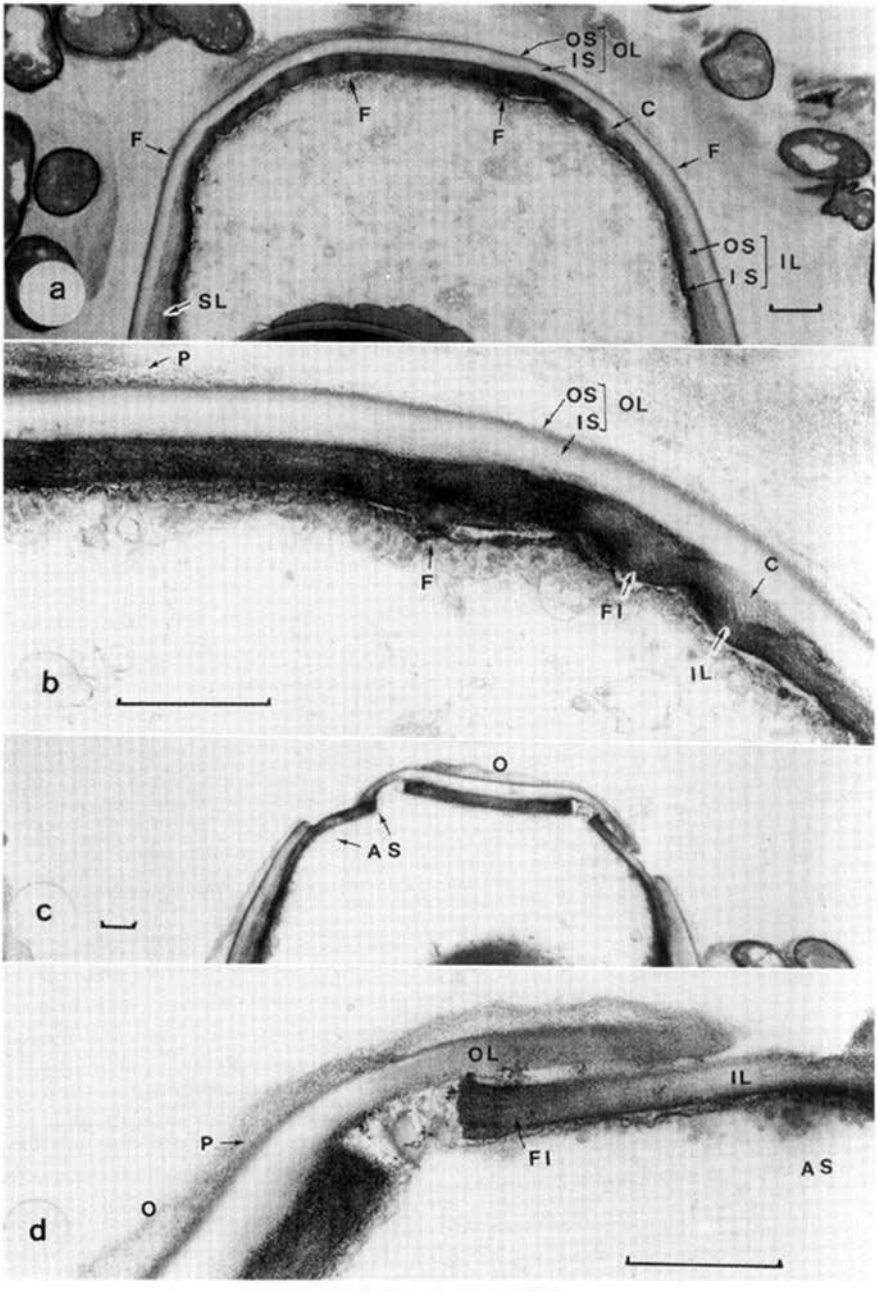

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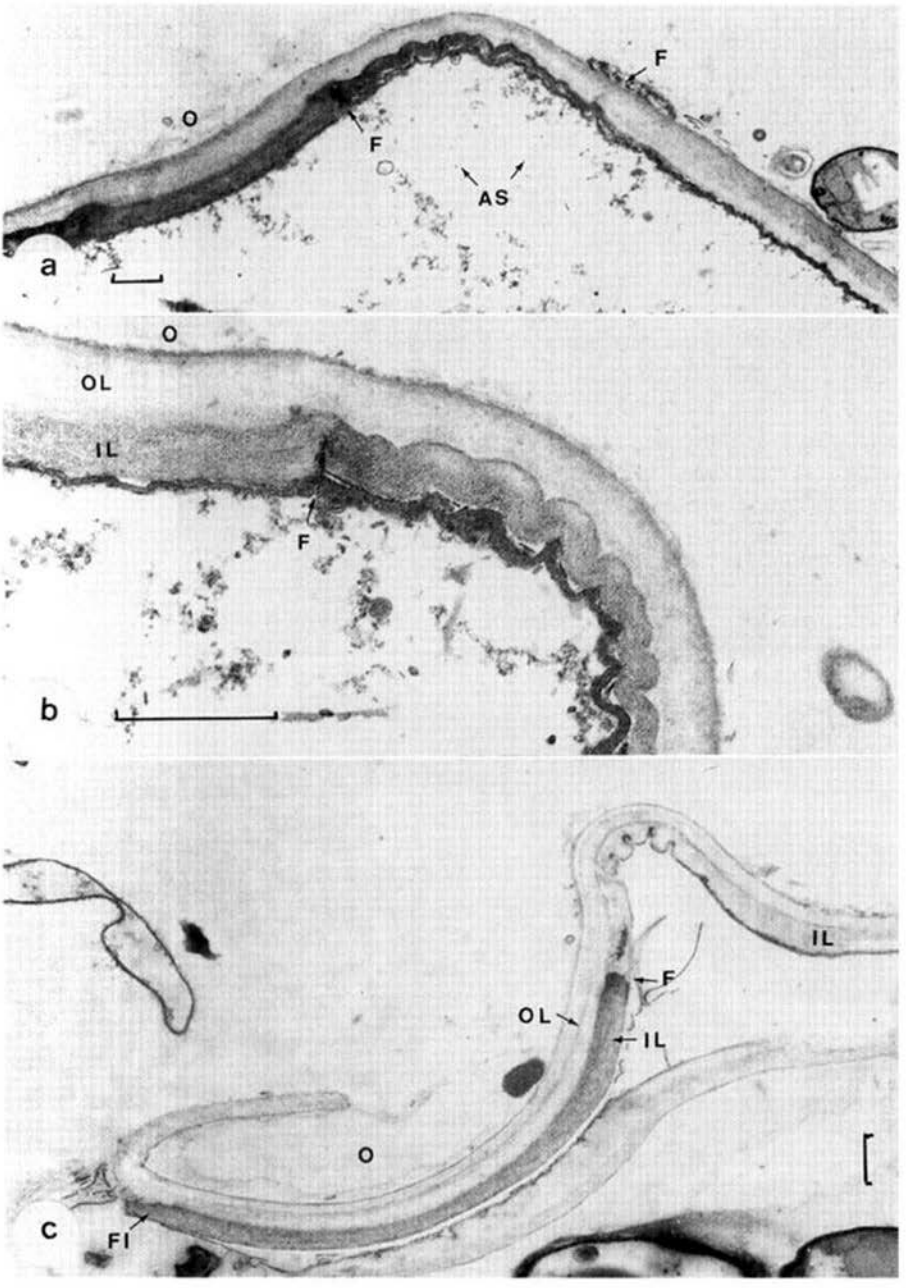

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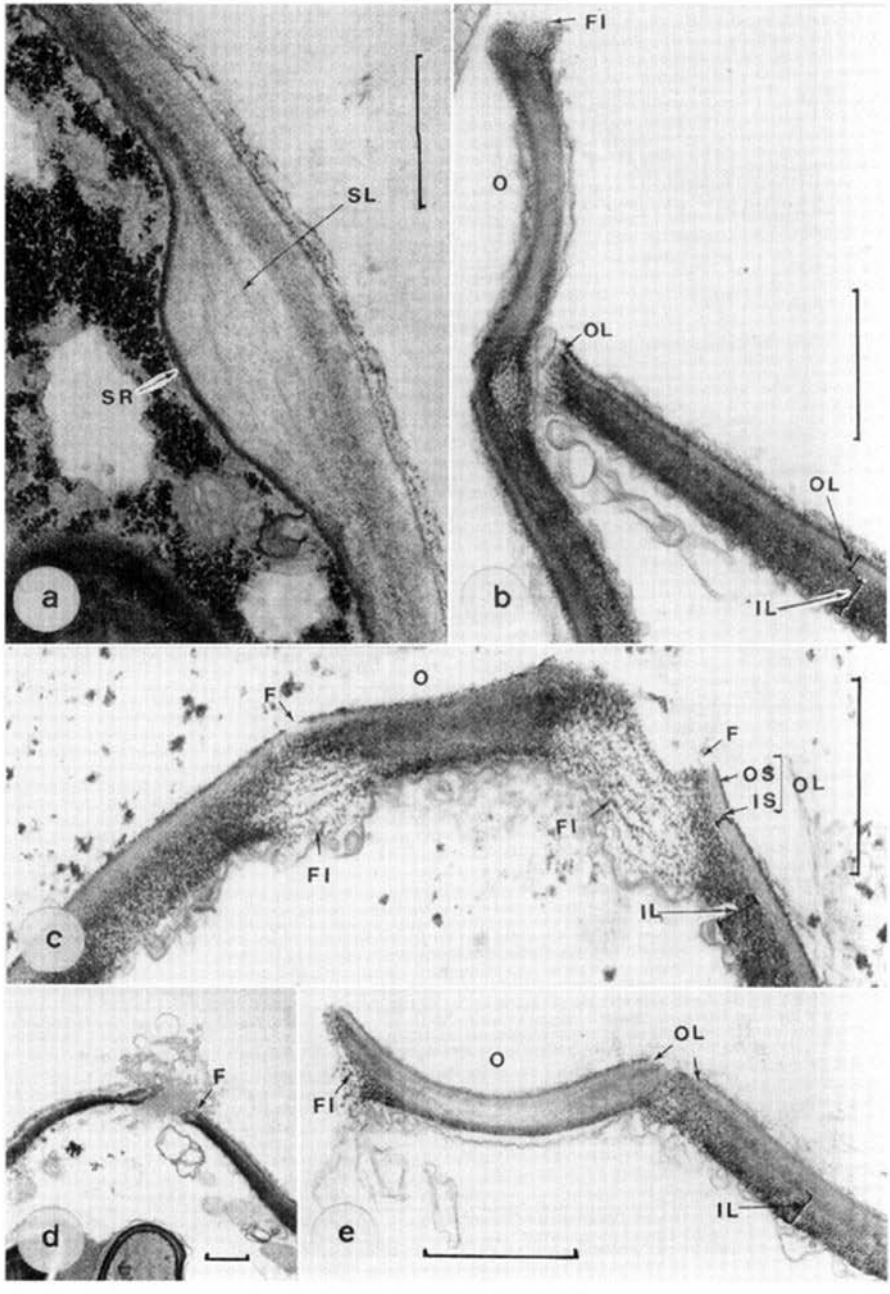

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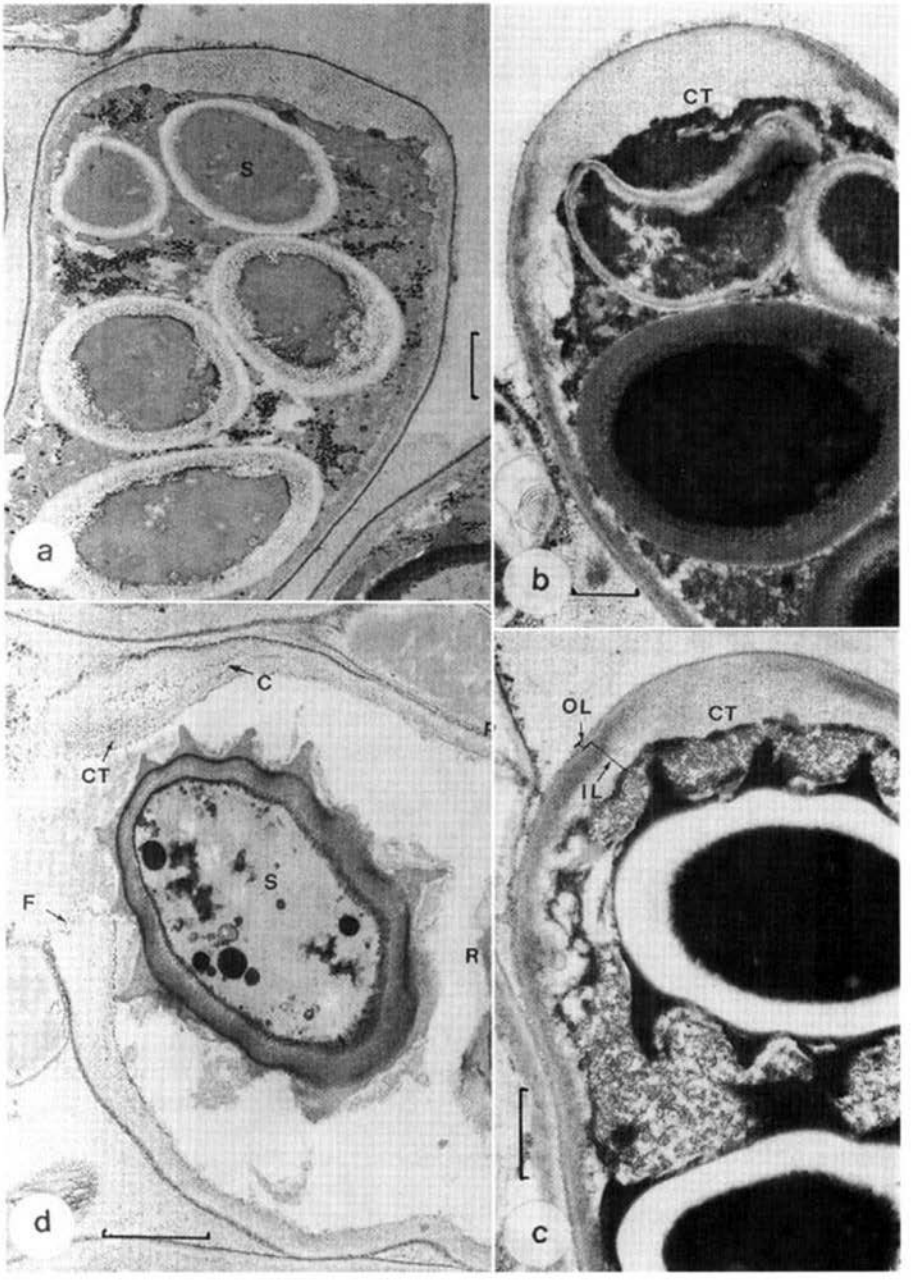

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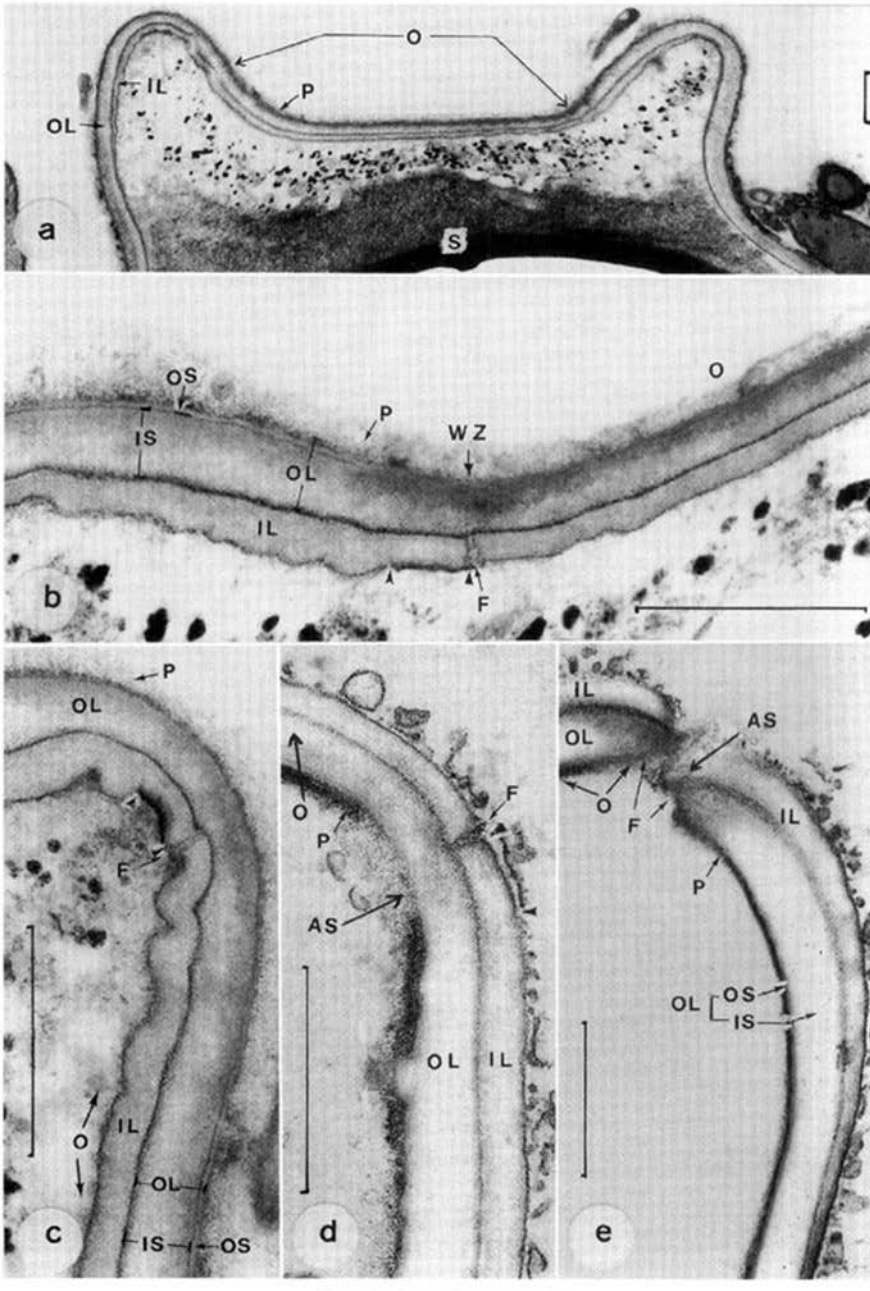


Fig. 12 (legend on page 434)

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. Secondary 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).

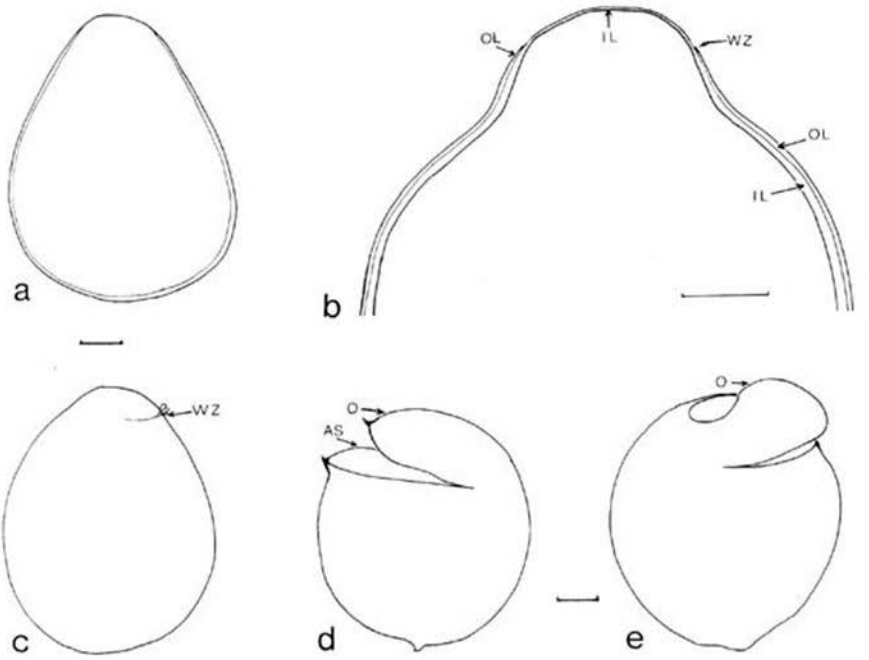


Fig. 13. *Trichobolus zukalii*, ascus development viewed by light microscopy. a. Mature ascus. b. Idem, detail, showing discontinuity of outer wall layer in the top. c. Beginning of opening along circular line. d, e. dehisced asci showing a large operculum. All scale markers represent 50 μm.

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.

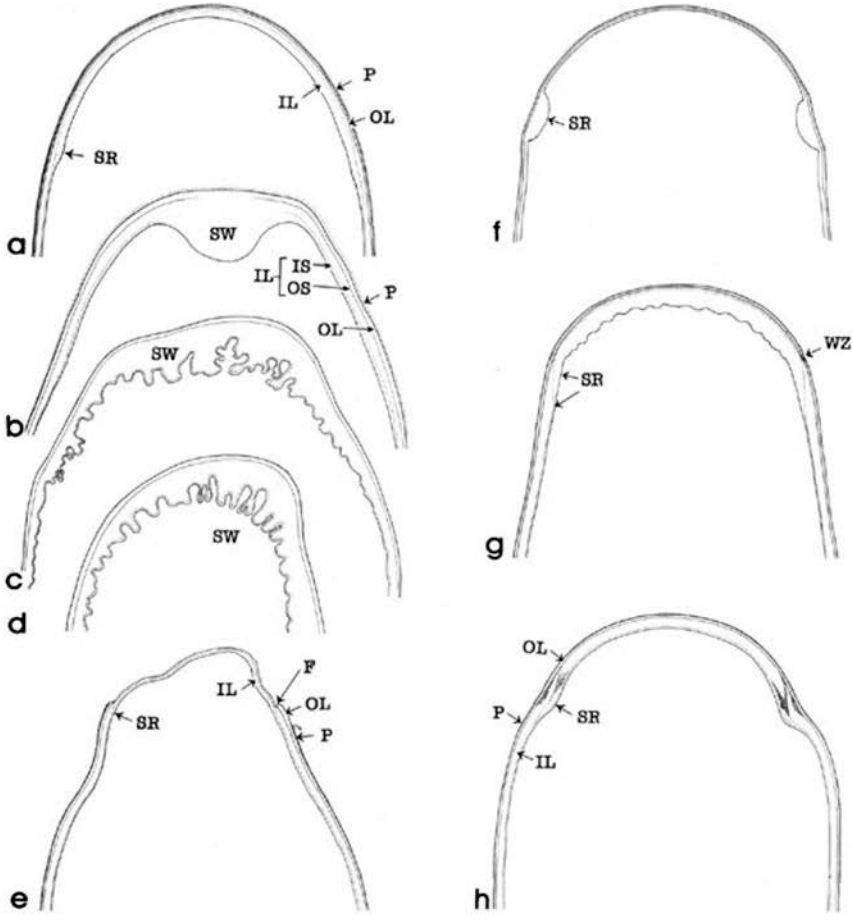


Fig. 14. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Figs. a–e. *Thelebolus microsporus* (a, b, fixed by ultra-rapid freezing and freeze substitution and contrasted with Thiéry technique; c–e, idem, but fixed in 1% glutaraldehyde). a. Almost mature ascus. b. Ripening ascus 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.

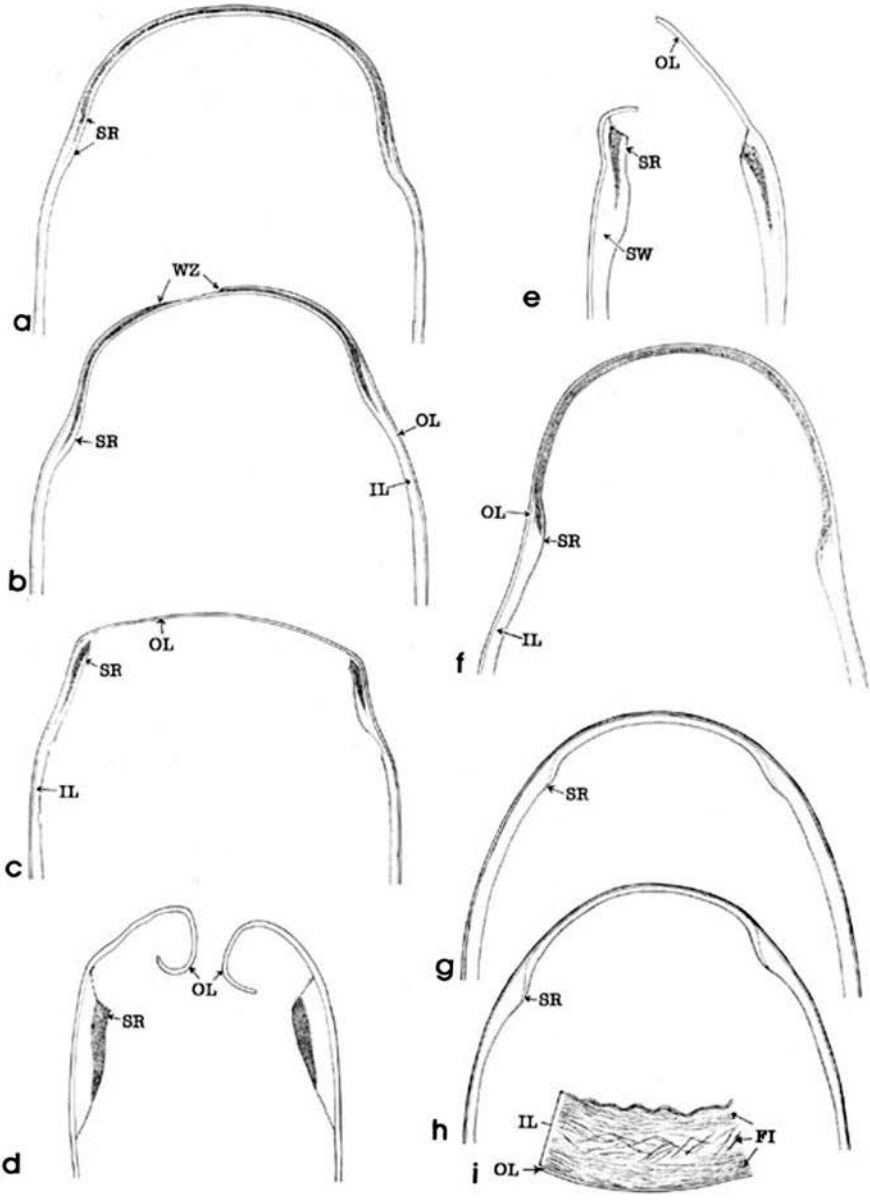
At the earliest stages the ascus shows only a single thin wall layer, which is already differentiated into an inner and an outer stratum. It is then that wall material is deposited at the inner face of the wall at the level of the future subapical ring (Fig. 2a), with a process very similar to that observed 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*.

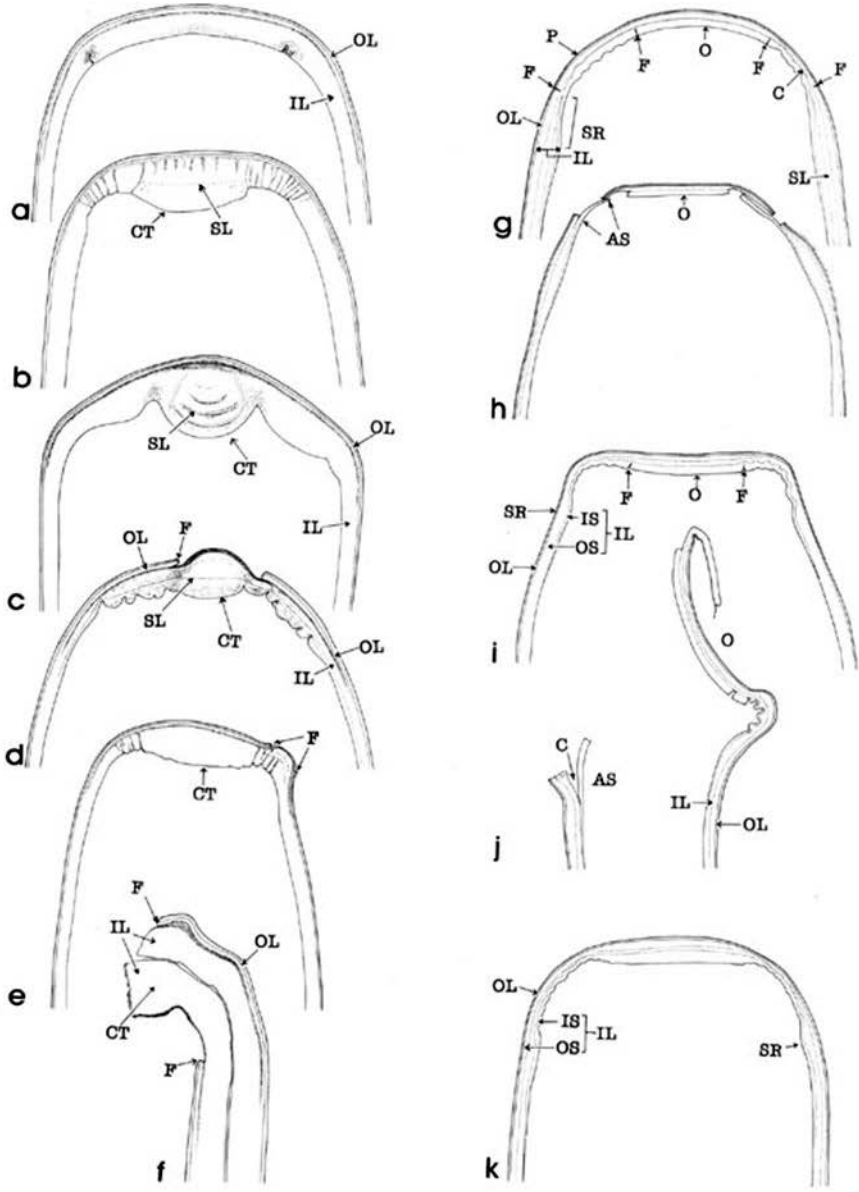
The banded appearance of the internal region of the inner layer in *T. stercoreus* was considered of great importance for the taxonomy of that genus by Samuelson & Kimbrough (1978a), since this was considered an important criterium for the 'bitunicate ascus' (Reynolds, 1971; 1989). When, however, the 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.

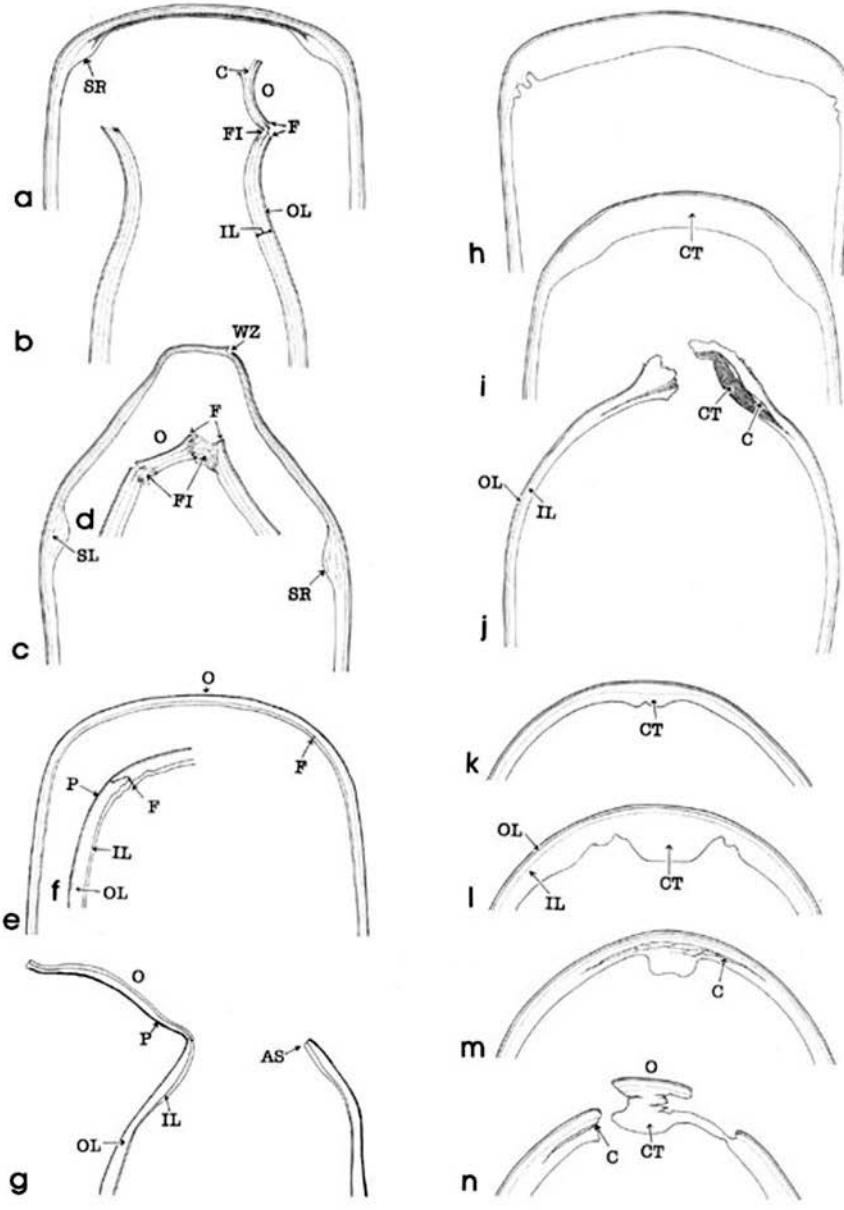
Sublayering of the inner layer is observed in the central thickening over a varying period. When treated with Waterman's blue-black ink during this period fine lines and cavities are stained blue. The staining with this dye, reported for the study of asci as a 'chitinoid' stain (Kimbrough & Korf, 1967; 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.

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CONTRIBUTIONS TOWARDS A MONOGRAPH OF PHOMA (COELOMYCETES)–I

3. Section Phoma: Taxa with conidia longer than 7 µm

J. DE GRUYTER[1], M.E. NOORDELOOS[2] & G.H. BOEREMA[3]

Eighteen species in section *Phoma* capable of producing conidia longer than 7 µm are keyed out and described according to their characteristics in vitro. A new combination *Phoma aliena* (Fr.: Fr.) v.d. Aa & Boerema is proposed. Indices of host-fungus and fungus-host relations and short comments on their ecology and distribution are given.

This is a continuation of Contributions I–1 (de Gruyter & Noordeloos, 1992) and I–2 (de Gruyter et al., 1993), referring to species with smaller conidia.

Phoma Sacc. sect. *Phoma* includes species which only produce one-celled conidia. However, septation may occur preceding germination. Pycnidia with only continuous conidia can also be found in some species of other sections of *Phoma*, such as sect. *Peyronellaea*, which is differentiated by the production of multicellular chlamydospores (Contr. II; Boerema, 1993) and sect. *Plenodomus*, which is characterized by the ability to produce scleroplectenchyma in the pycnidial wall (Contr. III–1/III–2; Boerema et al., 1994, 1996). In some species of sect. *Heterospora*, which is distinguished by a large-sized *Ascochyta/Stagonospora*-like conidial dimorph, the *Phoma*-phenotype may produce only one-celled conidia (Contr. IV; Boerema et al., 1997).

In species of sect. *Phoma* the conidial dimensions vary considerably, especially in vivo. The present paper deals with species, able to produce conidia longer than 7 µm. In some of these species most mature conidia in a pycnidium remain shorter than this but in other species the majority of mature conidia become longer.

MATERIALS AND METHODS

The isolates and herbarium specimens were studied as described in the previous Contributions I–1 and I–2 of this series (de Gruyter & Noordeloos, 1992 and de Gruyter et al., 1993). As mentioned in one of our previous Contributions (de Gruyter et al., 1993), the conidiogenous cells are rather variable, in young pycnidia more or less globose later becoming bottle-shaped, which means doliiform to ampulliform or lageniform. The growth-rate has been indicated by diameter of the colony.

1) Plant Protection Service, P.O. Box 9102, NL-6700 HC Wageningen, The Netherlands.
2) Rijksherbarium / Hortus Botanicus, P.O. Box 9514, NL-2300 RA Leiden, The Netherlands.
3) Karel Doormanstraat 4[5], NL-2041 HD Zandvoort, The Netherlands.

KEY TO THE SPECIES TREATED IN THIS PAPER

1a. Growth-rate very slow on OA, up to 25 mm in one week 2
b. Growth-rate moderate to fast on OA, at least 35 mm in one week 4
2a. NaOH causing a red-brown discoloration especially on MA, colonies dull green to greenish glaucous, conidia 5–7.5(–10.5) × 2–4 µm, on fruits and leaves of *Olea europaea* ..... 1. *P. fallens*
b. NaOH reaction negative 3
3a. Colonies dark herbage green on OA, conidia cylindrical, 3.5–8.5 × 1–2 µm, saprophyte on leaves of Gramineae, New Zealand, probably also Australia 2. *P. pratorum*
b. Colonies smoke grey to grey olivaceous/dull green on OA, conidia oblong to ellipsoidal, 5–7(–8) × 2.5–4.5 µm, hyaline with a greenish/yellow tinge, a pathogen of *Nerium oleander* ..... 3. *P. glaucispora*
4a. Colonies producing a diffusable pigment on MA, staining the agar yellowish or ochre 5
b. Colonies not producing a diffusable pigment on MA 8
5a. On MA diffusable pigment staining the agar yellowish to greenish 6
b. On MA diffusable pigment staining the agar ochre 7
6a. Yellow-green crystals are formed on MA, NaOH reaction on OA and MA rosy vinaceous to coral (not an E+ reaction), growth-rate moderate, 40–60 mm in one week, conidia 7–12 × 2.5–4 µm, soil fungus in North and South America 4. *P. humicola*
b. Crystals absent, NaOH reaction negative on OA, weak greenish-yellow on MA, (not an E+ reaction), growth-rate fast, 60–80 mm in one week, conidia 3.5–10.5 × 2–4 µm, necrophyte on *Daucus carota* (Europe), but also on *Spinacia oleracea* 5. *P. obtusa*
7a. NaOH reaction on OA greenish, then red (E+ reaction), on MA an orange discoloration of the original ochraceous pigment also occurs, conidia 4–8.5 × 2–4 µm, a pathogen of *Dracaena* and *Cordyline* spp. ..... 6. *P. draconis*
b. NaOH reaction on OA and MA red, then blue (not E+ reaction), conidia 4–12 × 2.5–4.5 µm, a saprophyte in South America, also recorded in New Zealand 7. *P. huancayensis*
8a. NaOH causing initially a yellow-green discoloration, gradually changing to red (E+ reaction) 9
b. NaOH reaction negative 10
9a. Growth-rate very fast, completely filling a Petri dish in one week, with coarsely floccose to woolly, white to olivaceous grey aerial mycelium on OA and MA, pycnidia scattered, mostly (partly submerged) in the agar, saprophyte or weak wound parasite on Gramineae (*Paspalum, Dactylis* and *Lolium* spp.) New Zealand .. 8. *P. paspali*
b. Growth-rate fast, 60–80 mm in one week, with powdery to finely floccose, white to pale olivaceous grey aerial mycelium on OA and MA, pycnidia abundant both on and in the agar, after three weeks the colony on OA discolours to saffron as pycnidia ripen, pathogen of *Lotus* spp., New Zealand ..... 9. *P. lotivora*
10a. Growth-rate fast on OA, 70–85 mm in one week 11
b. Growth-rate moderate on OA, 40–60 mm in one week 12

11a. Chlamydospores abundant on OA, mainly in the aerial mycelium, with a typical distinct 'envelope', saprophyte, Eurasia and North America, recorded as probably weakly parasitic on *Heterodera glycines* eggs ..... 10. *P. heteroderae*
b. Chlamydospores absent, saprophyte, New Zealand, probably also Australia ..... 11. *P. nigricans*

12a. Average Q > 3.5, conidia 7.5–12.5 × 1.5–3.5 µm, average 8–10 × 2–2.5 µm, on stems of *Astragalus* spp. ..... 12. *P. astragali*
b. Average Q < 3.5 13

13a. Colonies on OA mainly colourless, with darker zones due to the development of pycnidia, sometimes with some dark herbage green sectors 14
b. Colonies on OA distinct greenish or greyish 15

14a. Colonies on MA colourless to luteous due to concentric zones of yellow-brown pycnidia, reverse similar, a saprophytic fungus in North America ... 13. *P. herbicola*
b. Colonies on MA olivaceous buff to greenish olivaceous, reverse olivaceous buff honey with olivaceous black sectors at centre, pseudothecia may be present, on dead stems of species of *Urtica*, esp. *U. dioica* ..... 14. *P. urticicola*

15a. On MA growth-rate rather fast, 60–70 mm, colonies greenish, with rosy buff-honey sectors at the margin, on CA colonies olivaceous to vinaceous buff-fawn, conidia 4–12 × 2.5–4.5 µm, average 6.3–8.2 × 3.0–3.5 µm, an opportunistic parasite on various woody plants, e.g. *Buxus* spp. and *Berberis vulgaris* ..... 15. *P. aliena*
b. On MA growth-rate 25–50 mm, colonies on MA and CA greenish, brownish or greyish 16

16a. Growth-rate on OA and MA similar, 40–50 mm 17
b. Growth-rate on OA 35–45 mm, but less on MA, 25–37 mm, conidia 4.5–10.5 × 2–4 µm, average about 6.2 × 2.9 µm, an opportunistic parasite of *Vitis vinifera* ..... 16. *P. negriana*

17a. Colonies on OA colourless to grey olivaceous, without or with sparse grey olivaceous aerial mycelium, on MA olivaceous buff-greenish olivaceous to olivaceous, reverse similar, growth-rate on CA 40–50 mm, similar as those on OA and MA, conidia 3.5–8 × 1.5–3 µm, average 4.8–5.5 × 2.1–2.4 µm, plurivorous saprophyte or opportunistic parasite in New Zealand and Australia ..... 17. *P. plurivora*
b. Colonies on OA grey olivaceous to dull green, with appressed-felted to finely floccose, white to olivaceous grey aerial mycelium, on MA dull green, reverse leaden grey with grey olivaceous-dull green tinges, growth-rate rather slow on CA, 29–35 mm, (on OA and MA 40–45 mm) conidia 5–10 × 1.5–3 µm, average 6.7 × 2.2 µm, seed borne pathogen on *Aubrietia* hybrids, Europe ..... 18. *P. aubrietiae*

HOST/SUBSTRATUM–FUNGUS INDEX

Plurivorous (but sometimes with a favoured host, see below): *P. aliena* (no. 15), *P. herbicola* (13), *P. heteroderae* (10), *P. humicola* (4), *P. nigricans* (11), *P. plurivora* (17).

Soil borne: *P. heteroderae* (10), *P. humicola* (4), *P. paspali* (8).

On seeds and fruits: *P. aliena* (15).

Isolated from water: *P. herbicola* (13).

Frequently found on particular plants:

Agavaceae (*Cordyline* and *Dracaena* spp.)	*P. draconis* (6)
Astragalus spp. (Leguminosae)	*P. astragali* (12)
Aubrietia hybrids (Cruciferae)	*P. aubrietiae* (18)
Berberidaceae (*Berberis vulgaris* and *Mahonia aquifolium*)	*P. aliena* (15)
Buxus sempervirens (Buxaceae)	*P. aliena* (15)
Chenopodium quinoa (Chenopodiaceae)	*P. huancayensis* (7)
Daucus carota (Umbelliferae)	*P. obtusa* (5)
Euonymus europeus (Celastraceae)	*P. aliena* (15)
Gramineae e.g. *Dactylis, Lolium* and *Paspalum* spp.	*P. paspali* (8), *P. pratorum* (2)
Humulus lupulus (Cannabaceae)	*P. aliena* (15)
Lonicera spp. (Caprifoliaceae)	*P. aliena* (15)
Lotus spp. (Leguminosae)	*P. lotivora* (9)
Nerium oleander (Apocynaceae)	*P. glaucispora* (3)
Olea europaea (Oleaceae)	*P. fallens* (1)
Paspalum dilatum (Gramineae)	*P. huancayensis* (7)
Solanum spp. series *Tuberosa* (Solanaceae)	*P. huancayensis* (7)
Spinacia oleracea (Chenopodiaceae)	*P. obtusa* (5)
Urtica dioica (Urticaceae)	*P. urticicola* (14)
Vitis vinifera (Vitaceae)	*P. negriana* (16)

FUNGUS–HOST INDEX

P. aliena (15)	*Berberis vulgaris* and *Mahonia aquifolium* (Berberidaceae), *Buxus sempervirens* (Buxaceae), *Euonymus europeus*, (Celastraceae), *Humulus lupulus* (Cannabaceae)
P. astragali (12)	*Astragalus* spp. (Leguminosae)
P. aubrietiae (18)	*Aubrietia* hybrids (Cruciferae)
P. draconis (6)	*Cordyline* and *Dracaena* spp. (Agavaceae)
P. fallens (1)	*Olea europaea* (Oleaceae)
P. glaucispora (3)	*Nerium oleander* (Apocynaceae)
P. huancayensis (7)	*Chenopodium quinoa* (Chenopodiaceae), *Solanum* spp. series *Tuberosa* (Solanaceae), *Paspalum dilatum* (Gramineae)
P. lotivora (9)	Lotus spp. (Leguminosae)
P. negriana (16)	*Vitis vinifera* (Vitaceae)
P. obtusa (5)	*Daucus carota* (Umbelliferae), *Spinacia oleracea* (Chenopodiaceae)
P. paspali (8), *P. pratorum* (2)	Gramineae (*Paspalum, Dactylis* and *Lolium* spp.)
P. urticicola (14)	*Urtica dioica* (Urticaceae)

DESCRIPTIVE PART

Section Phoma

1. **Phoma fallens** Sacc. — Fig. 1

Phoma fallens Saccardo, Sylloge Fung. 10 (1892) 146.

Description in vitro

OA: growth-rate 18–20 mm, (14 days: 35–40 mm), somewhat irregular, with velvety, pale olivaceous grey aerial mycelium, colony dull green to greenish glaucous; reverse similar.

MA: growth-rate 15–20 mm, (14 days: 30–35 mm), regular or with undulating margin, with velvety, dull green aerial mycelium; colony dull green; reverse greenish olivaceous to olivaceous.

CA: growth-rate 18–20 mm, (14 days: 35–40 mm), with felted to finely floccose, greenish glaucous aerial mycelium; colony dull green, sometimes with flesh tinges; reverse dull green with flesh or fulvous tinges.

Pycnidia scattered, mainly in centre of colony, on or partly in the agar, 100–210 µm diam., globose to irregularly shaped, solitary or confluent, glabrous, with 1 papillate ostiole, citrine to honey, later olivaceous to olivaceous black; walls made up of 2–5 layers of cells, outer layer(s) pigmented; conidial exudate white. Conidiogenous cells 3–6 × 3–6 µm, globose or bottle-shaped. Conidia 5–7.5(–10.5) × 2–4 µm, av. 6.1 × 2.8 µm, Q = 1.7–2.8, av. Q = 2.2, oblong to ellipsoidal, with or without several indistinct guttules.

Chlamydospores absent.

NaOH spot-test: weak red on OA, distinct red-brown on MA (not E+ reaction).

Crystals absent.

Ecology and distribution. Probably widespread in olive-growing (*Olea europaea*) regions of the world, particularly southern Europe. Occurs in association with spots on fruits and leaves, but so far no pathogenicity tests have been done.

Culture studied. CBS 161.78 (LEV 11302) ex *Olea europaea* (Oleaceae), New Zealand.

2. **Phoma pratorum** Johnston & Boerema — Fig. 2

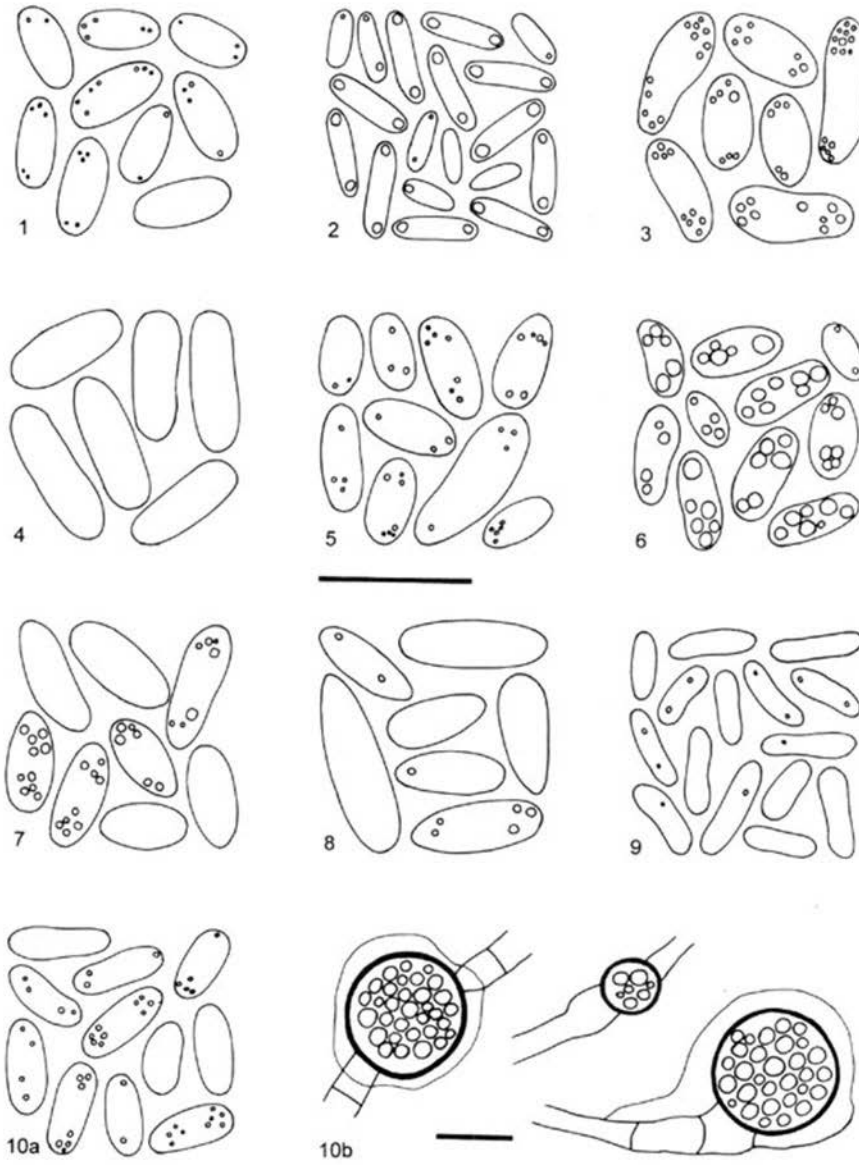
Phoma pratorum Johnston & Boerema, N. Z. Jl Bot. 19 (1981) 395–396.
Selected literature. Johnston & Boerema (1981).

Description in vitro

OA: growth-rate 20–23 mm (14 days: 42–51 mm), regular, with felted to finely floccose, white to olivaceous grey aerial mycelium; colony dark herbage green; reverse dull green to olivaceous.

MA: growth-rate 14–20 mm (14 days: 31–32 mm), regular, with compact, finely floccose, white to pale olivaceous grey aerial mycelium; colony pale olivaceous grey to olivaceous grey; reverse olivaceous with buff at margin.

CA: growth-rate 17–20 mm (14 days: 40–41 mm), regular, with finely felted to finely floccose, white to smoke grey aerial mycelium; colony dark herbage green with rosy buff at margin; reverse leaden grey at centre, dark herbage green with rosy buff towards margin.



Figs. 1–10a. Conidia. 1. *Phoma fallens*; 2. *P. pratorum*; 3. *P. glaucispora*; 4. *P. humicola*; 5. *P. obtusa*; 6. *P. draconis*; 7. *P. huancayensis*; 8. *P. paspali*; 9. *P. lotivora*; 10a. *P. heteroderae*. — Fig. 10b. Chlamydospores, *P. heteroderae*. — Bar = 10 µm.

Pycnidia abundant, mainly immersed in the agar, 90–250 µm diam., globose, solitary or confluent, glabrous, with 1–3 non-papillate or slightly papillate ostioles, buff-honey, later olivaceous to olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate white. Conidiogenous cells 3.7 × 3.7 µm. Conidia 3.5–7 (–8.5) × 1–2 µm, av. 4.1–6.0 × 1.4–1.6 µm, Q = 2.5–4.9, av. Q = 2.9–3.9, cylindrical with distinct, polar guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. Frequently isolated from the surface of living leaves of various grasses in New Zealand. This epiphytic saprophyte is probably also common in Australia. The substrates include species of *Dactylis*, *Lolium* and *Paspalum*.

Cultures studied. CBS 445.81 ex *Lolium perenne* and CBS 286.93 (PD 80/1252) ex *Dactylis glomerata* (Gramineae), the Netherlands.

3. **Phoma glaucispora** (Delacr.) Noordel. & Boerema — Fig. 3

Phoma glaucispora (Delacr.) Noordeloos & Boerema, Versl. Meded. plziektenk. Dienst Wageningen 166 (Jaarb. 1987) (1988) 108. — *Phyllosticta glaucispora* Delacroix, Bull. Soc. mycol. Fr. 9 (1893) 266.

Phyllosticta oleandri Gutner, Trudy bot. Inst. Akad. Nauk SSSR Leningrad Ser. II [Plantae Cryptogamae] 1 (1933) 306.

Description in vitro

OA: growth-rate 20–21 mm, (14 days: 40–43 mm), regular, with finely woolly to finely floccose, smoke grey to dull green aerial mycelium; colony smoke grey to grey olivaceous or dull green; reverse similar with olivaceous black sectors.

MA: growth-rate 18–19 mm, (14 days: 29–32 mm), regular or with undulating outline, with compact, finely floccose to finely woolly, white to smoke grey or greenish grey aerial mycelium; colony greenish grey/grey olivaceous to olivaceous/olivaceous grey; reverse iron grey to olivaceous black at centre, buff with small iron grey to grey olivaceous sectors at margin.

CA: growth-rate 15–17 mm, (14 days: 26–27 mm), with crenate margin, and with compact, finely floccose, white to greenish glaucous/greenish grey aerial mycelium; colony greenish glaucous to dark herbage green/grey olivaceous, with white near margin; reverse greyish blue to dark slate blue, with rosy buff at margin.

Pycnidia scattered, more obviously concentrically arranged at margin, partly submerged in the agar, 80–240 µm, globose to subglobose, solitary or confluent, glabrous, non-ostiolate, olivaceous buff to citrine green, later olivaceous to olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate rosy buff. Conidiogenous cells 5–8 × 4–7 µm, globose to bottle-shaped. Conidia 5–7(–8) × 2.5–4.5 µm, av. 6.2 × 3.5 µm, Q = 1.4–2.3, av. Q = 1.8, oblong to ellipsoidal, with several small guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. Common and widespread on oleander, *Nerium oleander*, in southern Europe (e.g. Italy, Spain). Leaf Spot. Also reported from glasshouse ornamental cultures (Russia, the Netherlands)

Culture studied. CBS 284.70 (PD 97/2400) ex *Nerium oleander* (Apocynaceae), Italy.

4. **Phoma humicola** Gilman & Abbott — Fig. 4

Phoma humicola Gilman & Abbott, Iowa State Coll. J. Sci. 1 (3) (1927) 266.
Selected literature. Boerema (1985).

Description in vitro

OA: growth-rate 40–45 mm, regular, without aerial mycelium; colony pale luteous due to the release of a diffusable pigment; reverse similar to grey olivaceous.

MA: growth-rate 40–58 mm, regular, with scanty woolly/floccose, olivaceous grey to grey olivaceous aerial mycelium; colony citrine with yellowish marginal zone, due to production of a diffusable pigment; reverse leaden grey and olivaceous at margin.

CA: growth-rate 45–53 mm, irregular, with finely floccose, olivaceous grey aerial mycelium; colony ochraceous, apricot particularly at centre, due to production of a diffusable pigment; reverse similar.

Pycnidia abundant, mainly in the agar, 80–150 µm diam., globose, solitary, rarely confluent, glabrous, with 1–2 non-papillate ostioles, olivaceous to olivaceous black; walls made up of 2–5 layers of cells, outer layer(s) pigmented; conidial exudate white to buff. Conidiogenous cells 4–8 × 5–9 µm, globose to bottle-shaped. Conidia (7–)8–10.5(–12) × 2.5–4 µm, av. 9.5 × 3.3 µm, Q = 2.5–3.3, av. Q = 2.9, oblong to cylindrical, without guttules.

Chlamydospores absent.

NaOH spot test: rosy vinaceous to coral on OA and MA (not an E+ reaction).

Crystals formed on MA, citrine green (anthraquinone pigments).

Ecology and distribution. Soil fungus in North America (Utah, Nevada, Wyoming) and South America (Andes, Peru). Isolated from a variety of organic substrates. A saprophytic fungus which may be an opportunistic pathogen (rotting of potatoes, Boerema, l.c.). It can be distinguished from *Phoma herbicola* in vivo (no. 13) by its broader conidia.

Culture studied. CBS 220.85 (PD 71/1030) ex *Franseria* sp. Nevada, USA.

5. **Phoma obtusa** Fuckel — Fig. 5

Phoma obtusa Fuckel, Jb. nassau. Ver. Naturk. 23–24 (= Symb. mycol.) (1870) 378.
Phoma carotae Diedicke, Krypt.-Fl. Mark Brandenb. 9, Pilze 7 (1912) 136–137.

Description in vitro

OA: growth-rate 60–70 mm, regular, with or without scarce, finely floccose, white aerial mycelium; colony colourless to rosy buff; reverse similar.

MA: growth-rate 76–79 mm, regular, with velvety-felted to woolly, white aerial mycelium; colony colourless to olivaceous buff due to production of a diffusable pigment; reverse similar.

CA: growth-rate 53–66 mm, regular, with velvety to finely woolly, pale olivaceous grey aerial mycelium; colony olivaceous to fawn; reverse similar.

Pycnidia abundant, on and in the agar, 50–180 µm diam., globose, solitary, covered with mycelial outgrowths, with 1 non-papillate ostiole, citrine-honey, later olivaceous to olivaceous black; walls made up of 2–4 layers of cells, outer layer(s) pigmented; conidial exudate white to sordid white. Conidiogenous cells 4–6 × 4–6 µm, globose. Conidia (3.5–)5–8.5(–10.5) × 2–4 µm, av. 5.3–5.6 × 2.4–3.0 µm, Q = 1.2–3.0, av. Q = 1.9–2.2, ellipsoidal, ovoid, usually with a few, small guttules.

Chlamydospores absent.

NaOH spot test: on OA negative, on MA a weak greenish-yellow discoloration of the exudate.

Crystals absent.

Ecology and distribution. In Europe this fungus is repeatedly recorded on dead stems of *Daucus carota*. Occasionally it has also been isolated from necrotic tissue of *Spinacia oleracea*. In this connection it is worth noting that the carrot-fungus has been interpreted as an overripe stage of a *Phoma* species commonly occurring on Chenopodiaceae (Allescher, 1898).

Cultures studied. CBS 391.93 (PD 80/87) ex *Spinacia oleracea* (Chenopodiaceae); CBS 377.93 (PD 80/976) ex *Daucus carota* (Umbelliferae), the Netherlands.

6. **Phoma draconis** (Berk. ex Cooke) Boerema — Fig. 6

Phoma draconis (Berk. ex Cooke) Boerema, Versl. Meded. plziektenk. Dienst Wageningen 159 (Jaarb. 1982) (1983) 24. — *Phyllosticta draconis* Berkeley, Welw. Crypt. Lusit. (1853) 51 [nomen nudum] ex Cooke, Grevillea 19 (1891) 8. — *Macrophoma draconis* (Berk. ex Cooke) Allescher, Rabenh. Krypt.-Flora (ed. 2) Pilze 7 (Lief. 88) (1903) [misapplied].

Phyllosticta maculicola Halsted, Rep. New Jers. St. agric. Exp. Stn 14 (1894) 412.

Phyllosticta dracaenae Griffon & Maublanc, Bull. Soc. mycol. Fr. 25 (1909) 238; not *Phyllosticta dracaenae* P. Hennings, Hedwigia 48 (1908) 111 [= *Asteromella* sp.].

Selected literature. Boerema (1983).

Description in vitro

OA: growth-rate 50–59 mm, regular, with tufted, finely floccose, white to grey olivaceous aerial mycelium, colony colourless to rosy buff-honey, with weak greenish tinge near margin; reverse similar.

MA: growth-rate 45–53 mm, regular, with velvety to finely floccose, white to dull green aerial mycelium; colony dull green to grey olivaceous, staining the agar ochraceous due to the release of a diffusable pigment, with greenish olivaceous margin; reverse olivaceous black with leaden grey patches, with ochraceous to greenish olivaceous margin.

CA: growth-rate 55–69 mm, regular, with finely woolly, grey olivaceous aerial mycelium; colony olivaceous grey, staining the agar red to scarlet due to the release of a pigment; reverse similar.

Pycnidia abundant, concentrically zoned, mostly partly in the agar, 90–220 µm diam., globose, solitary or confluent, glabrous, with 1(–2) non-papillate ostioles, olivaceous, around ostiole olivaceous black; walls made up of 2–5 layers of cells, outer layer(s) pigmented; conidial exudate white to buff. Conidiogenous cells 4–9 × 4–8 µm, globose to

bottle-shaped. Conidia 4–8.5 × 2–4 µm, av. 5.7 × 2.7 µm, Q = 1.7–2.8, av. Q = 2.1, ellipsoidal to ovoid, with numerous, large guttules.

Chlamydospores absent.

NaOH spot test: positive, greenish, then red (E+ reaction), on MA the ochraceous pigment discolours to orange.

Crystals absent.

Ecology and distribution. Recorded from wild *Dracaena* spp. in Africa (Rwanda) and from cultivated species of *Dracaena* in Europe, India and North America. Also reported from *Cordyline* spp. Leaf Spot. Dieback.

Culture studied. CBS 186.83 (PD 82/47) ex *Dracaena* sp. (Agavaceae), Rwanda.

7. **Phoma huancayensis** Turkensteen — Fig. 7

Phoma huancayensis Turkensteen, Fitopatologia 13 (1978) 68 [as '*hyancayense*'].
Selected literature. Johnston (1981).

Description in vitro

OA: growth-rate 40–57 mm, regular, with finely to coarsely floccose, smoke grey to olivaceous aerial mycelium; colony olivaceous buff to primrose, due to the release of a diffusable pigment, or umber to bay; reverse similar or with peach at the margin.

MA: growth-rate 30–54 mm, irregular, with floccose to woolly, compact, greyish-green to grey olivaceous aerial mycelium; colony ochraceous to fulvous with olivaceous buff to primrose patches due to a diffusable pigment production; reverse primrose to ochraceous and fulvous/bay to sepia.

CA: growth-rate 49–66 mm, regular, with velvety to floccose, grey olivaceous aerial mycelium; colony bay to rust due to a diffusable pigment production; reverse bay to chestnut-rust.

Pycnidia scarce to abundant, both on and in the agar, 95–240 µm diam., globose to irregular, solitary or confluent, glabrous, with 1, sometimes papillate ostiole, occasionally with an elongated neck, honey to sienna, later olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate white to salmon. Conidiogenous cells 4–8 × 4–6 µm, globose to bottle-shaped. Conidia (4–)5–8(–12) × 2.5–4.5, av. 7.0–7.1 × 3.0–3.6 µm, Q = 1.5–3.7, av. Q = 2.0–2.4, ellipsoidal to ovoid or subcylindrical, with or without polar guttules.

Chlamydospores absent, chains of swollen cells may occur.

NaOH spot test: positive, on OA and MA a red, then a blue discoloration of the pigment (not E reaction).

Crystals absent.

Ecology and distribution. A saprophytic fungus, probably indigenous to South America. It is originally described from dead leaves of *Chenopodium quinoa* and a wild species of *Solanum* spp. series *Tuberosa* in the Andes region of Peru (prov. Huancayo). The fungus is further recorded in New Zealand where it has been isolated from necrotic tissue of various dicotyledonous and monocotyledonous plants, e.g. often from *Paspalum dilatatum*, a grass originating from South America.

Cultures studied. CBS 105.80 (PD 75/908) ex *Solanum* sp. series *Tuberosa*; CBS 390.93 (PD 77/1179) ex *Chenopodium quinoa* (Chenopodiaceae), Peru.

8. **Phoma paspali** Johnston — Fig. 8

Phoma paspali Johnston, N. Z. Jl Bot. 19 (1981) 181.

Description in vitro

OA: growth-rate 42–60 mm after 5 days, regular to irregular, with scanty to abundant, floccose, white to olivaceous grey aerial mycelium; colony colourless with faint green tinge to grey olivaceous; reverse dark herbage green to grey olivaceous.

MA: growth-rate 60–72 mm after 5 days, regular with woolly, white to olivaceous grey aerial mycelium; colony colourless to olivaceous buff with primrose or greenish olivaceous tinges, or olivaceous grey; reverse similar or leaden grey/leaden black.

CA: growth-rate 53–70 mm after 5 days, regular, with woolly, white aerial mycelium; colony olivaceous to vinaceous buff; reverse similar.

Pycnidia scattered, sometimes on the agar, but mostly (partly submerged) in the agar, 100–140 µm diam., solitary, or confluent, up to 1150 µm in diam., globose to irregularly shaped, glabrous, with 1 to many papillate ostiole(s), later often developing an elongated neck, olivaceous black; walls made up of 4–7 layers of cells, outer layer(s) pigmented; conidial exudate white to straw. Conidiogenous cells 4–6 × 4–6 µm, globose to bottle-shaped. Conidia 5.5–8.5(–11) × 2.5–4 µm, av. 7.5 × 3.0 µm, Q = 1.8–3.2, av. Q = 2.5, obclavate-ovoid to ellipsoidal, without guttules.

Chlamydospores absent.

NaOH spot test: positive; green to bluish/green, then red (E+ reaction).

Crystals absent.

Ecology and distribution. Isolated from soil and various grasses (*Paspalum, Dactylis* and *Lolium* spp.) in New Zealand. Probably widespread in Australasia. Saprophyte or weak wound parasite.

Culture studied. CBS 560.81 (PDDCC 6614) ex *Paspalum dilatum* (Gramineae), New Zealand.

9. **Phoma lotivora** Johnston — Fig. 9

Phoma lotivora Johnston, N. Z. Jl Bot. 19 (1981) 178–179.
Selected literature. Johnston (1981).

Description in vitro

OA: growth-rate 58–80 mm, regular, with finely floccose, white aerial mycelium; colony colourless or with olivaceous grey spots, reverse similar. After three weeks the colony develops a saffron colour due to ripe pycnidia.

MA: growth-rate 60–77 mm, regular or irregular, with powdery to finely floccose, pale olivaceous grey aerial mycelium; colony olivaceous grey at centre, becoming pale olivaceous grey towards margin and finally buff to greenish olivaceous at margin; reverse leaden grey to leaden black with buff to honey tinges margin.

CA: growth-rate 64–69 mm, regular, with compact, powdery to finely floccose olivaceous grey aerial mycelium; colony scarlet to bay, due to the release of a diffusable pigment; reverse similar, with olivaceous centre.

Pycnidia abundant, both on and in the agar, 110–250 µm in diam., globose to irregular, solitary, glabrous, with 1(–2), non-papillate or papillate ostiole(s), honey to saffron,

later olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate white to pale buff. Conidiogenous cells 5–8 × 5–8 µm, globose to bottle-shaped. Conidia 4–7.5(–9) × 1.5–2(–2.5) µm, av. 5.6–5.7 × 1.6–1.7 µm, Q = 2.6–4.6, av. Q = 3.4–3.6, allantoid to subcylindrical, with or without a few, small guttules.

Chlamydospores absent.

NaOH spot test: positive on OA and MA, greenish/blue, then red (E+ reaction).

Crystals absent.

Ecology and distribution. Apparently a common pathogen of *Lotus* spp. in New Zealand. Stem and Leaf Spot. The fungus probably also occurs in Australia. It differs from the European *Phoma loticola* Died. in both cultural appearance and conidial dimensions.

Cultures studied. CBS 562.81 (PDDCC 6884) ex *Lotus pedunculatus* and CBS 628.97 (PDDCC 3870) ex *Lotus tenuis* (Leguminosae), New Zealand.

10. **Phoma heteroderae** Chen, Dickson & Kimbrough — Fig. 10a, b

Phoma heteroderae Chen, Dickson & Kimbrough, Mycologia 88 (6) (1996) 885–891.

Description in vitro

OA: growth-rate 70–82 mm, regular, with or without velvety grey olivaceous aerial mycelium; colony colourless to grey olivaceous/olivaceous or citrine; reverse similar.

MA: growth-rate 80–85 mm, regular, with scanty to abundant finely floccose, grey olivaceous aerial mycelium; colony citrine to olivaceous; reverse olivaceous, sometimes with leaden grey or greenish olivaceous sectors.

CA: growth-rate 80–85 mm, regular, with or without floccose, pale olivaceous grey to olivaceous grey aerial mycelium; colony olivaceous grey to olivaceous; reverse similar with brown vinaceous tinges.

Pycnidia abundant, mainly on, partly also in the agar, 70–250 µm diam., globose to irregular, solitary or confluent, glabrous, or with setae-like hyphal outgrowths (semi-pilose), with 1–4 non-papillate or slightly papillate ostioles, honey to olivaceous, later olivaceous black; walls made up of 2–6 layers of cells, outer layer(s) pigmented; conidial exudate buff to vinaceous buff. Conidiogenous cells 5–8 × 5–8 µm, globose to bottle-shaped. Conidia 3.5–7.5(–12) × 2–3.5(–4.5) µm, av. 5.3–6.5 × 2.4–2.5 µm, Q = 1.5–3.3, av. Q = 2.2–2.7, ellipsoidal to ovoid to cylindrical with or without small, polar guttules.

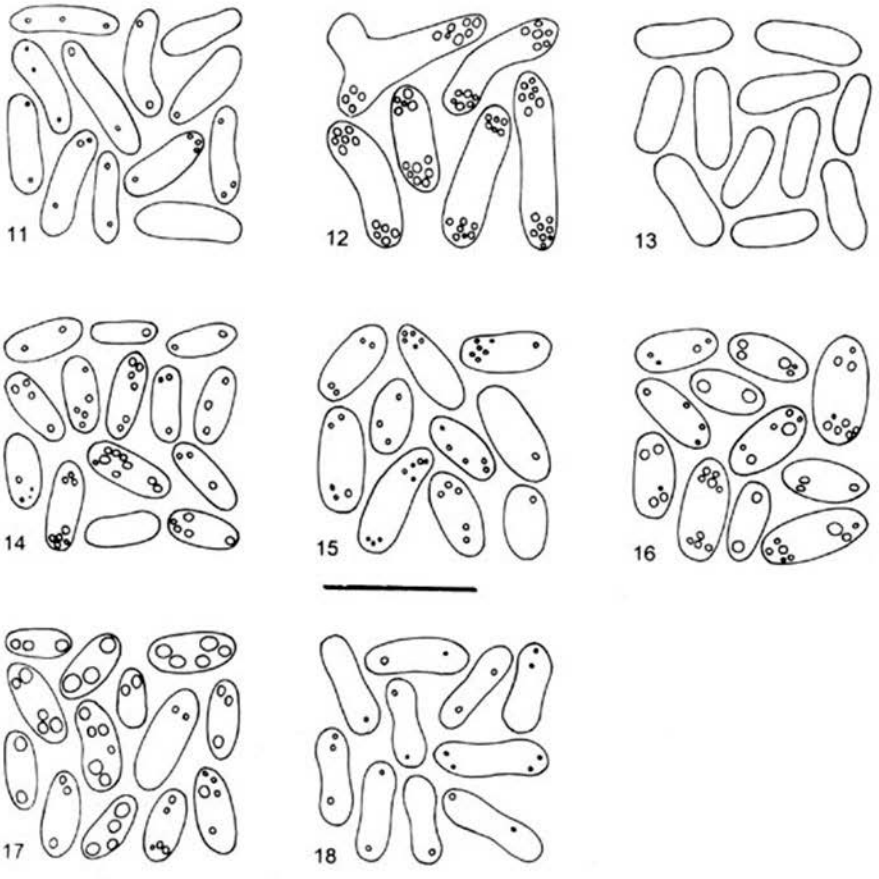
Chlamydospores abundant on OA, mainly in aerial mycelium, globose, intercalary, 7.5–25.5 µm diam., with a distinct 'envelope.'

NaOH spot test: negative, or weakly yellow-green to reddish on OA and MA, not specific.

Crystals absent.

Ecology and distribution. A saprophyte, isolated from various organic and inorganic substrata in Eurasia and North America. Apparently it is a soil-inhabiting fungus (fitting well with the abundant production of chlamydospores). Recorded as a probable weak parasite on eggs of *Heterodera glycines.*

Culture studied. CBS 96/2022 (ATCC 96683, IMI 361196) ex Eggs of *Heterodera glycines*, USA; CBS 875.97 (PD93/1503) ex cold storage chamber, USA.



Figs. 11–18. Conidia. 11. *Phoma nigrificans*; 12. *P. astragali*; 13. *P. herbicola*; 14. *P. urticicola*; 15. *P. aliena*; 16. *P. negriana*; 17. *P. plurivora*; 18. *P. aubrietiae*. — Bar = 10 µm.

11. **Phoma nigricans** Johnston & Boerema — Fig. 11

Phoma nigricans Johnston & Boerema, N. Z. Jl Bot. 19 (1981) 394–395.
Selected literature. Johnston & Boerema (1981).

Description in vitro

OA: growth-rate 70–82 mm, regular, with finely floccose, white to pale olivaceous grey aerial mycelium; colony grey olivaceous, rosy buff at margin; reverse similar.

MA: growth-rate 70–82 mm, regular, with grey olivaceous, woolly-floccose aerial mycelium; colony grey olivaceous becoming honey-citrine to greenish olivaceous near margin; reverse olivaceous black with honey to greenish olivaceous margin.

CA: growth-rate 70–78 mm, regular, with velvety to woolly, grey olivaceous to olivaceous grey aerial mycelium; colony olivaceous grey to grey olivaceous; reverse olivaceous black with olivaceous at margin.

Pycnidia abundant, mostly on, but also immersed in the agar, 80–310 µm diam., globose to irregular, solitary to confluent, glabrous, with 1 papillate ostiole, citrine, later olivaceous to olivaceous black; conidial exudate white to saffron; walls made up of 3–5 layers of cells, outer layer(s) pigmented. Conidiogenous cells 4–8 × 5–8 µm, globose to bottle-shaped. Conidia (3.5–)4.5–8(–10) × (1.5–)2–3(–4) µm, av. 4.9–5.9 × 2.4–2.8 µm, Q = 1.3–2.7, av. Q = 2.0–2.1, allantoid to subcylindrical, with or without a few small guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. Isolated from various woody and herbaceous plants in New Zealand apparently as an ubiquitous saprophyte; probably also common in Australia. The epithet refers to the conspicuous mature black pycnidia.

Culture studied. CBS 444.81 (PDDCC 6546) ex *Actinidia chinensis* (Actinidiaceae), New Zealand.

12. **Phoma astragali** Cooke & Harkn. — Fig. 12

Phoma astragali Cooke & Harkness, Grevillea 13 (1885) 111. — *Phoma astragali* Ellis & Kellerman, nomen nudum [herbarium name, Mycol. Coll., NY].

Description in vitro

OA: growth-rate 49–55 mm, regular, with or without scarce, finely floccose, white aerial mycelium; colony colourless to olivaceous grey or greenish grey; reverse similar.

MA: growth-rate 45–55 mm, regular, with floccose-woolly olivaceous grey aerial mycelium; colony greenish black, with dull green margin; reverse leaden grey to greenish black.

CA: growth-rate 45–55 mm, with appressed floccose, pale olivaceous grey aerial mycelium; colony colourless with dull green to greenish black sectors; reverse similar.

Pycnidia scattered all over the colony, on and (partly) in the agar, 80–180 µm diam., mainly globose, sometimes irregular, solitary or confluent, glabrous, with 1 rarely up to 3 non-papillate or papillate ostiole(s), honey to citrine with olivaceous tinges around ostiole; walls made up of 2–5 layers of cells, outer layer(s) pigmented; conidial exudate white to buff. Conidiogenous cells 4–10 × 5–8 µm, globose to bottle-shaped. Conidia 7.5–12.5 × 2–3.5 µm, av. 8.0–10.0 × 2.0–2.5 µm, Q = 2.9–5.0, av. Q = 3.9–4.0, cylindrical to allantoid, distorted shapes occur, with numerous small guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. This fungus is often recorded in North America (United States, Canada) on stems of various species of *Astragalus*. Probably an opportunistic parasite.

Culture studied. CBS 178.25 ex *Astragalus* sp. (Leguminosae), USA.

13. **Phoma herbicola** Wehm. — Fig. 13

Phoma herbicola Wehmeyer, Mycologia 38 (1946) 319–320 [description in accordance with DAOM 120225: herb. Wehmeyer '*Phoma herbicola* nov. sp.', with the indication 'Type' in pencil (original paper packet marked '*Phoma*' written by Wehmeyer); another specimen DAOM 120226, indicated in print with type (original packet marked '*Macrophoma*' by Wehmeyer) is in conflict with the original description: conidia larger, resembling those of *P. humicola*, no. 4].

Phoma pedicularis Wehmeyer, Mycologia 38 (1946) 319 [description checked with original collection in herb. Wehmeyer, filed under '*Apiosporella alpina* 1095', DAOM]; not *Phoma pedicularis* Fuckel in Von Heuglin, Reisen Nordpolarmeer III Beitr. Fauna Fl. Geol. (1874) 318–319 [as '*pedicularidis*'; belongs to sect. *Plenodomus*, treated in Contribution III–1; Boerema, de Gruyter & van Kesteren, 1994].

Selected literature. Wehmeyer (1946).

Description in vitro

OA: growth-rate 45–52 mm, regular, with scanty, fluffy, white aerial mycelium, particularly in marginal zone; colony colourless with darker concentric zones due to pycnidia; reverse similar.

MA: growth-rate 43–50 mm, irregular with undulating margin, with floccose, white aerial mycelium; colony colourless to luteous due to concentric zones of yellow-brown pycnidia; reverse colourless with pale luteous concentric zones.

CA: growth-rate 40–53 mm, regular, with finely floccose, white aerial mycelium; colony colourless to weak rosy buff; reverse similar.

Pycnidia abundant, on and partly in the agar, 120–340 µm diam., globose to irregular, solitary or confluent, glabrous, with 1–2 ostiole(s), with long elongated neck, citrine to honey, later olivaceous black; walls made up of 3–7 layers of cells, outer layer(s) pigmented; conidial exudate buff to saffron. Conidiogenous cells 3–9 × 5–8 µm, bottle-shaped. Conidia 5–7(–8.5) × 2–3 µm, av. 6.2 × 2.3 µm, Q = 2.4–3.3, av. Q = 2.7, cylindrical to subcylindrical, without guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. A saprophytic fungus indigenous to North America. The records so far are from northwestern United States (Montana and Wyoming) and refer to old dead stems of quite different herbaceous plants and to polluted lake water. The conidia in vivo are usually longer than those in vitro (up to 10.5 µm). Collections of this fungus may have been confused with the cosmopolitan *Phoma herbarum* Westend., the type species of sect. *Phoma*, treated in Contribution I–2, de Gruyter, Noordeloos & Boerema (1993).

Culture studied. CBS 629.97 (PD 76/1017) ex water, head of Seeley Lake, Missoula in Montana, USA.

14. **Phoma urticicola** v.d. Aa & Boerema — Fig. 14

Teleomorph: *Didymella urticicola* v.d. Aa & Boerema.

Phoma urticicola van der Aa & Boerema in Boerema, Trans. Brit. mycol. Soc. 67 (1976) 303–306. *Selected literature.* Boerema (1976).

Description in vitro

OA: growth-rate 55–61 mm, regular, with or without scanty, fluffy, white aerial mycelium; colony colourless, but with grey tinge due to development of pycnidia, sometimes with dark herbage green sectors; reverse similar.

MA: growth-rate 55–69 mm, regular, with compact, floccose to woolly, white aerial mycelium; colony olivaceous buff to greenish olivaceous; reverse olivaceous buff to honey, with olivaceous black sectors at centre.

CA: growth-rate 55–64 mm, regular, with finely floccose, white to pale olivaceous grey aerial mycelium; colony olivaceous grey to grey olivaceous; reverse leaden grey with olivaceous tinges, vinaceous buff towards margin.

Pycnidia abundant, formed on or in the agar, 110–500 µm, globose to bottle-shaped, solitary or confluent, glabrous, with 1–2 papillate ostiole(s), citrine to honey later ochraceous to olivaceous; walls made up of 2–5 layers of cells, outer layer(s) pigmented; with pale buff to saffron conidial exudate. Conidiogenous cells 5–9 × 4–8 µm, globose to bottle-shaped. Conidia (3–)4–6.5(–8.5) × (1.5–)2–3(–3.5) µm, av. 5.6 × 2.2 µm, Q = 1.7–3.4, av. Q = 2.5, ellipsoidal, with or without small guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Pseudothecia 150–440 × 125–300 µm, subglobose to pyriform. Asci 40–65 × 8–12 µm, 8-spored. Ascospores 12–18 × 5.5–7.5 µm, 2-celled (on ageing rarely 3–4 celled), the lower cell usually slightly tapering to the the base, the upper cell widest near the septum, tapering gradually to a broadly rounded apex.

Ecology and distribution. Common in Europe on dead stems of nettle, especially *Urtica dioica*; usually with simultaneous production of anamorph and teleomorph. In the past, i.e. before 1976, this fungus has always been confused with other species of *Phoma* and *Didymella* occurring on *Urtica* spp.

Culture studied. CBS 121.75 (ATCC 32164, IMI 194767, PD 73/584) ex *Urtica dioica* (Urticaceae), the Netherlands.

15. **Phoma aliena** (Fr.: Fr.) v.d. Aa & Boerema, *comb. nov.* — Fig. 15

Sphaeria aliena Fries: Fries, Syst. mycol. 2 [Sect. 2] (1823) 502. — *Perisporium alienum* (Fr.: Fr.) Fries, Syst. mycol. 3 [Sect. 1] (1829) 252. — *Phyllosticta aliena* (Fr.: Fr.) Saccardo, Michelia 2 (2) (1881) 342 [neotype sub *Perisporium alienum* '(Fr.) Desm.' on branches of *Euonymus europeus*, Vosges, France, coll.: B.D. Mougeot, in Rouméguère, Fungi gall. exs. No. 765, Herb. P.A. Saccardo, PAD].

Phoma berberidicola Vestergren, Öfvers. K. Svensk Vet.-Akad. Förh. (1897) 38; non *Phoma berberidicola* Brunaud, Act. Soc. Linn. Bordeaux (1898) 12 [= *Phoma enteroleuca* Sacc.; belongs to sect. *Plenodomus*, treated in Contribution III–1; Boerema, de Gruyter & van Kesteren, 1994].

Description in vitro

OA: growth-rate 52–55 mm, regular, with or without scanty floccose, pale olivaceous grey aerial mycelium; colony smoke grey; reverse smoke grey with olivaceous grey tinge.

MA: growth-rate 60–69 mm, regular, with rather coarse, floccose to woolly, white to grey olivaceous aerial mycelium; colony greenish olivaceous to dull green, with rosy buff to honey in sectors and at the margin; reverse similar.

CA: growth-rate 60–70 mm, regular, with velvety to finely floccose, pale olivaceous grey aerial mycelium; colony olivaceous to vinaceous buff/fawn; reverse similar.

Pycnidia on and in the agar, also in aerial mycelium, 80–260 µm diam., globose to irregular, usually solitary, glabrous, without or with 1(–2) non-papillate ostiole(s), citrine to olivaceous, later olivaceous black; walls made up of 3–7 layers of cells, outer layer(s) pigmented; conidial exudate rosy buff to salmon. Micropycnidia also present, 40–80 µm diam. Conidiogenous cells 5–8 × 4–7 µm, globose to bottle-shaped. Conidia (4–)5.5–10.5(–12) × 2.5–4.5 µm, av. 6.3–8.2 × 3.0–3.5 µm, Q = 1.4–3.5, av. Q = 2.1–2.3, ellipsoidal to slightly ovoid, with or without some small guttules.

Chlamydospores absent.

NaOH spot test: negative or weakly reddish on MA, not specific.

Crystals absent.

Ecology and distribution. Isolated from quite different woody plants, especially shrubs (including evergreens and conifers) in Europe. The fungus is most frequently encountered on *Buxus* spp., *Berberis vulgaris, Euonymus europeus, Mahonia aquifolium, Lonicera* spp. and *Humulus lupulus* (commonly growing together as wild plants in the Dutch dunes). Characteristically it is an opportunistic parasite often occurring in association with leaf necrosis and wood discoloration (sometimes flesh-coloured). It is commonly seed-borne on *Berberis* spp. Collections of this fungus are sometimes confused with *Phoma macrostoma* Mont., another opportunistic pathogen of woody plants [sect. *Phyllostictoides*, i.e. always producing some septate conidia].

Cultures studied. CBS 379.93 (PD 82/945) ex *Berberis* sp. (Berberidaceae); CBS 877.97 (PD 94/1401) ex *Buxus sempervirens* (Buxaceae), the Netherlands.

Note: The holotype of the basionym *Sphaeria aliena* Fr.: Fr., 'ad ramos languescentes *Evonymi*. Mougeot (v.s.)' is not known to exist. The designated neotype probably represents an isotype collection.

16. **Phoma negriana** Thümen — Fig. 16

Phoma negriana Thümen, Pilze Weinst. (1878) 185 [as '*negrianum*']. — *Phyllosticta negriana* (Thümen) Allescher, Rabenh. Krypt.-Flora [ed. 2], Pilze 6 [Lief. 60] (1898 [vol. dated '1901']) 135.

Phyllosticta vitis Saccardo, Michelia 1 (2) (1878) 135; not *Phoma vitis* Bonorden, Abh. naturforsch. Ges. Halle 8 (1864) 14.

Selected literature. Boerema & Dorenbosch (1979).

Description in vitro

OA: growth-rate 37–45 mm, regular to irregular, with scanty woolly, pale olivaceous grey aerial mycelium; colony greenish olivaceous with colourless margin at first, later greenish due to the development of pycnidia; reverse similar.

MA: growth-rate 25–37 mm, regular to irregular, with compact, floccose to woolly, dull green to olivaceous grey aerial mycelium; colony dull green to olivaceous grey; reverse leaden grey to olivaceous black with grey olivaceous to dull green margin.

CA: growth-rate 47–50 mm, regular to irregular, with floccose, white to pale olivaceous grey aerial mycelium; colony grey olivaceous to olivaceous, buff at margin; reverse similar.

Pycnidia abundant, in concentric rings, mainly on the agar, sometimes (partly) in the agar, 70–220 µm, globose or irregular, solitary or confluent, glabrous, with 1–2(–4) papillate ostiole(s), citrine-honey to sienna-olivaceous, later olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate saffron to pale vinaceous. Conidiogenous cells 5–8 × 5–8 µm, globose to bottle-shaped. Conidia 4.5–8.5 (–10.5) × 2–4 µm, av. 6.2 × 2.9 µm, Q = 1.7–2.7, av. Q = 2.1, ellipsoidal to oblong, with several, distinct guttules.

Chlamydospores absent.

NaOH spot test: on MA a weak reddish-brown discoloration, not specific.

Crystals absent.

Ecology and distribution. A common opportunistic pathogen of vine (*Vitis vinifera*) in southern Europe. It may be associated with disease symptoms on leaves, fruits or stems. On stems it has often been misidentified as *Phoma viticola* Sacc., a name referring to a quite different pathogen of vine: *Phomopsis viticola* (Sacc.) Sacc., believed by some workers to be the cause of Dead arm disease.

Culture studied. CBS 358.71 ex *Vitis vinifera* (Vitaceae), Germany.

17. **Phoma plurivora** Johnston — Fig. 17

Phoma plurivora Johnston, N. Z. Jl Bot. (1981) 181.
Selected literature. Johnston (1981).

Description in vitro

OA: growth-rate 40–50 mm, regular, with or without sparse, grey olivaceous aerial mycelium; colony colourless to grey olivaceous; reverse similar.

MA: growth-rate 40–50 mm, regular, with or without floccose, white to greenish olivaceous aerial mycelium; colony olivaceous buff-greenish olivaceous to olivaceous; reverse similar.

CA: growth-rate 40–50 mm, regular, with scanty, floccose, white to greenish olivaceous aerial mycelium; colony pale olivaceous to greenish olivaceous; reverse olivaceous mixed with fawn and hazel.

Pycnidia abundant, both on and (partly submerged) in the agar, 80–260 µm diam., globose to irregular, solitary to confluent, glabrous, with 1 papillate ostiole, honey-citrine, later olivaceous black; walls made up of 3–6 layers of cells, outer layer(s) pigmented; conidial exudate buff to pale saffron. Conidiogenous cells 5–9 × 4–8 µm, globose to bottle-shaped. Conidia 3.5–6(–8) × 1.5–2.5(–3) µm, av. 4.8–5.5 × 2.1–2.4 µm, Q = 1.7–2.9, av. Q = 2.2–2.3, ellipsoidal to oblong, usually with several, distinct guttules.

Chlamydospores absent.

NaOH spot test: negative.

Crystals absent.

Ecology and distribution. Typically a plurivorous saprophyte or opportunistic parasite, probably indigenous to Australasia. In New Zealand it has been isolated from various dicotyledonous and monocotyledonous herbaceous plants, as well as from trees and shrubs.

Cultures studied. CBS 558.81 (PDDCC 6873) ex *Setaria* sp. (Gramineae), New Zealand; CBS 284.93 (PD 75/907) ex *Medicago sativa* (Leguminosae), Australia.

18. **Phoma aubrietiae** (Moesz) Boerema — Fig. 18

Phoma aubrietiae (Moesz) Boerema in Boerema & Valckx, Gewasbescherming 1 (1970) 66. — *Sclerophomella aubrietiae* Moesz, Balkan-Kutat. Tud. Eredm. 3 (1926) 144–145.

Selected literature. Boerema & Valckx (1970).

Description in vitro

OA: growth-rate 42–44 mm, regular, with appressed-felted to finely floccose, white to olivaceous grey aerial mycelium; colony grey olivaceous to dull green; reverse similar.

MA: growth-rate 40–45 mm, regular, with compact, felted-velvety dull green aerial mycelium; colony dull green with grey olivaceous marginal zone; reverse leaden grey with grey olivaceous or dull green tinges.

CA: growth-rate 29–35 mm, regular, with finely velvety, white to dull green aerial mycelium; colony dull green to greenish olivaceous at the margin; reverse leaden grey to grey olivaceous or dull green.

Pycnidia abundant, mainly on the agar, 80–160 µm diam., globose to subglobose, solitary, glabrous, with 1 non-papillate or papillate ostiole, citrine to olivaceous, later olivaceous black; walls made up of 3–5 layers of cells, outer layer(s) pigmented; conidial exudate white. Conidiogenous cells 6–10 × 6–11 µm, globose to bottle-shaped. Conidia (5–) 6–7.5(–10) × 1.5–3 µm, av. 6.7 × 2.2 µm, Q = 2.3–3.9, av. Q = 3.1, cylindrical to allantoid, with some small, polar guttules.

Chlamydospores absent, typical hyphal swellings may be formed.

NaOH spot test: negative or weakly reddish on OA, not specific.

Crystals absent.

Ecology and distribution. A common seed borne pathogen of *Aubrietia* hybrids in Europe. The fungus causes Damping-off of seedlings and Decay of stems and leaves of older plants.

Cultures studied. CBS 383.67 (PD 65/223) and CBS 627.97 (PD 70/714) ex *Aubrietia* hybrid (Cruciferae), the Netherlands.

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ERRATA

Gruyter, J. de, M.E. Noordeloos & G.H. Boerema: Contributions towards a monograph of *Phoma* (Coelomycetes) I–2. Section *Phoma*: Additional taxa with very small conidia and taxa with conidia up to 7 µm long. Persoonia 15 (3) (1993) 369–400.

Page 390: line 19 from bottom: add *(occasionally a small percentage larger conidia may be produced, up to 9 × 3 µm).*
line 11 from bottom: replace 'conidia' with *conidial diversity.*

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MULTIVARIATE ANALYSIS OF THE SCUTELLINIA UMBRORUM COMPLEX (PEZIZALES, ASCOMYCETES) FROM FIVE ECOTOPES IN THE NETHERLANDS

JAANUS PAAL[1], BELLIS KULLMAN[2] & HENK A. HUIJSER[3]

The multivariate structure of the *Scutellinia umbrorum* complex (*Pezizales, Ascomycetes*), based on the morphometrical parameters of 81 specimens from five ecotopes in the Netherlands, was analysed. According to conventional expert estimation, five putative taxa resp. species were established: *S. patagonica* (Rehm) Gamundi, *S.* aff. *subhirtella* Svrček, *S. umbrorum* (Fr.) Lambotte, *S. parvispora* J. Moravec and *S. subhirtella* s. Kullman. These taxa form a taxonomic continuum hardly separable by traditional taxonomy. Five clusters obtained by UPGMA (with the generalized J-distance for mixed data as a measure of resemblance) are more distinct and in good accordance with ecological factors; some of them, however, are statistically not well separated. The revision of the clusters' structure by k-means approach yields highly discontinuous clusters. The morphometric characters of specimens differ when going from open habitats to the forest. Differences are also revealed in phenology: the growing season starts in the forest later than in open habitats. The data are divided into two subsets according to spore ornamentation and spore width, the withingroup variation of either subset is caused mainly by the length of marginal hairs. On the basis of several statistical methods a supposition was introduced that the *S. umbrorum* complex probably consists of two polymorphic species, *S. umbrorum* (Fr.) Lambotte and *S. subhirtella* Svrček s.l., with the mean value of marginal hairs longer than 450 µm and shorter than 450 µm, respectively. The UPGMA clusters can be interpreted as ecodemes of respective species.

INTRODUCTION

The systematics of the order *Pezizales* (*Ascomycetes*) is disputable and rather unstable (Kimbrough & Gibson, 1989; van Brummelen, 1994; Bunyard et al., 1995). The separation of some species is based on the morphological description of one or two specimens only. The result is a multitude of putative taxa. When studying more abundant material of closely related species in *Pezizales*, an extensive variation of morphological characters becomes evident.

Taking into account that the *Pezizales* have "a virtually invariant haplophase with predominantly homomictic mating" (Burnett, 1987; Weber, 1992) it may be considered that these fungi exist in natural conditions as lineages, i.e. as groups of clones with the same DNA haplotype. In the genus *Scutellinia* (Cooke) Lambotte emend. Le Gal there are homothallic species, e.g. *Scutellinia scutellata* (Fr.) Lambotte (Gwynne-Vaughan & Williamson, 1933).

The evolutionary pattern of *Scutellinia* follows continuous divergence, the substrate being the most important natural selection factor for these saprotrophic fungi (Kullman,

1) Institute of Botany and Ecology, University of Tartu, Lai St. 40, EE-2400 Tartu, Estonia.
2) Institute of Zoology and Botany, Riia St. 181, EE-2400 Tartu, Estonia.
3) Frederikstraat 6, 5671 XH Nuenen, The Netherlands.

1979, 1982, 1986; Kullman & Rahi, 1988a, 1988b, 1989, 1990; Schumacher, 1990). Sometimes response to the substrate and other environmental conditions is expressed also by local ecotypical variability which is revealed in pronounced morphological difference (Stebbins, 1950; Kullman, 1977, 1995).

The present paper aims to (i) analyse the structure of a comparatively large data set of the *Scutellinia umbrorum* complex, hardly distinguishable morphologically, (ii) test the statistical significance of clusters obtained by different phenetic approaches of classification of these data, and (iii) analyse the dependence of data structure on substrate and habitat conditions.

MATERIAL AND METHODS

Study sites

The material was collected in the Netherlands during 1979–89. Five localities, constituting a trophic gradient from ecotopes poor in nutrients to richer ones were studied:

(i) Best-Son (denoted as B). Former loam-pit of brick-work. The habitat represents a deposition of loam or loamy sand being permanently wet or drying slowly after the removal of loam. Only pioneer vegetation has formed, dominated by *Equisetum* spp., *Salix* spp., *Alnus glutinosa*, mosses, etc. 16 specimens collected.

(ii) Nuenen (N). Abandoned loam-pit. Habitat and vegetation quite similar to that of Best Son. 16 specimens collected.

(iii) Eindhoven, Urkhoven (E). Paludified meadow with a sparse field layer on sandy-loamy soil. Traversed by car-tracks. Transition zone between wet heathland and reed swamp. The main vascular species are: *Pedicularis* spp., *Gentiana pneumonanthe*, *Dactylorhiza maculata*, *Platanthera bifolia*, *Carex* spp., *Phragmites australis*, *Sphagnum* spp. 7 specimens collected.

(iv) Helmond, De Schouw (H). Rather open moderately eutrophic moist meadow (hayland) on sandy-loamy soil. Traversed by car-tracks. Dominant vasculars are: *Carex* spp., *Equisetum* spp., and *Dactylorhiza majalis*. 25 specimens collected.

(v) Elsloo-Geulle, Bunderbos (L). Eutrophic forest slope (with calcareous springs) on slowly drying black mud along a regularly overflowing brooklet. Abundant vasculars are: *Equisetum telmateia*, *Impatiens noli-tangere*, *Chrysosplenium* sp., and *Allium ursinum*. 16 specimens collected.

Measurements

The characteristics of fungal fruit-bodies were measured with the microscope 'Olympus BH-2', magnification up to 1000 ×. Tap water was used as an observation medium. For all 81 specimens, 4 morphological parameters were measured: length of marginal hairs on the apothecium (PILL), width of marginal hairs on the apothecium (PILW), spore length (SPOL), spore width (SPOW). For every specimen all morphological parameters were measured in not less than ten replications. The mean values were used as initial data for multivariate analysis. A subtracted parameter, the ratio of spore length to spore width (Q), was also included in the data matrix. Additionally, the type of spore ornamentation (OR) was identified as (i) tuberculate, (ii) verrucose, and treated as a binary variable. The type of ornamentation was studied by staining the spore wall in cotton blue solution in lactophenol as well as from photographs of the scanning electron microscope (Figs. 1 and 2).

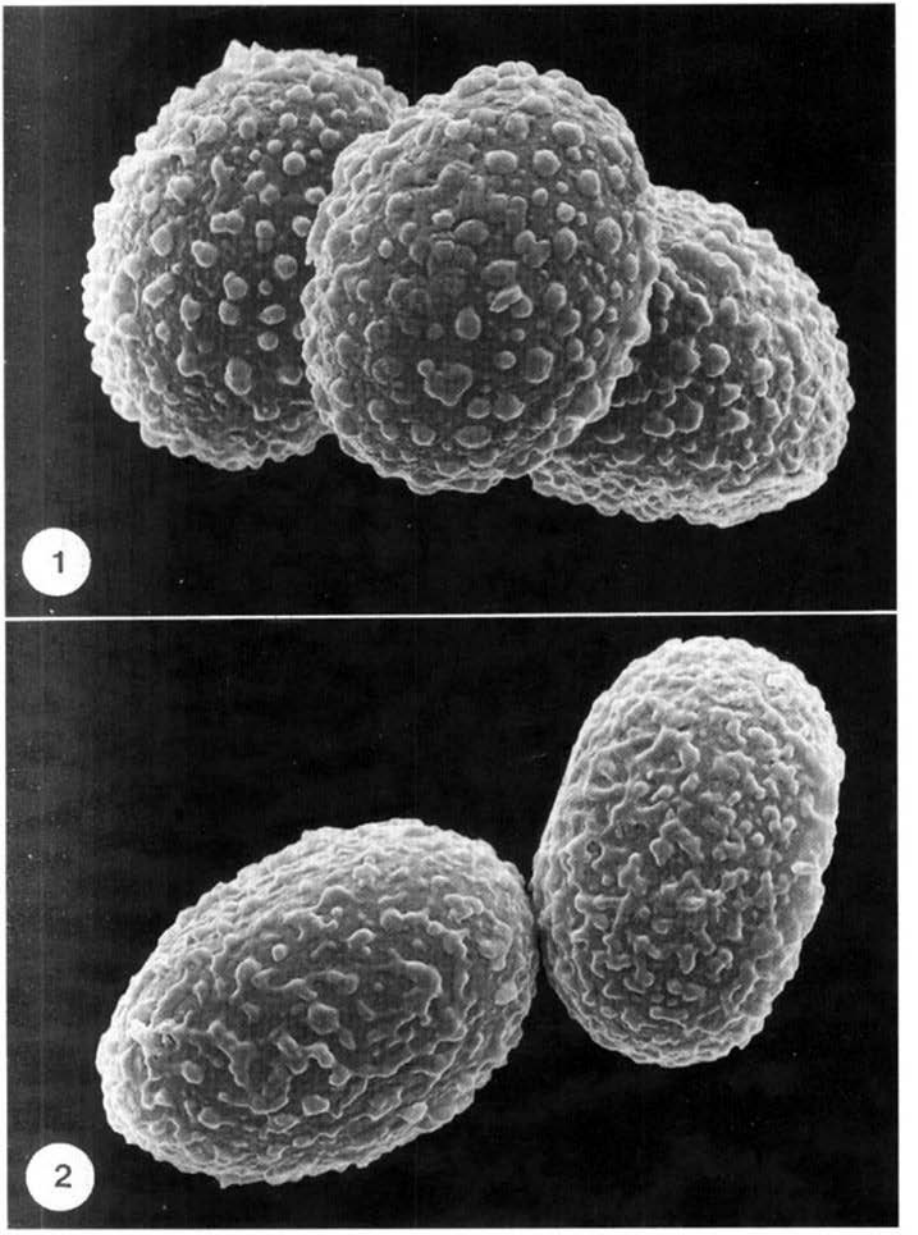


Fig. 1. Spores with a tuberculate type of ornamentation. Specimen of *S. parvispora* according to the expert estimation. Magnification 3000 × (15 mm = 5 µm). — Fig. 2. Spores with a verrucose type of ornamentation. Specimen of *S. subhirtella* s. Kullman according to the expert estimation. Magnification 3000 × (15 mm = 5 µm).

Two nominal characteristics were used as environmental parameters. One describes the type of the ecotope according to its trophicity and openness from the loam pit to the eutrophic forest slope (see study sites: B, N, E, H, and L). The other corresponds to the substrate: S = mineral soil, O = humous soil or decaying wood, W = wood.

Data

At first the specimens (OTUs) were classified traditionally (see diagnoses below). The results of this were further taken as a basis for comparison and search for an optimal solution by numerical methods.

1. *Scutellinia patagonica* (Rehm) Gamundi (denoted as Pt)

Diagnosis: Apothecia 3–12(–20) mm. Marginal hairs 200–1160 × 18–40(–45) µm, sample means between 535–695 × 25–32 µm, average 600 × 28.8 µm, 3–28 septate; walls (2–)3–6 µm thick; base with 1–3 roots, the longest hairs often more complex. Ascospores (17.5–)18–22 × 15–18.5 µm, sample means between 19.4–20.7 × 15.7–17.5 µm, average 20.1 × 16.7 µm; Q = 1.1–1.3, means between 1.14–1.27, average 1.20; ornamentation tuberculate, warts of different size rarely confluent 0.5–1.2(–1.5) µm high and 0.3–1.7(–2.5) µm wide. Habitat H.

These characteristics are in good agreement with *S. patagonica* as described by Gamundi (1975) and Schumacher (1990), but also correspond with *S. arenosa* (Velen.) Le Gal (Le Gal, 1966b and Moravec, 1974).

2. *Scutellinia* aff. *subhirtella* Svrček (denoted as Sb)

Diagnosis: Apothecia 1.5–10(–15) mm. Marginal hairs 110–650(–720) × 12–35 µm, sample means between 255–465 × 19–27.4 µm, average 360 × 23.6 µm, (0–)2–14 septate; walls 1–5 µm thick; base usually with 1–3 roots, exceptionally more complex. Ascospores (17–)18–23(–24) × (13–)14–16.5(–17.5) µm, sample means between 18.9–22.5 × 14.3–16.3 µm, average 20.5 × 15.3 µm; Q = 1.2–1.5(–1.65), means between 1.26–1.46, average 1.34; ornamentation heterogeneous tuberculate(-verrucose), variable warts of different size and shape 0.3–1.0 µm high and 0.3–2.0 µm wide, often isolated but sometimes confluent, forming short ridges and irregular complexes 1–1.5 µm high and 2–3(–4) µm wide. Habitats B and N.

The marginal hairs fit well Schumacher's (1990) description of *S. subhirtella* Svrček, but spore shape and ornamentation deviate too much and look more like his *S. umbrorum*. Because of this resemblance such collections have certainly been conceived as *S. umbrorum* (cf. Le Gal, 1947; Maas Geesteranus, 1969; they might even represent the real *Peziza umbrorum* Fries, 1823).

3. *Scutellinia umbrorum* (Fr.) Lambotte (denoted as Um)

Diagnosis: Apothecia 3–16(–20) mm. Marginal hairs 200–1120 × 16–43 µm, sample means between 430–745 × 23–30.4 µm, average 568 × 25.9 µm, 2–29 septate; walls 2–7 µm thick; base with 1–3 conspicuous roots but longest hairs also multifurcate. Ascospores 18.5–24 × (13–)13.5–17.5 µm, sample means between 20.4–22.5 × 14.1–16.4 µm, average 21.3 × 15.5 µm; Q = 1.25–1.55(–1.7), means between 1.30–1.47, average 1.38; ornamentation tuberculate, mostly isolated semiglobose or slightly angular warts of different size 0.5–1.5(–2.5) µm high and 0.5–2(–3) µm wide, sometimes crateriform.

Habitats H and E. In H often growing together with *S. patagonica* which starts fruiting one or two weeks earlier and differs mainly in the lower Q-value for the ascospores. During the research period no mixing of the two species could be observed.

These characteristics correspond well with the descriptions of *S. umbrorum* by Denison (1959) and Kullman (1982), which are based on the neotypification of this species by the material of 'Ellis & Everhart's North American Fungi' No. 2911 in CUP. According to both authors the ascospores of the neotype clearly show a tuberculate ornamentation. Le Gal (1966a), however, found the ornamentation to be more confluent warty, like in *Scutellinia ampullacea* (Limm. ex Cooke) O. Kuntze and therefore rejected Denison's neotype. The diagnosis above does also correspond with the descriptions by Le Gal (1966a) and Schumacher (1990), which are based on another neotypification of *S. umbrorum* by Le Gal (1966a) with the material of Boudier's Icones mycologicae 2: plate 369, 1906 (leg. Boudier, Montmorency, 1883, "No. 369", in Herb. Boudier, PC). Unfortunately this shows different or poorly developed marginal hairs.

4. *Scutellinia parvispora* J. Moravec (denoted as Pr)

Diagnosis: Apothecia 3–13 mm. Marginal hairs 200–950 × 15–38 µm, sample means between 385–665 × 21.3–30.6 µm, average 510 × 26.3 µm, (0–)2–22 septate; walls (1–)2–7 µm thick; base with 1–3 roots sometimes more complex. Ascospores 17–22 × 13.3–16.6 µm, sample means between 18.1–20.6 × 14.1–15.5 µm, average 19.9 × 14.6 µm; Q = 1.25–1.50, means between 1.27–1.40, average 1.36; ornamentation rather homogeneous tuberculate, mostly isolated semiglobose warts 0.3–1.2 µm high and 0.5–1.5(–2) µm wide. Habitat L.

Except for the habitat and the somewhat smaller spores, the differences with *S. umbrorum* are marginal.

5. *Scutellinia subhirtella* Svrček s. Kullman (denoted as Sk)

Diagnosis of the first morph: Apothecia (2–)4–17 mm. Marginal hairs 150–1000 × 11–33 µm, sample means between 340–650 × 18.3–25.6 µm, average 505 × 22.8 µm, 3–31 septate; walls (1.5–)2–6 µm thick; base with 1–3 roots, the longest hairs more complex. Ascospores 17.5–23.5 × 12.5–16 µm, sample means between 19.4–21.8 × 13.7–15.1 µm, average 20.4 × 14.2 µm; Q = 1.3–1.6(–1.7), means between 1.38–1.50, average 1.43; ornamentation confluent verrucose, like *S. scutellata* up to 1.0(–1.5) µm high. Habitat mostly wet woodland with *Alnus* and *Salix* surrounding B, H and E, but also in H.

Diagnosis of the second morph: Apothecia 2–6(–8) mm. Marginal hairs 100–465 × 14–28 µm, sample means between 245–365 × 17.5–22 µm, average 295 × 20.6 µm, 2–10 septate; walls 1.5–5 µm thick; base with 1–3 roots very rarely more complex. Ascospores 19–21.8 × 13.4–15 µm, sample means between 20.0–20.7 × 13.9–14.3 µm, average 20.3 × 14.1 µm; Q = 1.3–1.6, means between 1.40–1.47, average 1.44; ornamentation confluent verrucose up to 0.5(–1) µm high. Habitat L on drier mossy wood of *Fraxinus*(?).

These morphs correspond well with Kullman's (1982) interpretation of *S. subhirtella* Svrček. According to Schumacher (1990) who studied the type, the spore ornamentation is tuberculate instead of confluent verrucose. In his monograph no description could be found which fully fits the above mentioned characteristics. Perhaps it has been included in his conception of *S. olivascens* (Cooke) O. Kuntze (= *S. ampullacea* (Limm. ex Cooke)

O. Kuntze) which shows broader hairs and larger spores. Recent type studies of both *S. olivascens* and *S. ampullacea* by Yao & Spooner (1996) also demonstrated somewhat larger ascospores. The larger apothecia of the Dutch specimens with hairs up to 1000 µm look very much like *S. umbrorum*, but differ in the slightly more slender marginal hairs and spore ornamentation.

DATA PROCESSING

Cluster analysis

For classification the unweighted average linkage method (UPGMA) was used, where the process of specimens fusion is based on the minimum average distance between specimens and clusters. By this method cluster sizes are considered while recalculating values in the distance matrix. If cluster i has 1, cluster j has 4 and cluster h has 2 specimens, then for calculating the distance between newly formed cluster ij and cluster h, d_{jh} must be considered twice as much as d_{ij} (Podany, 1994).

For optimization of UPGMA-clusters as well as, for testing the stability or invariantness of classification, the k-means clustering (MacQueen, 1967) was used. This method minimizes the within-cluster variation (sum of squares). The process is iterative: as initial centers (means) of clusters the centroids of UPGMA-clusters were exploited. Then, in each step of the procedure it is examined if the relocation of any specimen from one cluster to another provides decrease of the sum of squares. The object for which maximum decrease may be achieved is moved to the new group. The iterations stop if no further reduction is possible.

To measure the fit of hierarchical UPGMA dendrogram to the originating distance matrix **D**, the matrix of ultrametric distances **C** was computed. The last matrix consists of elements c_{ij}, which are defined to be the first level in the dendrogram at which specimens i and j occur in the same cluster (Everitt, 1993). Then, the product moment correlation or the cophenetic correlation (Sokal & Rohlf, 1962) between these two matrices was calculated.

Both classifications, as well as estimation of cophenetic correlation were realized by SYN-TAX 5.0 program package (Podany, 1993).

Ordination

To visualize graphically the specimens' mutual relationship in multidimensional character space, principal components analysis (PCA) of $\ln (1 + x_{ij})$ transformed data was exploited. By means of PCA new 'artificial' variables (scores) are computed on the bases of the original data in attempt to achieve a more efficient representation of data in few dimensions (principal components, shown in figures as axes of the ordination). For ordination CANOCO package, version 3.1 (ter Braak, 1988, 1990), and CANODRAW package, version 3.0 (Smilauer, 1992) were used.

Estimation of adjacency

When focusing on taxonomic continuum we usually do not mean all possible transitions between clusters but only relations between clusters which are most similar, or adjacent in the character space. Thus, the number of clusters to which a specimen or an operational taxonomic unit (OTU) with intermediate characteristics can belong is always smaller than the total number of clusters. Numerical analysis requires a formal criterion for deciding whether the clusters should be regarded as adjacent.

One can postulate: the j-th cluster is treated as adjacent to the i-th cluster if the distance between at least one of the OTUs of the i-th cluster and the centroid of the j-th cluster is smaller than the distance to the centroids of all other clusters (Paal & Kolodyazhnyi, 1983; Paal, 1994). This definition of adjacency is non-symmetric: if the j-th cluster is adjacent to the i-th cluster, the latter need not necessarily be adjacent to the OTUs belonging to cluster j.

According to such a criterion the distance of all OTUs from all centroids (except the cluster to which the OTU belongs) can be calculated and the adjacent clusters estimated. The results are presented in the form of the adjacency matrix.

Testing of clusters' distinctness

In order to measure the degree of distinctness the α-criterion (Duda & Hart, 1976) was used. To acquire a better interpretation of the estimates, it is more convenient to apply the corresponding probabilities as coefficients of indistinctness (CI) instead of direct values (Paal, 1987, 1994):

$$CI = 100 / \sqrt{2 \pi \int \exp(-x^2/2) dx}. \quad (1)$$

To visualize the distribution of OTUs located between the centroids of two adjacent clusters in the character space, the split window method (Parzen, 1962) appears appropriate. The density of the OTUs projection probability distribution on a straight line passing through the centroids of both clusters can be calculated as

$$p(x) = 1/n \sum_{i=1}^{n} (1/h) \Phi [(x-x_i)/h], \quad (2)$$

where p(x) is the distribution density at point x, Φ – the window function, h – the smoothing parameter or window breadth, n – the number of OTUs in the cluster, x_i – the projection of the i-th OTU on the line. The density of the normal distribution was regarded as the window function.

The smoothing parameter h was determined according to the formula:

$$h = 2s (0.05 + 1/\sqrt{n}), \quad (3)$$

where s is the standard error of the projections. The density of projection probability for the OTUs of either cluster was calculated for the line segment ± 3s for every 0.1 unit of the standard error. Normalization to the standard error makes it possible to estimate the expression of distinctness independently of the number of OTUs in the cluster.

The transition zone between centroids, denoted by two dotted lines perpendicular to the line connecting the centroids in figures is estimated so that its length is exactly half the distance between the centroids but, in depending on the within-group dispersion rate, the transition zone shifts toward one or the other centroid (Paal & Kolodyazhnyi, 1983; Paal, 1987).

RESULTS

Expert classification

Adjacent taxa for *S. patagonica* (cluster 1) are *S. umbrorum* (cluster 3) and *S. parvispora* (cluster 4) (Table I). All these three taxa are mutually insufficiently separated (Table II). The distribution of OTUs projection probability on a line joining the centroids of these clusters is multimodal and largely overlapping. An example is shown in Fig. 3.

Table I. Adjacency matrices of clusters obtained by different classification procedures. Figures in the matrix indicate the percentage of OTUs of the analysed cluster for which the centroid of the compared cluster is the nearest in the character space.

Cluster analysed	Cluster compared				
	1	2	3	4	5
Expert classification					
1	–	–	66.7	33.3	–
2	–	–	–	–	96.2
3	56.3	31.3	–	12.5	–
4	12.5	25.0	37.5	25.0	–
5	–	40.9	22.7	31.8	–
UPGMA classification					
1	–	–	100	–	–
2	–	–	–	76.8	21.4
3	50.0	–	–	9.1	36.4
4	–	100	–	–	–
5	7.7	7.7	84.6	–	–
k-means classification					
1	–	–	100	–	–
2	–	–	–	75.9	24.1
3	52.4	–	–	9.5	38.1
4	–	100	–	–	–
5	7.7	7.7	84.6	–	–

Table II. Coefficients of indistinctness between the taxa of *S. umbrorum* complex. Below the diagonal: evaluation of clusters estimated by the expert classification, above the diagonal: evaluation of clusters obtained by UPGMA.

Cluster	1	2	3	4	5
1	X	0.0	60.7	0.0	0.7
2	0.0	X	0.0	10.2	0.0
3	63.5	0.0	X	0.0	76.0
4	10.9	0.0	48.0	X	0.0
5	0.2	9.8	2.1	48.7	X

For specimens of the *S.* aff. *subhirtella* Svrček (cluster 2), a single adjacent cluster is 5, *S. subhirtella* s. Kullman (Table I), from which it is separated non-significantly (Table II).

The main neighbour in the character space for cluster 3 is cluster 1; adjacency is seen also with *S.* aff. *subhirtella* Svrček, and with *S. parvispora,* i.e. with clusters 2 and 4 (Table I).

Cluster 4, *S. parvispora*, is the most diffuse, varying in different directions and being adjacent to all other clusters to an almost equal degree (Table I). It is distinctly separated only from cluster 2, *S.* aff. *subhirtella* Svrček (Table II).

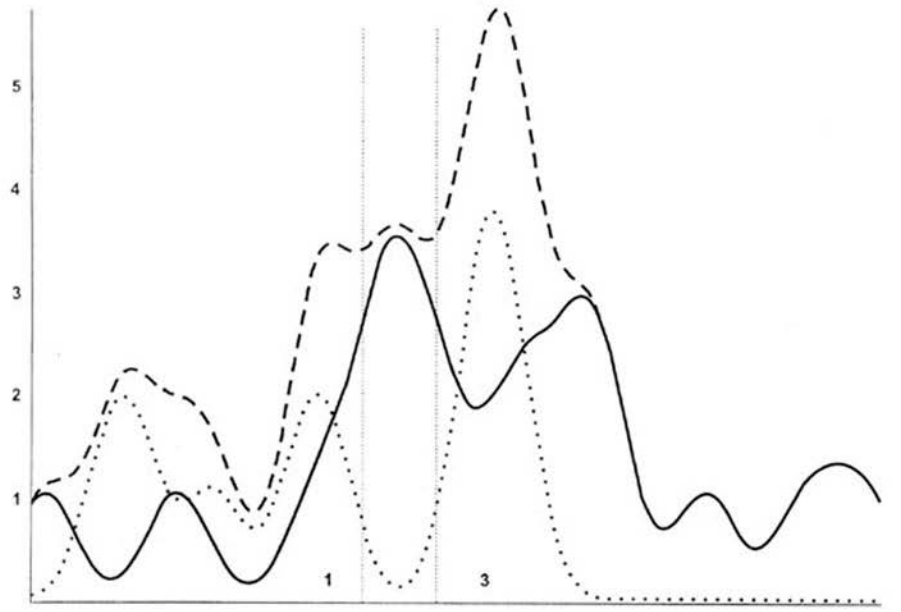


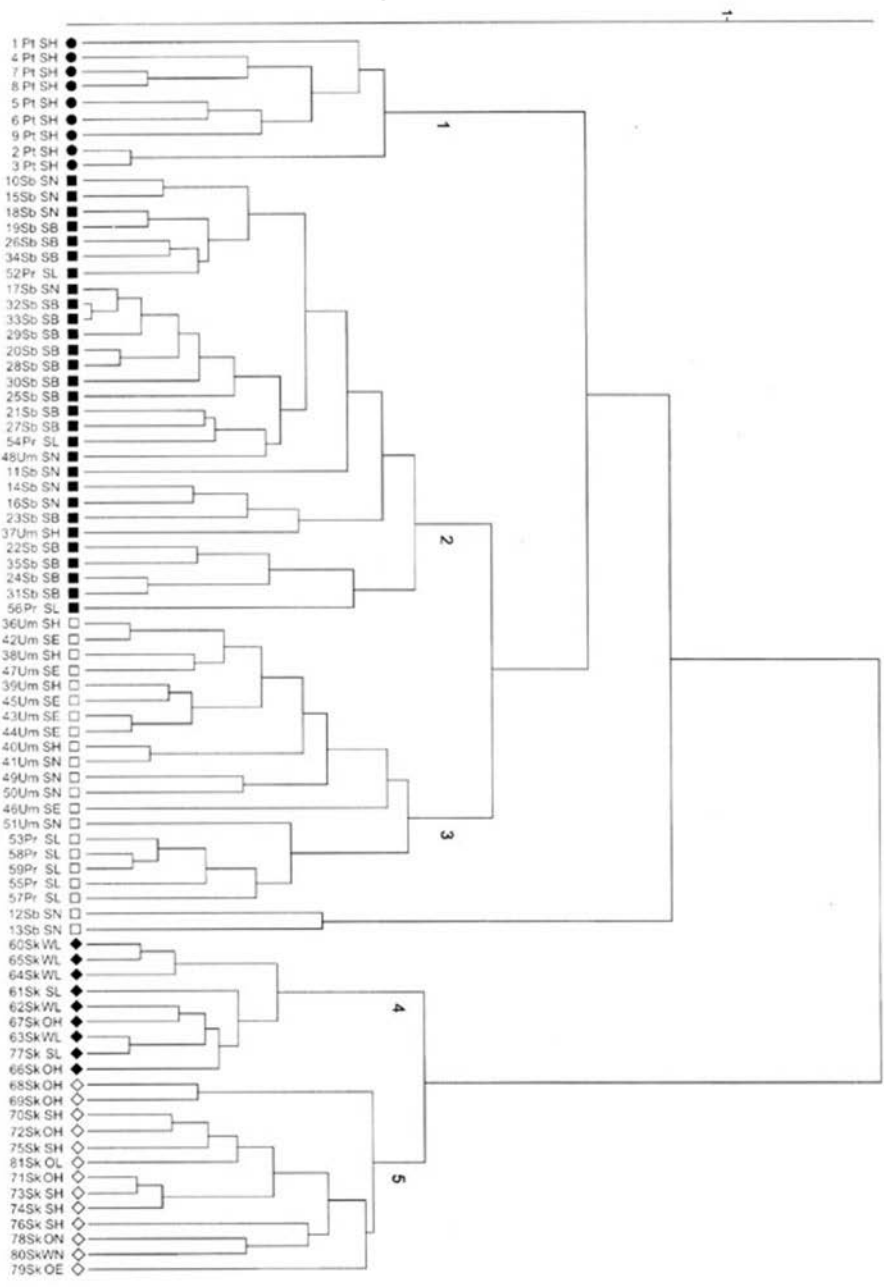
Fig. 3. OTUs projections probability distribution according to the split window method. The curve marked with dots portrays the left-hand cluster, the continuous curve corresponds to the right-hand cluster. The curve with short lines above them represents the OTUs projection probability distribution of the joint cluster. Clusters 1 and 3 of the expert classification.

For the OTUs of cluster 5, *S. subhirtella* s. Kullman, cluster 2, *S.* aff. *subhirtella* Svrček, cluster 3, *S. umbrorum*, and cluster 4, *S. parvispora* are adjacent (Table I). For cluster 5 indistinctness appears with respect to cluster 2 and cluster 4 (Table II).

These results allow to conclude that the adjacency matrix generally conforms with coefficients of indistinctness. On the basis of the adjacency matrix asymmetric relationship between clusters becomes apparent. Thus, cluster 5 is adjacent for 96% of the specimens of cluster 2, whereas cluster 2 is adjacent only for 41% of the specimens of cluster 5. It appears, too, that the taxa estimated according to the expert classification are rather insufficiently separated. Only *S.* aff. *subhirtella* Svrcek has no more than one indistinct relation with other taxa, whereas the other taxa have two, or even three, indistinct relationships like cluster 4, *S. parvispora*.

UPGMA *classification*

The dendrogram received by UPGMA is split into two large subsets at very high level of dissimilarity (Fig. 4). Further, the first of them is divided into three clusters, two OTUs (12 and 13) providing an additional separate branch. (Development of these apotheciums


1
1 Pt SH
4 Pt SH
7 Pt SH
8 Pt SH
5 Pt SH
6 Pt SH
9 Pt SH
2 Pt SH
3 Pt SH
10Sb SN
15Sb SN
18Sb SN
19Sb SB
26Sb SB
34Sb SB
52Pr SL
17Sb SN
32Sb SB
33Sb SB
29Sb SB
20Sb SB
28Sb SB
30Sb SB
25Sb SB
21Sb SB
27Sb SB
54Pr SL
48Um SN
11Sb SN
14Sb SN
16Sb SN
23Sb SB
37Um SH
22Sb SB
35Sb SB
24Sb SB
31Sb SB
56Pr SL
36Um SH
42Um SE
38Um SH
47Um SE
39Um SH
45Um SE
43Um SE
44Um SE
40Um SH
41Um SN
49Um SN
50Um SN
46Um SE
51Um SN
53Pr SL
58Pr SL
59Pr SL
55Pr SL
57Pr SL
12Sb SN
13Sb SN
60Sk WL
65Sk WL
64Sk WL
61Sk SL
62Sk WL
67Sk OH
63Sk WL
77Sk SL
66Sk OH
68Sk OH
69Sk OH
70Sk SH
72Sk OH
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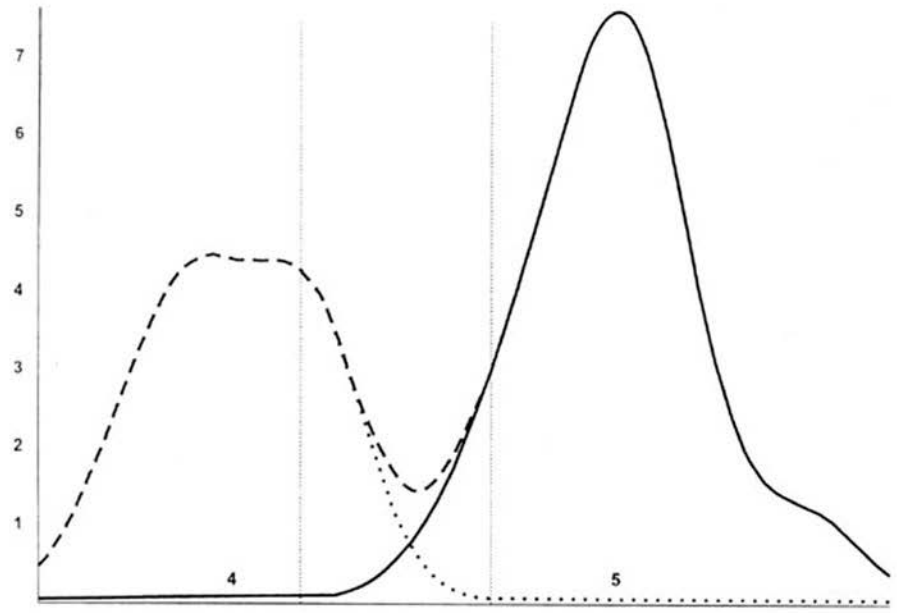

Fig. 5. OTUs projection probability distribution according to the split window method. Clusters 4 and 5 of the UPGMA classification.

is somewhat disturbed, many asci contain only 2–6 mature spores.) The second subset comprises two clusters. Cluster 1 incorporates all specimens of the preliminarily estimated *S. patagonica* but no specimens of the other putative taxa. Cluster 2 includes mainly specimens of *S.* aff. *subhirtella* Svrček of the expert identification, cluster 3 (plus OTUs 12 and 13) merges specimens of two previously recognized taxa, *S. umbrorum* and *S. parvispora*. The specimens assumed to belong to *S. subhirtella* s. Kullman are divided into clusters 4 and 5.

Changes in cluster structure are reflected plainly in the adjacency matrix (Table I). For the objects of cluster 1 (*S. patagonica*) the only neighbour is now cluster 3 (*S. umbrorum* + *S. parvispora*). On the other hand, cluster 1 is also the main neighbour for the specimens of cluster 3, but only for 50% of its OTUs. These clusters are separated non-significantly ($CI_{1,3} = 60.7$, Table II).

For the objects of cluster 2 (*S.* aff. *subhirtella* Svrček) cluster 4 becomes the most adjacent. For the OTUs of cluster 4 only cluster 2 is adjacent, but not cluster 5 as we could

Fig. 4. Dendrogram of UPGMA clustering based on the generalized J-distance for mixed data. The specimen's label includes its number in the sample, abbreviation of empirically estimated taxon name, denotation of substrate, and denotation of habitat. The cluster numbers are marked beside the branches of the dendrogram, the specimens of different clusters are denoted with various symbols.

Table III. Clusters obtained by k–means classification procedure. For the specimen's label cf. Fig. 4.

Cluster	Specimen				
1	1Pt SH	4 Pt SH	7 Pt SH	8 Pt SH	46Um SE
	49Um SN	50Um SN	51Um SN	57Pr SL	81Sk HL
2	14Sb SN	16Sb SN	17Sb SN	20Sb SB	21Sb SB
	22Sb SB	23Sb SB	25Sb SB	26Sb SB	28Sb SB
	29Sb SB	32Sb SB	33Sb SB	35Sb SB	52Pr SL
	56Pr SL	63Sk WL	66Sk OH	67Sk OH	77Sk SL
3	2Pt SH	3Pt SH	5Pt SH	6Pt SH	9Pt SH
	36Um SH	38Um SH	39Um SH	40Um SH	41Um SN
	42Um SE	43Um SE	44Um SE	47Um SE	53Pr SL
	58Pr SL	59Pr SL	68Sk OH	70Sk SH	71Sk OH
	72Sk OH	73Sk SH	75Sk SH	76Sk SH	78Sk ON
4	10Sb SN	11Sb SN	12Sb SN	13Sb SN	15Sb SN
	18Sb SN	19Sb SB	34Sb SB	60Sk WL	61Sk SL
	62Sk WL	64Sk WL	65Sk WL		
5	24Sb SB	27Sb SB	30Sb SB	31Sb SB	37Um SH
	45Um SE	48Um SN	54Pr SL	55Pr SL	69Sk OH
	74Sk SH	79Sr OE	80Sk WN		

expect, because both clusters (4 and 5) were referred to in the expert classification as belonging to *S. subhirtella* s. Kullman. Clusters 2 and 4 represent another pair of indistinct clusters ($CI_{2,4} = 10.2$); at the same time, clusters 4 and 5 are separated even more significantly ($CI_{4,5} = 0.0$, Table II) than several of the putative species. The probability of OTUs projection distribution of both clusters is almost normal, and the overlapping of the corresponding curves quite limited (Fig. 5). According to the adjacency matrix (Table I), cluster 5 is now closely related to cluster 3, both constituting the third indistinct pair of clusters ($CI_{3,5} = 76.0$, Table II).

If the OTUs 12 and 13 are excluded from cluster 3 as outliers, the classification structure will not be considerably more distinct. The same cluster pairs will remain indistinct, only for clusters 3 and 5 the coefficient of indistinctness will be lower ($CI_{3,5} = 29.4$, Table II).

Taking into account that "hierarchical clustering techniques impose a hierarchical structure on data and it is usually necessary to consider whether this is merited or whether it introduces unacceptable distortions of the original relationships amongst the individuals, as implied by their observed proximities" (Everitt, 1993: 72), the cophenetic correlation coefficient was calculated. In the present case the coefficient is 0.90. This value is close to the upper bound of the range (0.74–0.90) of most frequently occurring cophenetic correlations (Sneath & Sokal, 1973). Rohlf & Fisher (1968) stated that values above 0.8 are sufficient to reject the null hypothesis that "The specimens represent a random sample from a single multivariate normal distribution." In that way, we can conclude that the obtained UPGMA dendrogram is in good accordance with the real structure of data, and the loss of information due to arranging the specimens into a hierarchical classification system is rather limited.

K-means classification

A further attempt to optimize the classification by the k-means procedure, using the previous result as the initial group membership vector, enables to get clusters which are all well distinct, with the coefficient of indistinctness close to zero. However, now correspondence with the empirical classification is much weaker than in the case of UPGMA results (Table III). Still, adjacency relations for clusters obtained on the basis of the k-means algorithm will remain rather similar to the relations established for UPGMA clusters (Table I).

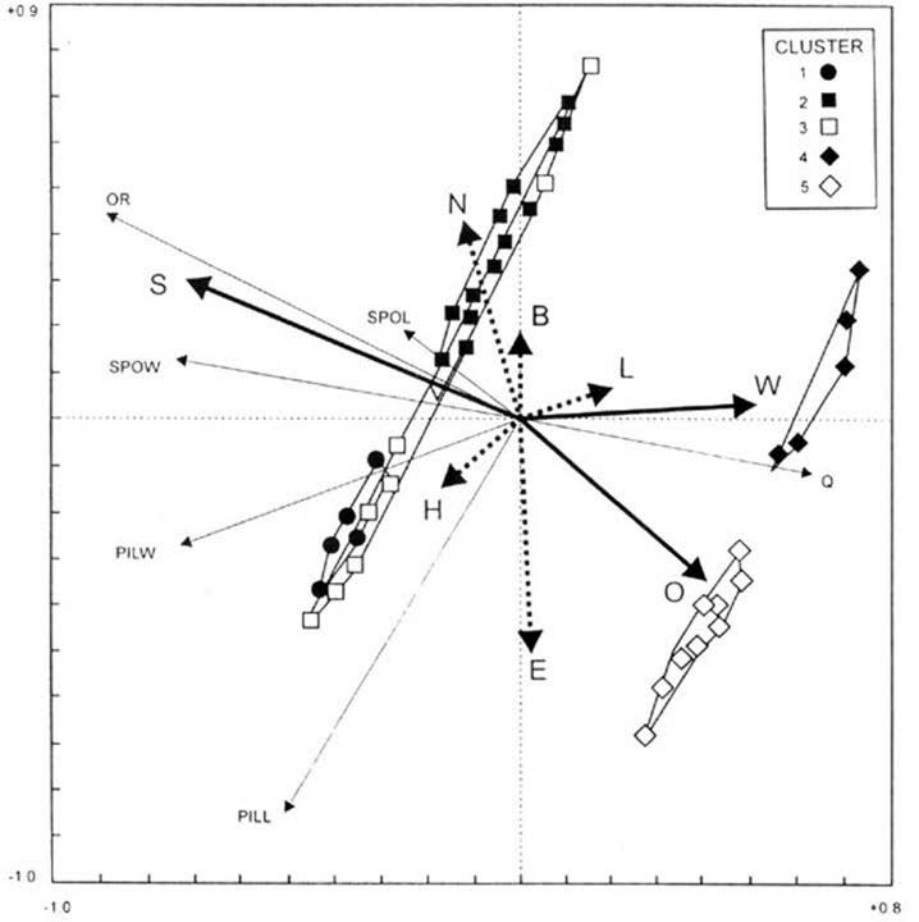

Fig. 6. Classification polygons superimposed onto a PCA ordination and specimen-character-environmental variables triplot. Clusters obtained by UPGMA. The first component (abscissa) explains 55.0% of the total variation and the second component (ordinate) 39.7%.

Ordination analysis

The PCA demonstrates that the whole sample consists of two obviously separated subsets, both having parallel variation (Fig. 6). The two-dimensional solution of PCA is well acceptable, as the first axis accounts for 55.0% of total variance, and the second axis for 39.7%. Amount of variation connected with the next axes is much smaller: the third axis explains 3.3% of variation and the fourth 1.2%. Therefore, these axes are not considered further. The subsequent superposition of clusters onto the ordination plot (Figs. 6 & 7) enables to visualize the issue of clusters estimated by different methods.

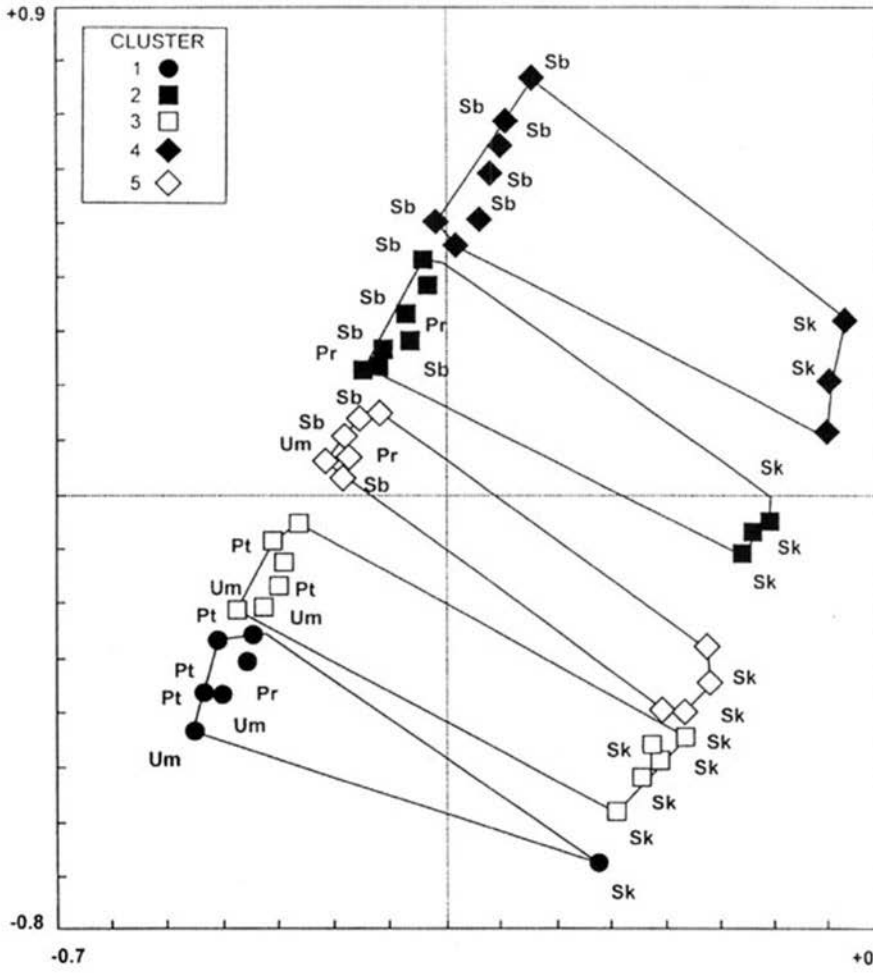

Fig. 7. Classification polygons superimposed onto a PCA ordination. Clusters obtained by the k-means algorithm.

The principal similarity between the classification structure achieved empirically and the one achieved by UPGMA is conspicuous. In both cases the specimens of the bigger subset are separated into clusters parallel to the axis of within-group variation, while the OTUs of several putative taxa are to some extent intermixed (Fig. 6). The OTUs of the smaller subset remain all in a separate cluster or clusters. Parallel variation explains also the large overlapping of the OTUs projection probability distribution (cf. Fig. 3).

K-means clustering provides spherical clusters; in this case we cannot expect that revision of the cluster structure obtained by the hierarchical UPGMA algorithm will produce the same solution as the sum of squares criterion followed by k-means clustering (Podany, 1994). According to the k-means solution, the elongated subsets of OTUs are divided perpendicularly to the direction of their main variation, and there is no overlapping of classification polygons (Fig. 7).

On the basis of the triplot method in which the distribution of the specimens and the loading of the characters for the principal axes are combined in the same figure (Fig. 6), we can get an understanding of the most important characteristics determining the data structure, and how they are related to environmental factors (Jongman et al., 1987; ter Braak, 1990). The specimens are divided clearly into two subsets according to the type of spore ornamentation (OR) and spore width (SPOW) (Figs. 6 & 8). The larger subset of

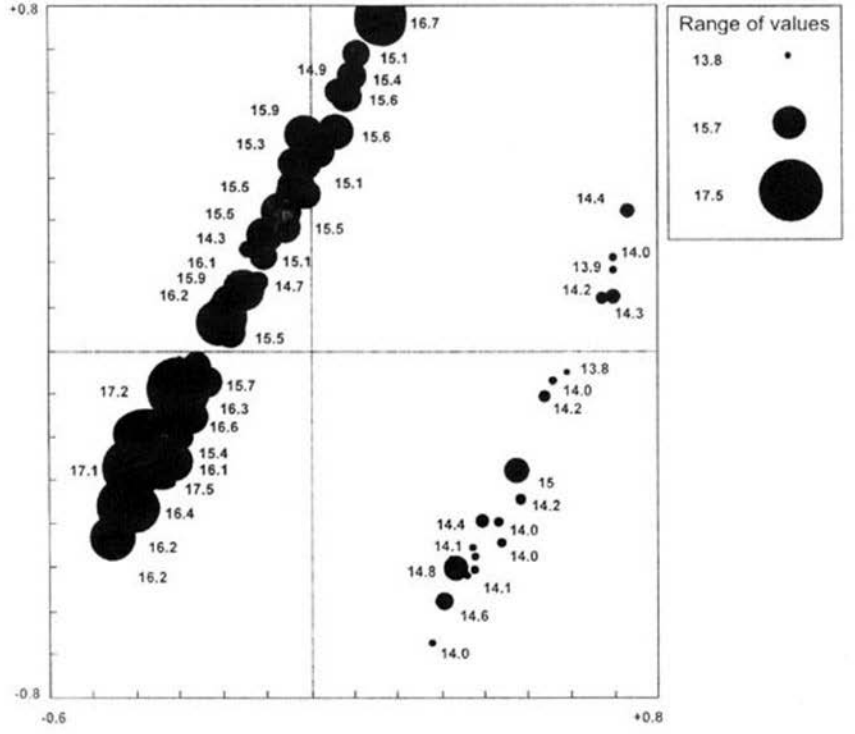

Fig. 8. Distribution of spore width values.

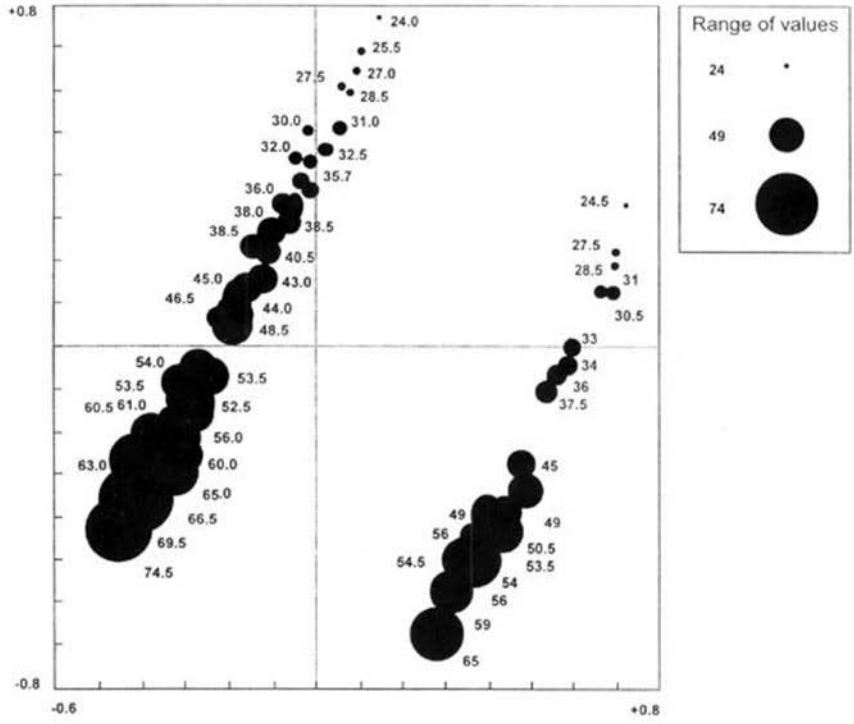

Fig. 9. Distribution of the values of length of marginal hairs. Values in the figure are 10 times lower than real values.

specimens includes only specimens with tuberculate ornamentation and wide spores; to the smaller subset belong specimens with verrucose ornamentation and much narrower spores. These variables are opposed by the ratio of spore length to spore width (Q), but the values of this substracted parameter do not have such a clear distribution pattern. The within-group variation of either subset is caused mainly by the length of marginal hairs (PILL, Figs. 6 & 9).

These results indicate that in this case UPGMA clusters are in a much better accordance with the character of the parameters' variation than the clusters obtained by k-means procedure. Now, the separation of the empirically estimated *S. subhirtella* s. Kullman into two distinct clusters also finds an explanation: it is based mainly on the length of marginal hairs. Cluster 4 includes specimens with short hairs (245–375 µm), while specimens with longer hairs (450–650 µm, Fig. 9) belong to cluster 5. In this way, these clusters correspond well to two morphs of *S. subhirtella* s. Kullman distinguished on the basis of marginal hairs length (Kullman, 1982). Besides, specimens of these clusters are evidently connected with different substrata: specimens with short hairs grow on wood under drier

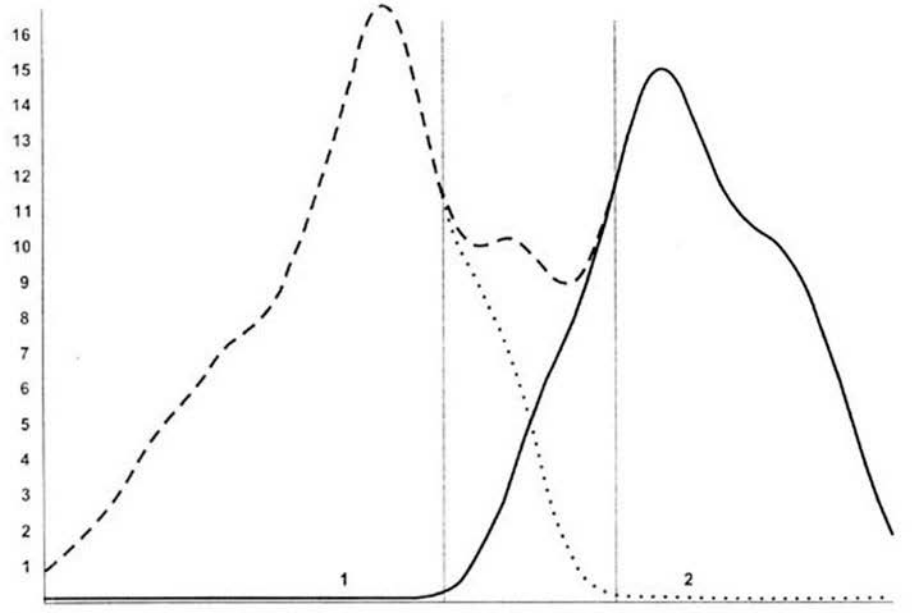

Fig. 9. Distribution of the values of length of marginal hairs. Values in the figure are 10 times lower than real values.

conditions, specimens with long hairs are related to humous soil saturated with water or wet decayed wood (Fig. 6). The specimens of three other UPGMA-clusters all grow on mineral soil with various content of humus.

The data structure is not so clearly associated with general habitat features. Still, the specimens of UPGMA-clusters 1, 3, and 5 (*S. patagonica, S. umbrorum*, and the *S. subhirtella* s. Kullman morph with long hairs) grow mainly on moist meadows (Fig. 6, H & E). Specimens of cluster 2 (*S.* aff. *subhirtella* Svrcek) are associated with loam-pits (N & B). With forest habitat (L) above all the specimens of cluster 4 (*S. subhirtella* s. Kullman morph with short hairs) are related.

According to field records, there is also a remarkable divergence in the phenology of the studied fungi, depending on the habitat type. The growth period in open habitats lasts from May till July, in the forest from June till October.

Suboptimal solution

Considerably different results obtained by two cluster analyses used as well as indistinctness between several pairs of UPGMA-clusters indicate a need to search for a suboptimal solution at a more generalized level. For this purpose we merged mutually continuous UPGMA clusters 1, 3 and 5 into one cluster, and clusters 2 and 4 into another. Testing of the reliability of these joint clusters confirms the significance of their distinctness ($CI_{1,2} = 0.0$). Reorganization of these clusters by k-means procedure did not cause such a remarkable discrepancy as it did at the five-cluster level. Now only three

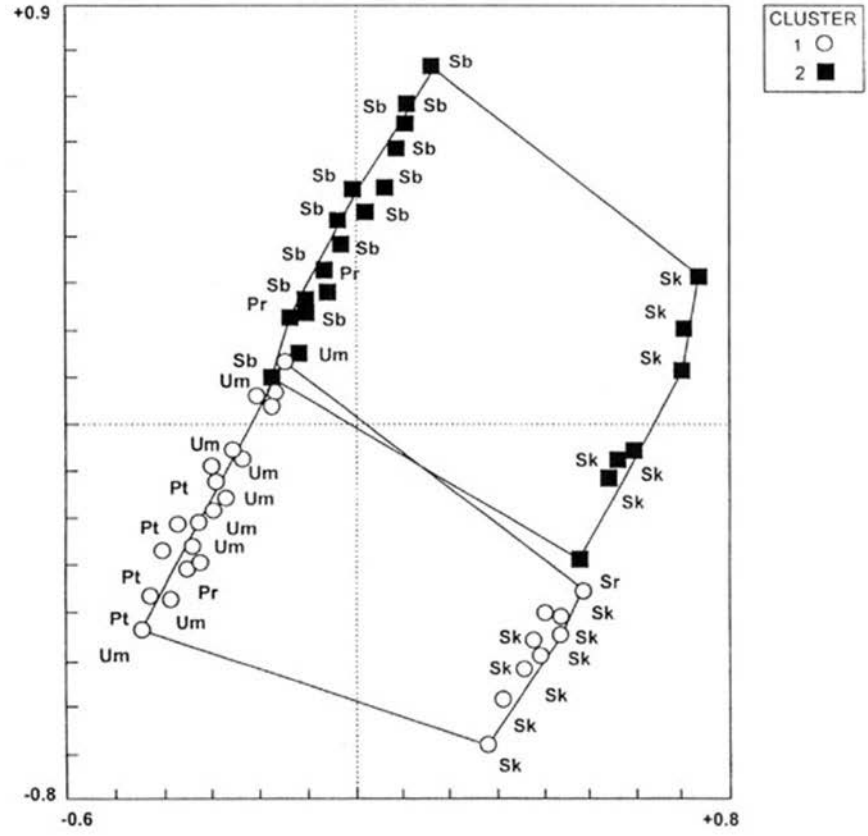

Fig. 11. Classification polygons superimposed onto a PCA ordination. Clusters obtained by the k-means algorithm at the two-cluster level.

OTUs from either cluster are shifted to another one. The coefficient of indistinctness for k-means clusters is very close to zero; the good separation of clusters is revealed by the OTUs probability distribution curves (Fig. 10) as well as by the ordination plot (Fig. 11). Remarkable similarity of the results of two classifications, obtained by rather different clustering procedures, gives a good reason to assume that now we have caught some essential or invariant features in the data structure.

DISCUSSION

The statistical indistinctness of empirically estimated taxa demonstrates the dubiousness of the conventional taxonomy of the *S. umbrorum* complex. At the same time, the present analyses show the sophisticated structure of the data even in case they are collect-

ed from a rather limited area. Several methods of clustering and comparative analysis of their results were needed to disentangle the structure of the data. Only after superimposing clusters onto the ordination plot we were able to grasp mutual relationships between them.

It is not simple to say which classification is the most appropriate. The use of cluster analysis does not simply involve the application of one particular technique, but rather necessitates a series of steps each of which may be dependent on the results of the preceding one. The final judgement of the quality of a particular result depends very much on the fact how informative it is, and what kind of new information it will give (Everitt, 1993).

It became evident that at the five-cluster level k-means clustering best provides significantly discontinuous clusters. However, it should be considered that clusters obtained by k-means procedure are approximately isodiametric, and thus we lose information about the directional variation of subsets in the character space, which is another important feature of data structure On the other hand, if the 'chain'-like configuration of subsets, related to their variation, is taken into account, not all clusters are distinctly separated. Figs. 6 and 7 illustrate convincingly these statements.

Despite the lack of discontinuity at the five-cluster level, UPGMA-clusters are ecologically better justified than k-means clusters. Also, they are in good accordance with the main features of the data structure by reflecting their division into two subsets according to spore ornamentation and width, as well as taxonomic continuum caused by the length of marginal hairs.

Still, the decision about the effectiveness of the obtained classification depends most of all at which taxonomic level we attempt to interpret the results and what the aim of a given classification is. Contradictory results obtained by UPGMA and by k-means procedure at the five-cluster level indicate difficulties in regarding either cluster as species. If the result at the two-cluster level is considered as species, the taxonomy of the *Scutellinia umbrorum* complex should be revised. Then, the first UPGMA-cluster compiling the specimens of the putative species *S.* aff. *subhirtella* Svrček and *S. subhirtella* s. Kullman morph with short marginal hairs can be called *S. subhirtella* Svrček s.l. The other cluster then joins the specimens of the putative species *S. umbrorum, S. parvispora* and *S. subhirtella* s. Kullman morph with long hairs on the apothecium, and it corresponds to *S. umbrorum* s.l. (Fr.) Lambotte. Taking into account the good relation of UPGMA-clusters obtained at the five-cluster level with habitat conditions, these clusters can be interpreted as ecodemes of respective species.

Hence, the type of spore ornamentation should not be considered the most important character for identifying species within the *Scutellinia umbrorum* complex, as it is traditionally done (Svrček, 1971; Kullman, 1982; Schumacher, 1990); instead the length of marginal hairs comes on the first plane. Thus, to *S. subhirtella* s.l. belong specimens with hairs shorter than 450 µm, to *S. umbrorum* s.l. specimens with hairs longer than 450 µm. The limit of 450 µm should certainly be taken with precaution, only as a pilot point established on the basis of data of a recently studied sample.

Unambiguous estimation of the type of spore ornamentation is quite often questionable because this character does not have clearly fixed states but is varying continuously like almost all quantitative morphological parameters. Van Brummelen (1993) has shown that the formation of spores ornamentation varies only slightly between *Scutellinia* species. Moreover, as it was proved earlier (Kullman, 1982), one ascus can contain spores with

different types of ornamentation. Restriction of the estimation of the ornamentation type to only two classes is a rather rough simplification which would overemphasize this parameter in comparison with other parameters in classification. Therefore, OTUs on the dendrogram (Fig. 4) as well as on the ordination plot are divided into two subsets namely according to their spore ornamentation type. If we established at least one more ornamentation type, the result would certainly be different. It is remarkable that the taxonomic continuum of morphs established by UPGMA appears between the very clusters which merge specimens with different types of spore ornamentation (Table II). The result suggests also that two well separated subsets of OTUs on the ordination plot are not located too far from each other in the character space. Due to the reduction of dimensionality all ordination plots represent, to some extent, a simplification of the existing relations between objects and contain an error due to which larger distances become more distorted (Paal et al., 1989). Dubiousness of the spore ornamentation type as one basic parameter in the *Scutellinia umbrorum* complex systematics is confirmed by the results of k-means clustering at the five-cluster level, where all clusters include specimens with both types of spore ornamentation.

On the basis of the current analysis we can suppose that the number of species we can distinguish in the *Scutellinia umbrorum* complex is not as large as it was so far expected. At the same time, the morphological plasticity of the species is rather great. The appearance of different morphs, usually interpreted as sister species, seems to be determined by ecological conditions in the habitat.

The present study was not intended to revise the systematics of the *Scutellinia umbrorum* complex, but to elucidate, by means of different multivariate methods, the complicated structure of the data and their dependence on ecological factors. It is obvious that in order to establish more or less invariate systematics of *Scutellinia*, we still are in need of sufficient data representing the variation of taxa in different ecotopes and geographical regions. Furthermore, more variables should be taken into account. To establish the variation amplitude of variables by the uniparental species of *Scutellinia*, cultivation under controlled conditions is necessary.

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NOTES ON CYSTOLEPIOTA: SECTIONS CYSTOLEPIOTA AND PULVEROLEPIOTA

ELSE C. VELLINGA & HENK A. HUIJSER

A key to the species of *Cystolepiota* Sing., and descriptions of *C. cystidiosa* (A.H. Smith) M. Bon, *C. adulterina* (F. Møller) M. Bon, *C. hetieri* (Boud.) Sing., and *C. moelleri* Knudsen are given. Four taxonomic revisions were made: 1. type studies of *C. cystidiosa*, *C. luteicystidiata* (D. Reid) Knudsen, and *Lepiota lycoperdoides* Kreisel have revealed these three species to be conspecific; 2. *C. adulterina* var. *reidii* (M. Bon) M. Bon is synonymized with *C. adulterina*; 3. the type collection of *C. subadulterina* M. Bon appeared to be a mixed collection of *C. hetieri* and *C. adulterina*, and 4. the genus *Pulverolepiota* M. Bon, created to accommodate *C. pulverulenta* (Huijsman) Vellinga, is reduced to a section of *Cystolepiota*.

The European species of the genus *Cystolepiota* Sing. are assigned to two sections (Bon, 1993b), viz. section *Pseudoamyloideae* Sing. & Clémenç. accommodating the species with dextrinoid spores, and section *Cystolepiota* for species with non-dextrinoid spores. A third section is added here, to accommodate *C. pulverulenta* (Huijsman) Vellinga, for which Bon (1993a) created the genus *Pulverolepiota*.

Species delimitation in *Cystolepiota* section *Cystolepiota* is still problematic, though recently several books and keys on the genus have been published (Candusso & Lanzoni, 1990; Bon, 1993b; Kelderman, 1994). The authors of the past did not make things easy for the present-day taxonomist, with their diverse and differing interpretations of names, the frequent introduction of new names (often not according to the rules of the International Code of Botanical Nomenclature) and the use of quite similar epithets (e.g. *hetieri* and *hetieriana*). Some authors appear to attach major significance to small differences between collections, while neglecting gross and overall similarities. This has resulted in an undesirable proliferation of names.

Most problematic is the group to which the following taxa belong: *C. adulterina* (F. Møller) M. Bon with its variety *reidii* (M. Bon) M. Bon, *C. subadulterina* M. Bon, *C. hetieri* (Boud.) Sing., and *C. cystidiosa* (A.H. Smith) M. Bon, *C. luteicystidiata* (D. Reid) Knudsen and *L. lycoperdoides* Kreisel. The confusion is caused by the similarities in macroscopic characters between the species involved, and the fact that they often grow together, or quite close to each other. Mixed herbarium collections have been encountered more than once. As already pointed out by Candusso & Lanzoni (1990) it is extremely difficult to name species from pictures or in the field. Fortunately, microscopic characters give excellent clues for identification of the species involved.

Other characters which could prove to be very valuable are the colours of the lamellae of the exsiccates, the chemical reactions of (parts of) the basidiocarps with $FeSO_4$ and with NH_3 vapours, and the spore print colour. Unfortunately, there are as yet no systematic

1) Rijksherbarium / Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands.
2) Frederikstraat 6, 5671 XH Nuenen, The Netherlands.

studies of the chemical and spore print characters available, only some incidental observations.

Type collections have been studied to establish the differences and/or similarities of the taxa. A key to the species of the genus *Cystolepiota* occurring in the Netherlands and adjacent regions is included. The species are treated in alphabetical order.

Colour annotations in the descriptions are from Munsell Soil Color Charts (1975).

The notation [45, 4, 3] indicates that measurements were made on 45 spores from 4 basidiocarps in 3 collections.

The abbreviation avl stands for average length, avw for average width and avq for average quotient.

Shape and size of the cheilocystidia have been studied halfway between stipe and pileus margin.

Abbreviations of herbaria are according to Index herbariorum (Holmgren et al., 1990).

KEY TO THE SPECIES OF CYSTOLEPIOTA OCCURRING IN THE NETHERLANDS AND ADJACENT REGIONS

1. Elements of pileus covering elongate, oblong, inflated; clamp-connections absent; basidiocarps white, discolouring orange-brown, with (sub)conical pileus and big floccose-granulose warts; cystidia absent ..... *C. pulverulenta*
   (For a discussion on and description of this species see Vellinga, 1992.)
1. Elements of pileus covering globose; clamp-connections present; basidiocarps white, remaining white or changing colour, or distinctly coloured, with applanate, rounded, or umbonate pileus, with or without floccose warts; cystidia absent or present.
   2. Basidiocarps with distinct lilac or violaceous tinges; smell strong, like indole, unpleasant; spores (6.5–)7.0–9.0(–10.0) µm long, dextrinoid, though the reaction can be very slow or weak ..... *C. bucknallii*
      (Not treated here; for a description see e.g. Candusso & Lanzoni, 1990.)
   2. Basidiocarps white, pink, brownish, yellow, greyish etc.; smell not like indole; spores up to 7.0 µm long, not dextrinoid, or rarely dextrinoid (but then basidiocarps yellow).
      3. Cheilocystidia absent; pileus white, rarely pale pink or yellowish, not discolouring with age or when damaged ..... *C. seminuda*
         (For a discussion on and description of this species see Vellinga, 1987.)
      3. Cheilocystidia present; pileus either white and discolouring orange-brown, or with other colours.
         4. Basidiocarps white, cream or greyish, discolouring (orange-)brown with age or on damaging.
            5. Cheilocystidia and pleurocystidia with yellow contents and exudates ..... *C. cystidiosa*
            5. Cheilocystidia (and pleurocystidia) without yellow contents and exudates.
               6. Spores 5.0–6.5(–7.0) × 2.0–3.0 µm, Q = (1.8–)2.0–2.6, average Q = 2.1–2.4; pleurocystidia absent ..... *C. adulterina*
               6. Spores 4.0–6.0(–6.5) × 2.0–3.0(–3.5) µm, Q = (1.5–)1.6–2.1(–2.3), average Q = 1.8–2.0; pleurocystidia present, especially close to lamella edge ..... *C. hetieri*

4. Basidiocarps with greyish, pale lilac-brown, cream, yellow, yellowish-ochraceous or pink(-brown) covering, not discolouring orange-brown when damaged or with age.
   7. Basidiocarps yellow (sulphur-yellow or yellowish brown); spores dextrinoid *C. icterina*
   (Not yet known from the Netherlands; for a description of this species see Knudsen, 1978.)
   7. Basidiocarps with grey, brown or pink tinges, not yellow throughout; spores not dextrinoid.
      8. Pileus covered with small pink-coloured granulose warts, often resembling members of *Lepiota* section *Echinatae*; cheilocystidia narrowly clavate to obovate, usually with cylindrical, moniliform or branched, excrescence at apex; pleurocystidia absent ..... *C. moelleri*
      8. Pileus covered with a uniform, thick velar layer, or with pinkish grey-brown to greyish yellow, pyramidal warts; pleurocystidia present or absent, if present with distinct yellow contents.
         9. Cheilocystidia without yellow contents or exudate; pleurocystidia absent; spores 5.0–6.5(–7.0) × 2.0–3.0 µm, Q = (1.8–)2.0–2.6, average Q = 2.1–2.4; elements of pileus covering 15–45 µm ..... *C. adulterina*
         9. Cheilocystidia and pleurocystidia with yellow contents or exudate; spores 3.5–5.5(–6.0) × 2.0–3.0 µm, Q = 1.5–2.2(–2.4), average Q = 1.7–2.1; elements of pileus covering (10–)20–70(–95) µm ..... *C. cystidiosa*

Cystolepiota adulterina (F. Møller) M. Bon — Figs. 1, 2a, 3a

Lepiota adulterina F. Møller, Friesia 6 ('1957–1958', 1959) 23; *Cystolepiota adulterina* (F. Møller) M. Bon, Doc. mycol. 7 (27–28) (1977) 54. — *Cystolepiota subadulterina* M. Bon, Doc. mycol. 6 (24) (1976) 43; *Cystolepiota adulterina* var. *subadulterina* (M. Bon) M. Bon, Doc. mycol. 22 (88) (1993) 27 (not valid, basionym not mentioned). — *Cystolepiota adulterina* f. *reidii* M. Bon, Doc. mycol. 11 (43) (1981) 25; *Cystolepiota adulterina* var. *reidii* (M. Bon) M. Bon, Doc. mycol. 22 (88) (1993) 27.

Misapplied. Cystolepiota hetieri sensu J. Lange, Fl. agar. dan. 1 (1935) 35–36, pl. 14J.

Excluded. Cystolepiota adulterina sensu Kelderman, Coolia 31 (1988) 15 (= *C. cystidiosa*). — *Cystolepiota adulterina* f. *reidii* sensu Lanzoni & Zecchin, Riv. Micol. 31 (1988) 104; sensu Candusso & Lanzoni, Fungi eur. 4 (1990) 88–90 (= *C. moelleri* in both cases).

Selected icons. J. Lange, Fl. agar. dan. 1 (1935) pl. 14J (as *C. hetieri*); Rald et al., Svampe 26 (1992) 34 (dry specimens); Ryman & Holmåsen, Svampar (1984) 411.

Selected descriptions & figures. F. Møller, Friesia 6 ('1957–1958', 1959) 22–23, figs. a–d; D. Reid, Fung. rar. Ic. col. 6 (1972) 10–11, figs 18a & b.

Pileus 15–30(–50) mm, conico-convex, applanate to convex, when young covered with a thick cream whitish, to beige layer, later with cream-ochre to greyish yellow flocculose warts; margin with velar remnants. Lamellae crowded, free, sordid cream. Stipe 30–60 × 3–7 mm, cylindrical or tapering downwards, fistulose, at apex whitish, lower down covered with floccules, concolorous with pileus, brown discolouring at base. Smell and taste not known.

Spores [125, 8, 6] 5.0–6.5(–7.0) × 2.0–3.0 µm, Q = (1.8–)2.0–2.6, average Q = 2.1–2.4, cylindrical to slightly broadened in basal part, not colouring in Melzer's Reagent, with pink inner wall in Cresyl Blue. Basidia 14.5–23 × 5.0–9.0 µm, (2–)4-spored.

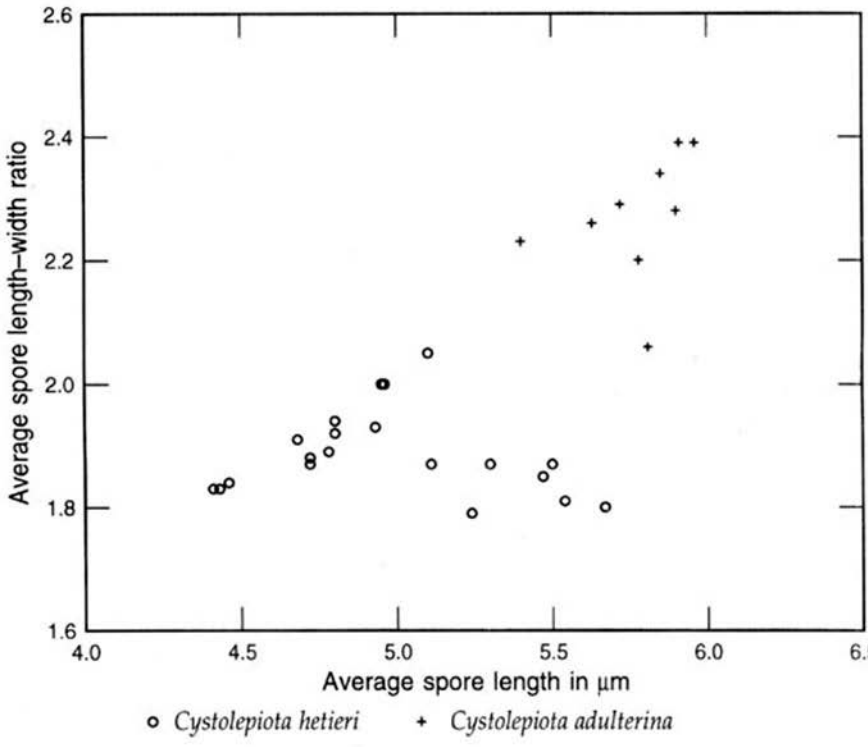

Fig. 1. Scatterdiagram of average spore length and average length-width ratio of the spores of *C. hetieri* and *C. adulterina*.

Cheilocystidia abundant, 17–32 × 5.5–9.0 µm, lageniform with abrupt, cylindrical to slightly moniliform, flexuous, 1.5–4.0 µm wide neck, rarely without neck or only with short excrescence, colourless. Pleurocystidia not observed. Velar elements on pileus globose, 13–46 µm in diam., thick-walled and slightly incrusted, colourless. Clamp-connections present in all tissues.

Habitat & distribution — In small groups, saprotrophical and terrestrial, often on mull (Knudsen, pers. comm., July 1997), in deciduous woods, often on calcareous soils. Sept.–Oct. Not yet known from the Netherlands, rare and scattered in Europe, from southern Scandinavia southwards, not yet recorded from Mediterranean countries.

Collections examined. DENMARK: Falster, Kohave, 10-X-1960, *F.H. Møller* (L); Sjaelland, Avnstrup near Osted, 7-IX-1980, *E. Bille Hansen* (C); Jaegersborg Dyrehave near Rådvad, 28-IX-1982, *T. Læssøe* TL 430 (C). — GERMANY: Nordrhein-Westfalen, Heesten near Detmold, X-1976, *G.A. de Vries* (L); Baden-Württemberg, Gottenheim, Wasenweiler Wald, 4-IX-1975, *M. Bon 750904* p.p. (herb. Bo lectotype of *C. subadulterina*); Ueberlingen, Affenberg, 17-IX-1987, *E. Ludwig* (L). — GREAT I TAIN: England, Gloucestershire, Stonehouse, Nymfsfield, Woodchester Park Field Station, 4-X-19(*E.C. Hemken* (K; holotype of *C. adulterina* var. *reidii* (M. Bon) M. Bon).

The short macroscopical description is based on the notes accompanying the collection made by Ludwig and the descriptions selected above.

Møller (1959) did not indicate a type collection, though he did call Sønder Kohave near Nykøbing on Falster (Denmark) the type locality. Knudsen (1978) chose one of the two collections still preserved in C as lectotype (viz. 1-X-1955, Sønder Kohave). A later collection, made by Møller himself, from the type locality was studied for this paper.

Reid (1972) described a collection of *C. adulterina*. This collection was the basis for Bon's (1993a) variety (first described as a forma in 1981), *C. adulterina* var. *reidii*. On account of the bigger spores and the more pink colours Bon (1993a) considers this to be a good variety. The colours of the pileus are, according to Reid (1972), "beige (Light Pinkish Cinnamon)" in young specimens, and "ochraceous (Light Ochraceous-Salmon)" in older specimens. The codes in brackets are taken from Ridgway (1912). According to Hamley (1949) they correspond to the following Munsell notations, resp. 1 YR 8.0/5.0, and 8 YR 8.0/5.5. Especially the colour of the young pilei of this collection seems to be a bit more pink than encountered in other collections. To judge whether this taxon deserves infraspecific rank the type collection of *C. adulterina* var. *reidii* (M. Bon) M. Bon was studied. The microscopical characters are as follows: spores 5.4–6.5 × 2.4–2.9 µm, avl × avw = 5.9 × 2. 6 µm, Q = (1.9–)2.05–2.4(–2.6), average Q = 2.3; basidia 4-spored; cheilocystidia 19–32 × 7.0–9.0 µm, lageniform, mostly with up to 17 µm long, cylindrical or slightly moniliform neck; velar elements of pileus globose, 13–28 µm in diam., thin-walled. The relatively long spores, the shape of the cheilocystidia, and the small elements of the pileus covering are all very characteristic for *C. adulterina*.

In all respects, this collection fits perfectly in *C. adulterina*, and there is no reason to keep the variety as a separate taxon.

Another taxon in this group was named *C. subadulterina* by Bon (1976a and b). Bon (1976a) gave a comprehensive description of this taxon (which he described as a new species later in the same year). His description clearly combines the macroscopic features of *C. adulterina* or *C. cystidiosa*, and the microscopic characteristics of *C. hetieri*. The figures reflect the same ambiguity. Examination of the type collection confirmed the suspicion that two species are involved.

The type collection consists of basidiocarps belonging to *C. hetieri* and basidiocarps representing *C. adulterina*. The microscopic characteristics of the former are as follows: spores 4.8–5.8 × 2.3–2.5(–2.8) µm, Q = 1.95–2.1(–2.3), average Q = 2.05; cheilocystidia abundant, fusiform and capitate to moniliform, colourless; pleurocystidia present; elements of velum universale globose and around 35 µm in diameter.

The basidiocarps belonging to *C. adulterina* are microscopically characterized in the following way: spores 5.0–5.8 × 2.3–2.5 µm, Q = 2.0–2.4, average Q = 2.25; cheilocystidia abundant, with long cylindrical and slightly flexuous neck; pleurocystidia absent; elements of velum universale globose, 18–30 µm in diameter. These specimens are chosen here as the lectotype of *C. subadulterina*.

The type collection was not in a state to reveal further details.

After we had notified Mr. Bon of the identity of this collection, he reduced the species in rank to a variety of *C. adulterina* (Bon, 1993a), though invalidly, citing the publication in which he gave a desription of the species (Bon, 1976a), rather than the paper in which the official publication had been made (Bon, 1976b).

Many authors had difficulties distinguishing *C. hetieri* from *C. adulterina* (for example Breitenbach & Kränzlin, 1995). This is probably due to the incomplete descriptions of Moser (1978, 1984), the abundance of names and taxa without a set of well-discriminating characters as given by Bon (1981, 1993b), and the fact that the original publication of Møller (1959) is not widely known.

The characters that differentiate *C. hetieri* most clearly from *C. adulterina*, are microscopical, and can easily be confirmed on herbarium material: the shape of the spores, the shape of the cheilocystidia, and the size of the velar elements. The spores of *C. adulterina* are longer than those of *C. hetieri*; the average length-width ratio in the former is 2.1–2.4, whereas it does not exceed 2.1 in *C. hetieri* (see Fig. 1, 2). The cheilocystidia in *C. hetieri* measure 14–32 × 7–12 µm, with up to 15 µm long, abrupt, capitulate and cylindrical or moniliform excrescence at apex, and the cystidia are widest just below the excrescence, whereas in *C. adulterina* the cheilocystidia have a very long, protruding neck (Fig. 3). But the exact shape of the cystidia in *C. adulterina* is often difficult to see; though the necks are quite obvious. Furthermore, pleurocystidia are often present in *C. hetieri*, and absent in *C. adulterina*. Velar elements of *C. hetieri* measure 20–60 µm; in *C. adulterina* they do not exceed 50 µm and are generally around 30 µm.

Some authors have taken *C. moelleri* for *C. adulterina* f. *reidii* (Lanzoni & Zecchin, 1988; Candusso & Lanzoni, 1990). The differences between the two taxa are very distinctive, and easily observed, even in the field.

Cystolepiota cystidiosa differs from *C. adulterina* in the presence of pleurocystidia, which are conspicuous and have yellow contents. The spores of *C. adulterina* are the longest and narrowest in this subsection, with an average length-width ratio of 2.1–2.4. The spores of *C. cystidiosa* are shorter, and the average length-width ratio is 1.7–2.1. Furthermore, there is a difference in the size of the velar elements: 13–46 µm in *C. adulterina*, and (10–)20–70(–95) µm in *C. cystidiosa*.

Cystolepiota cystidiosa (A.H. Smith) M. Bon — Figs. 2b–d; 3b, c

Lepiota cystidiosa A.H. Smith, Papers Mich. Acad. Sci., Arts Letters 27 ('1941', 1942) 58; *Cystolepiota cystidiosa* (A.H. Smith) M. Bon, Doc. mycol. 11 (43) (1981) 26. — *Lepiota luteicystidiata* D. Reid, Fung. rar. Ic. col. 2 (1967) 9; *Cystolepiota luteicystidiata* (D. Reid) M. Bon, Doc. mycol. 6 (24) (1976) 43. — *Lepiota lycoperdoides* Kreisel, Wiss. Z. Ernst Moritz Arndt-Univ. Greifswald 16 (1967) 238; *Cystolepiota luteicystidiata* var. *lycoperdoides* (Kreisel) M. Bon, Doc. mycol. 11 (43) (1981) 26. — *Lepiota huysmani* Wichanský, Mykol. Sbornik 37 (1960) 121 (not valid, no type collection indicated).

Misapplied. Lepiota rufescens sensu Huijsman, Meded. Ned. mycol. Vereen. 28 (1943) 47–48.

Selected icons. Lonati, Boll. Ass. micol. ecol. Romana 12 ('1987', 1988) 15 (as *C. luteicystidiata*); Migl. et al., Riv. Micol. 32 (1989) 103 (as *C. luteicystidiata*); D. Reid, Fung. Ic. rar. col. 2 (1967) pl. 10b (as *L. luteicystidiata*, basidiocarps exceptionally dark).

Selected descriptions & figures. Huijsman, Schweiz. Z. Pilzk. 39 (1961) 51; Kelderman, Coolia 31 (1988) 5, fig. II (as *C. adulterina*); Kreisel, Wiss. Z. Ernst Moritz Arndt-Univ. Greifswald 16 (1967) 237–238 (as *L. lycoperdoides*); D. Reid, Fung. rar. Ic. col. 2 (1967) 9–10, fig 3 (as *L. luteicystidiata*); A.H. Smith, Papers Mich. Acad. Sci., Arts Letters 27 ('1941'; 1942) 58–60, pl. 1, 2.; H.V. Smith, Lloydia 17 (1954) 318–319; Winterhoff & Bon, Carolinea 52 (1994) 6.

Pileus 20–40(–70) mm, when young spherical to hemispherical with inflexed margin, expanding to applanate-campanulate, plano-convex with or without broad umbo, covered

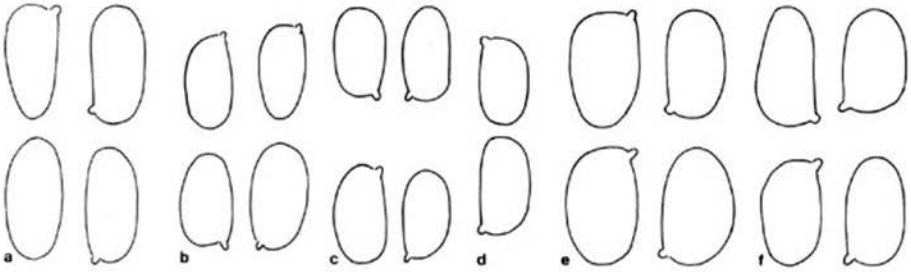

Fig. 2. Spores of a. *C. adulterina* (from holotype of *C. adulterina* f. *reidii*), b–d. *C. cystidiosa* (b. from holotype, *A.H. Smith 15268*; c. from holotype of *L. luteicystidiata*; d. from holotype of *L. lycoperdoides*), e. *C. hetieri* (from *E.C. Vellinga 1020*), and f. *C. moelleri* (from *E.C. Vellinga 666*). — 3000 ×.

with floccose, granular pyramidal warts, up to several millimeters high, varying in colour from whitish when very young, to cream discolouring pinkish, to pinkish grey-brown, pinkish-brownish (7.5 YR 6/4, 10 YR 4/3–7.5 YR 5/4, 10 YR 7/6, 10 YR 8/4 to 7.5 YR 7/6), often staying paler at margin, and there covering thinner, showing the whitish context; when warts are removed, a whitish 'scar' remains; margin exceeding lamellae. Lamellae, L = 30–45, l = 1–3, moderately crowded to crowded, free, segmentiform to slightly ventricose, up to 4.5 mm wide, cream coloured, and brown-spotted with age, with even or flocculose, white to concolourous edge, which is also brown-spotted with age. Stipe 15–70 × 2.5–5 mm, cylindrical, sometimes widened at apex, occasionally curved in lower part, fistulose, at apex whitish or cream, finely pubescent, glabrescent with age, in lower half or 3/4 of length, below an annular zone which is more or less distinct, granular floccose or with floccose patches, concolorous with pileus, often discolouring (e.g. pinkish brown, 7.5 YR 6/6) when touched. Context white or whitish cream and dull in pileus, shiny and cream in stipe, brownish or vinaceous downwards. Smell fungoid or 'lepiotoid', often like *Lepiota cristata*, but not always. Taste a bit unpleasant, musty fungoid, with unpleasant lingering aftertaste. Spore print cream.

Spores [252, 19, 18] 3.5–5.5(–6.0) × 2.0–3.0 µm, Q = 1.5–2.2(–2.4), average Q = 1.7–2.1, ellipsoid to cylindrical with parallel sides, sometimes in side-view slightly widened at base, often in tetrads, colourless, with slightly thickened wall, non-dextrinoid, non-amyloid, with cyanophilous wall, and inner wall pink in Cresyl Blue. Basidia 12.5–20 × 4.5–6.5 µm, 4-spored. Lamella edge sterile, in fresh specimens covered in a yellow exudate. Cheilocystidia 17–40(–50) × 6.5–12 µm, cylindrical-fusoid, narrowly clavate, rather variable in shape, without or with apical excrescence, varying from a small capitulum, 3.0–4.0 × 3.0 µm, to a long moniliform neck, up to 45 µm long; occasionally whole cystidium moniliform; often thick-walled, with yellow, often granular, contents. Pleurocystidia abundant, similar to cheilocystidia, but without or with short-moniliform neck; at a magnification of 400 × cystidia clearly visible as yellow dots, evenly distributed over lamella surface. Elements of velar covering on pileus (10–)20–70(–95) µm in diam., globose to slightly subglobose, thin-walled or slightly thick-walled, often with slightly brownish walls; in fresh specimens with very small yellow droplets or particles. Stipitipellis a

cutis made up of cylindrical narrow hyphae, 1.5–5.0 µm in diam.; below covered in elements as on pileus, and often more irregular in shape (pyriform, ellipsoid etc.). Clamp-connections present in all tissues.

Habitat & distribution — Gregarious, sometimes in big flocks, saprotrophical and terrestrial in deciduous woods on nutrient-rich soils, and in greenhouses. Known in the Netherlands from several localities in southern Limburg; also found in several regions of Germany; also growing in North America. Aug.–Oct. in the wild, throughout the year in greenhouses.

Collections examined. NETHERLANDS: prov. Utrecht, Baarn, Cantonspark, VIII-1943 and V-1945, *G.A. de Vries* (L); prov. Noord-Holland, Kortenhoef, tomato-greenhouse, I-1971, *J. Daams* (herb. Tjallingii); 's-Graveland, Boekesteyn, 10-X-1983, *J. Daams* (L); prov. Zuid-Holland, Leiden, Botanical Garden, 4-VIII-1958, *C. Bas* (L), 16-VI-1997, *E.C. Vellinga 2078* (L), and 8-VII-1997, *E.C. Vellinga 2086* (L); prov. Limburg, *Cadier & Keer*, Riesenberg, 4-X-1989, *E.C. Vellinga 1615* & *1619*, 9-X-1991, *E.C. Vellinga 1758*, 20-VIII-1993, *E.C. Vellinga 1899* (all in L); Valkenburg, Schaelsberg, *H.A. Huijser*, 23-IX-1989 (L). — BELGIUM: prov. Antwerpen, Antwerpen, Kruidtuin, 13-XII-1981, *A. de Meijer 539* (L). — GERMANY: Bayern, Erlangen, Botanical Garden, 4-I-1987, *G. Wölfel* (L); Mecklenburg, Greifswald, Botanical Garden, 6-XII-1964, *H. Kreisel* (GFW, holotype of *L. lycoperdoides*); Nordrhein-Westfalen, Mönchengladbach, 29-IX-1987, *H. Bender* (L); Neeosen Truppenbahn, 17-X-1984, *H. Bender* (herb. Bender). — GREAT BRITAIN: England, Surrey, Richmond, Kew, Royal Botanic Gardens, Palm House, 25-IV-1961, Mr. *Harrison* (K, holotype of *C. luteicystidiata*), and Princess of Wales Conservatory, 9-III-1993, *E.W. Brown* (L). — USA: Michigan, Washtenaw Co., Ann Arbor, 9-IX-1940, *A.H. Smith 15268* (MICH, holotype of *C. cystidiosa*).

The type collections of *C. cystidiosa*, *C. luteicystidiata* and *L. lycoperdoides* have been studied. Knudsen (1978) considered *C. luteicystidiata* and *L. lycoperdoides* synonymous, though he did not study the type collections. This opinion was not shared by Bon (1981), who lowered *L. lycoperdoides* in rank to a variety of *C. luteicystidiata*, on account of presumed bigger size of the basidiocarps and the smell like *Lycoperdon*. However, Kreisel (1967) gives the smell as very faint, reminiscent of the smell of *L. cristata*. The basidiocarps of the type collection of *L. lycoperdoides* are surprisingly small.

The microscopic characters of the three type collections are presented in Table I. Judging from this table and the macroscopic characters (see resp. Smith, 1942; Reid, 1967; and Kreisel, 1967), there are no reasons to keep them separate. Although American specimens of *C. cystidiosa* are sturdy and much bigger than the average European basidiocarps, pilei up to 7 cm in diameter can be encountered in Europe as well.

Table I. Type studies on *Cystolepiota cystidiosa*, *C. luteicystidiata*, and *L. lycoperdoides*.

type collection	spores	avl × avw	avq	pleurocystidia & cheilocystidia	velar elements
cystidiosa A.H. Smith	3.7–4.8 × 2.1–2.7 µm	4.2 × 2.4 µm	1.75	with yellow contents, capitulate	35–57 µm in diam.
luteicystidiata D. Reid	3.7–4.6 × 2.1–2.5 µm	4.1 × 2.3 µm	1.83	with yellow contents	up to 55 µm in diam.
lycoperdoides Kreisel	4.1–4.9 × 2.1–2.6 µm	4.3 × 2.4 µm	1.82	with yellow contents, some capitulate	32–65 µm in diam.

Cystolepiota cystidiosa can be confused with *C. hetieri*; the spores are of the same size in the two species. The yellow contents of the cystidia are the best character to distinguish between the two. The colours of young basidiocarps can be quite similar, but are different in mature specimens.

In the field *C. cystidiosa* shows a certain resemblance to *C. pulverulenta* as well. Both species may have pyramidal warts on the pileus, and also the colours can be rather similar. The pileus of *C. pulverulenta*, however, is often quite conical, whereas in *C. cystidiosa*, the pileus is rounded, or umbonate. The two species share certain chemical characters. Both colour bright yellow by NH_3 vapours and both discolour very dark violet-blue when rubbed with $FeSO_4$, whereas some other species in this group become more greenish blue. The microscopical characters, like the shape of the velar elements, and the presence of cystidia and clamp-connections, are in cases of confusion of decisive value. It is striking that in *C. cystidiosa* the velar elements close to the stipe surface show a tendency to become ellipsoid or even oblong, resembling the velar elements of *C. pulverulenta*.

It is often quite difficult to get a good view of the shape of the cystidia of *C. cystidiosa*.

The colour of exsiccates varies greatly; some specimens remained greyish-brownish, others have become orange-brown. Huijsman (1943) (mis)applied the name *L. rufescens* B. & Br. sensu Lange to the latter. In all specimens examined the lamellae are pinkish grey-brown (7.5 YR 6/3), except in freshly dried specimens, in which the colour is still cream.

Cystolepiota cystidiosa is known in Europe mostly from greenhouses, but in the woods of southern Limburg it has been known to the second author since 1977; more recently there are records by Kelderman (1994) and Winterhoff & Bon (1994) of occurrences in the wild.

Plate 210 (as *C. hetieri*) in Breitenbach & Kränzlin (1995) strongly resembles *C. cystidiosa*, although the microscopical characters, and especially the spore shape, excellently fit *C. adulterina*. Plate 212 (as *C. spec.*) in the same work, which is there considered to be different, on account of its yellow-cream coloured spores, resembles *C. adulterina*, both visually and microscopically. Little is known about the variability of the spore print colour in the taxa in this group, and the use of this character in species delimitation remains to be studied.

Cystolepiota hetieri (Boud.) Sing. — Figs. 1, 2e, 3d

Lepiota hetieri Boud., Bull. Soc. mycol. Fr. 18 (1902) 137; *Cystoderma hetieri* (Boud.) Sing., Schweiz. Z. Pilzk. 17 (1939) 53; *Cystolepiota hetieri* (Boud.) Sing., Beih. Sydowia 7 (1973) 67. — *Agaricus granulosus* var. *rufescens* B. & Br., Ann. Mag. nat. Hist. 5, Ser. VII (1881) 124 (Notic. Brit. Fungi 1834); *Lepiota rufescens* (B. & Br.) J. Lange, Dansk bot. Ark. 9 (6) (1938) 65, non *L. rufescens* Morgan, 1906; *Lepiota langei* Locq., Bull. mens. Soc. linn. Lyon 14 (1945) 87, non *L. langei* Knudsen, 1980; *Cystolepiota langei* (Locq.) M. Bon, Doc. mycol. 22 (88) (1993) 27.

Excluded. Lepiota hetieri sensu J. Lange, Fl. agar. dan. 1 (1935) 35–36, pl. 14J (= *C. adulterina*); *Cystolepiota hetieri* sensu Breitenb. & Kränzl., Pilze Schweiz 4 (1995) pl. 210 (= *C. adulterina* or *C. cystidiosa*). — *Lepiota rufescens* sensu Huijsman, Meded. Ned. mycol. Vereen. 28 (1943) 46–51 (= *C. cystidiosa*); sensu Kühner & Romagn., Fl. anal. Champ. sup. (1953) 396 (=? *C. cystidiosa*).

Selected icons. Boud., Bull. Soc. mycol. Fr. 18 (1902) pl. 6, fig. 1; J. Lange, Fl. agar. dan. 1 (1935) pl. 14I; Rald et al., Svampe 26 (1992) 34.

Selected descriptions & figures. Herink, Česká Mykol. 15 (1961) 226–233, figs 6–8; Kelderman, Parasolzw. Zuid-Limburg (1994) 36–37; Kühner, Bull. trimest. Soc. mycol. Fr. 52 (1936) 205–206; F. Møller, Friesia 6 ('1957–1958', 1959) 25.

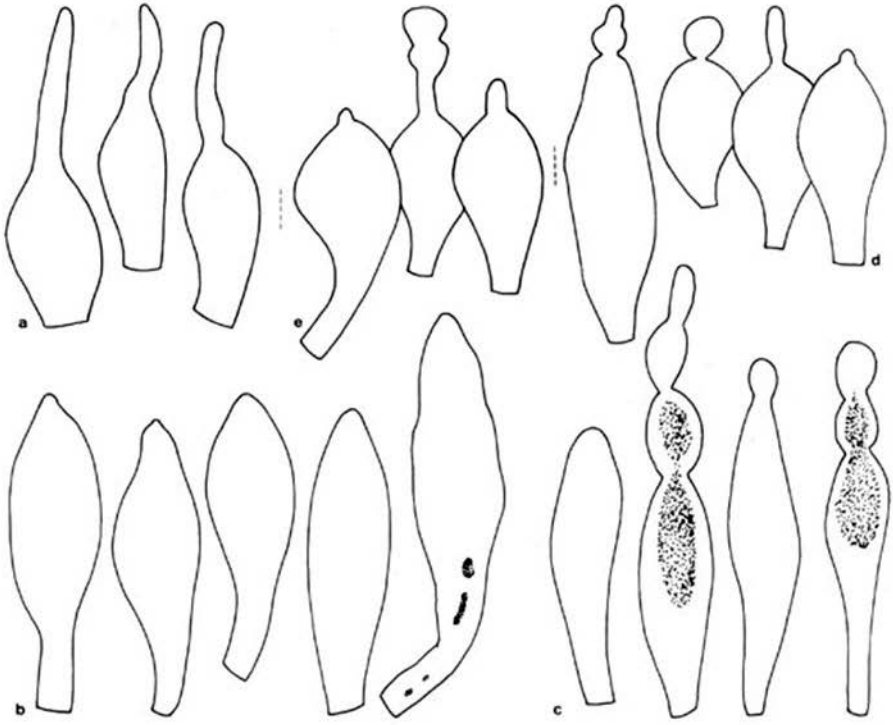

Fig. 3. Cheilocystidia of a. *C. adulterina* (from holotype of *C. adulterina* f. *reidii*), b. & c. *C. cystidiosa* (b. from Wölfel, 4-I-1987; c. from *E.C. Vellinga 1758*), d. *C. hetieri* (from *E.C. Vellinga 1020*), and e. *C. moelleri* (from *C. Bas 8305*). — 1500 ×.

Pileus 10–22(–30) mm, when young hemispherical, expanding to plano-convex with umbo, with velar remnants at margin, at first white to whitish cream, rarely greyish, soon discolouring to orange-brown in patches or totally discoloured (5 YR 5/6–8), granulose to more or less squamulose, when young often with low pyramidal warts on woolly background; warts at centre rather crowded. Lamellae, L = 35–60, l = 3, moderately crowded, free, segmentiform to subventricose, up to 3 mm broad, white to cream coloured, with age discolouring, especially at edge, to orange-brown or rarely to pale buff (7.5 YR 8/4) when touched, with white, concolorous even edge. Stipe 15–40(–50) × 2–4 mm, cylindrical, fistulose, with or without woolly ring-like zone, whitish cream, rarely greyish, at apex pruinose, below ring-like zone granulose to flocculose, often rather quickly discolouring orange-brown or red-brown, especially when touched, with white basal tomentum. Context whitish in pileus, in stipe apex cream coloured, and orange-brown (7.5 YR 4/6) at base, white around cavity. Smell unpleasant sweetish-fungoid, not resembling the smell of *L. cristata*, or according to other observations like the smell of *L. cristata*. Taste not recorded. Spore print colour not known.

Spores [210, 21, 21] 4.0–6.0(–6.5) × 2.0–3.0(–3.5) µm, Q = (1.5–)1.6–2.1(–2.3), average Q = 1.8–2.0, oblong, some (sub-)cylindrical, some slightly broadened at basal part, colourless, with slightly thickened wall, often in tetrads, non-dextrinoid, non-amyloid, with cyanophilous wall; spore wall pink in Cresyl Blue. Basidia 15–25 × 5–7 µm, 4-spored. Cheilocystidia usually abundant, 14–32 × 7–12 µm, with up to 15 µm long abrupt, capitulate and cylindrical or moniliform excrescence at apex, clavate or ellipsoid, thin-walled to slightly thick-walled especially in apical part, usually colourless, some with brownish granular contents. Pleurocystidia present, especially near lamella edge, sometimes rather rare, similar to cheilocystidia. Hymenium at lamella edge brown coloured. Pileus covered with a velar epithelium, made up of globose to ellipsoid and spheropedunculate elements, 20–60(–80) µm in diam., slightly thick-walled, with, rarely without, brownish parietal pigment. Stipitipellis at apex a cutis of cylindrical elements, 4–10 µm in diam., usually colourless, sometimes red-brown coloured, slightly thick-walled, lower down with chains of globose elements, 20–60 µm in diam., slightly thick-walled and brown coloured. Clamp-connections present in all tissues.

Habitat & distribution — Saprotrophical and terrestrial, gregarious in deciduous woods on clayey, loamy soils, rich in humus, often rich in lime; also in greenhouses. In the Netherlands widespread, but not common. Occurring from the end of Aug.–Oct.(–Nov.), but in greenhouses throughout the year. Known from temperate regions of Europe.

Collections examined. NETHERLANDS: prov. Gelderland, Neerijnen, estate Neerijnen, 6-IX-1980, *Th.W. Kuyper 1424* (L); prov. Noord-Holland, Amsterdam, Amsterdamse Bos, 5-X-1983, *C.B. Uljé 455* (herb. Uljé); 's-Graveland, Boekesteyn, 1-V-1971, *H.S.C. Huijsman 8* (L); prov. Zuid-Holland, Rotterdam, Kralinger Hout, 30-IX-1961, *C. Bas 2454a* (L); prov. Noord-Brabant, Eindhoven, Philips de Jong Park, 30-VIII-1979, Aug.–Sept. 1980, *H.A. Huijser* (herb. Huijser); prov. Limburg, Brunssum, mine stone heap of mine 'Hendrik', 13-IX-1980, *P.H. Kelderman 240* (L); Cadier en Keer, Örenberg, 4-X-1989, *E.C. Vellinga 1601* (L) and Riesenberg, 4-X-1989, *E.C. Vellinga 1616* (L); Gronsveld, Savelsbos, 4-IX-1977, *Th.W. Kuyper 895* (L); Gulpen, Wijlrebossen, 6-XI-1982 and 25-IX-1986, *P.H. Kelderman 1604 & 1630* resp. (L); Heerlen, Imstenrader Bosch, 21-IX-1988, *E.C. Vellinga 1402*; Heerlen, Putberg, 25-VIII-1982, *H.A. Huijser* (herb. Huijser) and 3-VIII-1988, *E.C. Vellinga 1323* (L). — BELGIUM: prov. Liège, Tilff, Vallon de la Chawresse, 11-IX-1995, *E.C. Vellinga 1928* (L); prov. Namur, Dourbes, Grand Mont, 22-IX-1986, *E.C. Vellinga 1020* (L); Dourbes, Tiêne-aux-Pauquis, 8-X-1982, *T. Boekhout 1032* (L); Rochefort, Fond des vaux, 10-IX-1975, *M.E. Noordeloos 128* (L). — CZECH REPUBLIC: Česky Kras, near Cernosiče, 5-IX-1981, *Th.W. Kuyper 1719* (L). — DENMARK: Falster, Kohaven, 10-X-1960, *F.H. Møller* (L). — GERMANY: Berlin, Berlin-Grünewald, Riemeister Fenn, 8-IX-1979, *E. Ludwig* (L); Berlin-Lichtenrad, 15-X-1987, *E. Ludwig* (L); Baden-Württemberg, Gottenheim, Wasenweiler Wald, 4-IX-1975, *M. Bon 750904* p.p. (herb. Bon; part of type collection of *C. subadulterina*), and 5-IX-1975, *C. Bas 6591* p.p. (L). — SWITZERLAND: ct. Neuchâtel, Planeyse, 16-IX-1965 and 16-IX-1968, *H.S.C. Huijsman* (L).

In the field, *Cystolepiota hetieri* can easily be confused with *C. pulverulenta*. The latter differs from *C. hetieri* in the oblong, inflated elements of the veil, the absence of cystidia and the absence of clamp-connections.

Cystolepiota seminuda is slender, does not discolour orange-brown and lacks cystidia, and is therefore easily recognized.

Cystolepiota adulterina is often confused with *C. hetieri*, and some authors doubt whether it is a separate species. For comments on differences and similarities, see under *C. adulterina*. *Cystolepiota cystidiosa* comes very close to *C. hetieri*, has similar spores (both in shape and size), but differs in the colour of the basidiocarps and the yellow contents and exudates of the cystidia and other parts of the basidiocarps.

Bon (1993b) distinguishes *C. langei* from *C. hetieri*, on account of the distinct ring-like zone on the stipe and the non-moniliform cystidia in the former. Both characters were found in the material studied, but are not correlated. The shape of the cystidia is very variable, and changes with the age of the basidiocarps.

Cystolepiota moelleri Knudsen — Figs. 2f, 3e

Lepiota rosea Rea, Trans. Br. mycol. Soc. 6 (1918) 61–62; *Cystolepiota rosea* (Rea) M. Bon, Doc. mycol. 6 (24) (1976) 43, non *Cystolepiota rosea* Sing., 1969; *Cystolepiota moelleri* Knudsen, Bot. Tidsskr. 73 (1978) 134; *Cystolepiota rosella* Mos., Röhrlinge Blätterpilze, 4. Aufl. (1978) 236 (superfluous name change).

Misapplied. Cystolepiota adulterina f. *reidii* sensu M. Bon, Doc. mycol. 11 (43) (1981) 25; sensu Lanzoni & Zecchin, Riv. Micol. 31 (3–4) (1988) 104; sensu Candusso & Lanzoni, Fungi eur. 4 (1990) 88–90; *Lepiota pseudoasperula* sensu Enderle & Krieglst., Z. Mykol. 55 (1989) 86.

Selected icons. Enderle & Krieglst., Z. Mykol. 55 (1989) opp. p. 96 (as *L. pseudoasperula*); R. Phillips, Paddest. Schimm. (1981) 30 (as *L. rosea*).

Selected descriptions & figures. Kelderman, Coolia 31 (1988) 12–14, fig. 1; P.D. Orton, Trans. Br. mycol. Soc. 43 (1960) 287 (as *L. rosea*).

Pileus 8–40 mm, when young hemispherical with inflexed margin, expanding to planoconvex with shallow central depression, or with applanate centre, at centre densely set with low acute and (sub-)pyramidical pink or pinkish red-brown squamules or warts (2.5 YR 3/5, 3–2.5/2, 2.5–5 YR 4/4), at margin with more widespread squamules, on a very pale pinkish-brownish background (7.5 YR 7/6), with velar remnants at margin when young. Lamellae, L = 30–40, l = 1–3, moderately crowded to crowded, free, segmentiform to ventricose, up to 3.5 mm wide, whitish cream, greyish cream with age, with concolorous to pinkish even to flocculose (under lens) edge. Stipe 15–40(–70) × 2–5 mm, cylindrical or slightly broadening towards base, curved at base, fistulose, at apex pinkish cream and pruinose at fibrillose background, lower down with scattered pink or reddish brown flocculose or lanate bands of warts (2.5 YR 6/4) on pale pink to vinaceous red background. Context in pileus white to cream, white to pale pinkish in stipe. Smell faint and sweetish, pleasant or like *Lepiota cristata*. Taste indistinct, fungoid. Spore print 'white'.

Spores [167, 16, 15] 4.0–5.5(–6.0) × 2.5–3.0 µm, Q = (1.5–)1.6–2.2(–2.3), average Q = 1.7–1.9(–2.0), oblong to cylindrical, some slightly phaseoliform in side-view, thin-walled and colourless, non-dextrinoid, cyanophilous, congophilous, metachromatic in Cresyl Blue; often in tetrads. Basidia 16–26 × (3.5–)5.0–7.5 µm, 4-spored. Lamella edge sterile; cheilocystidia abundant, 15–35(–39) × 7.0–15 µm, narrowly clavate to obovate, usually with cylindrical, moniliform or branched, excrescence at apex, up to 22 µm long, colourless and thin-walled. Pleurocystidia not present. Squamules on pileus made up of globose to ellipsoid elements, 15–70 µm in diam., slightly thick-walled, with brown parietal pigment. Stipitipellis at apex of stipe a cutis of cylindrical to inflated, 4.0–8.0 µm wide, not coloured elements, lower down with chains of globose to ellipsoid elements, 30–60 µm in diam., brown-coloured and slightly thick-walled. Clamp-connections present in all tissues.

Habitat & distribution — Gregarious or solitary, saprotrophical and terrestrial in mixed deciduous woods on loamy, calcareous or nutrient-rich soils; in the Netherlands rare,

mostly in southern Limburg, Aug.–Oct. Widespread and rather rare in temperate parts of Europe.

Collections examined. NETHERLANDS: prov. Utrecht, Breukelen, estate Nijenrode, 28-VIII-1983 and 25-IX-1983, *G. Immerzeel* (L); prov. Limburg, Bemelen, 9-X-1991, *E.C. Vellinga 1756* (L); Brunssum, mine stone heap of mine 'Hendrik', 9-X-1987, *P.H. Kelderman 1514* (L); Cadier en Keer, Örenberg, 9-X-1991, *E.C. Vellinga 1768* (L); Savelsbos, 6-VIII-1976, *H.A. Huijser* (L); Elsloo, Bunderbos, 7-IX-1996, *E.C. Vellinga 2010* (L). — BELGIUM: prov. Namur, Han-sur-Lesse, Grande Tinémont, 9-IX-1975, *F. & G. Tjallingii* (herb. Tjallingii); Matagne la Grande, Bois les Mires, 4-X-1984, *C. Bas 8305* (L); Nismes, 30-IX-1984, *E.C. Vellinga 666* (L). — FRANCE: dept. Haute Savoie, le Salève, 6-IX-1986, *E.C. Vellinga 993* (L); dept. Pas de Calais, Bois de Hardelot, 18-X-1991, *E.C. Vellinga 1801* (L). — GERMANY: Baden-Württemberg, Nerenstetten, 9-X-1984, *M. Enderle* (L); Bayern, Kissendorf, Bubesheimerwald, 19-IX-1988, *M. Enderle* (L); Nordrhein-Westfalen, Sauerland, Almequellen, 1-IX-1974, *F. & G. Tjallingii* (herb. Tjallingii); Mönchengladbach, Volksgarten, 16-X-1984, *H. Bender* (herb. Bender); Rheinland-Pfalz, Müllenborn, 20-IX-1990, *E.C. Vellinga 1689* (L); Nohn, Dreimüllerwald, 28-IX-1987, *E.C. Vellinga 1201* (L). — GREAT BRITAIN: Somerset, Higher Merridge, 15-IX-1960, *D.A. Reid* (neotype, K); Surrey, Mickleham, Norbury Park, 29-VIII-1992, *N.W. Legon* (K).

The type collection of *Lepiota rosea* Rea (Caughley Woods, Shropshire (Salop), 29-IX-1917) does not exist any more. The original water colour of Rea's collection, preserved at K, shows a pink *Cystolepiota* species; there is no discrepancy between this picture and the present-day interpretations of the species. A neotype has been selected, viz. Somerset, Higher Merridge, 15-IX-1960, *D.A. Reid* (K), a collection also seen by Orton.

Bon (1993b) cites this taxon as *C. rosea* (Rea) Sing. However, Singer (1969) validly describes the new species *Cystolepiota rosea*, a species in its own right, differing from *C. moelleri* in the absence of cheilocystidia and in the rather smooth surface of the pileus.

Cystolepiota moelleri might be confused with *Lepiota pseudoasperula*, but in the latter spores are dextrinoid and cheilocystidia absent.

Cystolepiota section **Pulverolepiota** (M. Bon) Vellinga, *comb. et stat. nov.*

Basionym: *Pulverolepiota* M. Bon, Doc. mycol. 22 (88) (1993) 30.

This section of *Cystolepiota* is characterized as follows: spores very slowly becoming red-brown in Melzer's Reagent, clamp-connections absent, and velar elements elongate and inflated.

Further research is necessary to determine whether *C. pseudogranulosa* (B. & Br.) Pegler, also belongs in this section, just as *C. pulverulenta*. *Cystolepiota pseudogranulosa* is provided with numerous, though small, clamp-connections (Pegler, pers. comm., Febr. 1996), and the spores are strongly dextrinoid (Dennis, 1952).

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FURTHER NEW SPECIES OF MYCENA AND A NEW SECTION FROM SPAIN

M. VILLARREAL[1], M. HEYKOOP[1], F. ESTEVE-RAVENTÓS[1] &
R.A. MAAS GEESTERANUS[2]

This short note directs the attention towards a new section and its type species, and two new species of section *Fragilipedes* (Fr.) Quél. *Hydropus flocculinus* is transferred to the genus *Mycena*.

Section *Fragilipedes* is the most numerous and complex group within the genus *Mycena* (Pers.) Roussel. Several new species belonging to this section have been described in recent times in Europe alone (Maas Geesteranus, 1988a, 1988b, 1988c, 1991a, 1991b, 1992, 1993, 1995; Aronsen & Maas Geesteranus, 1989; Maas Geesteranus & Schwöbel, 1989; Robich, 1992; Aronsen, 1994; Maas Geesteranus & Enderle, 1994; Maas Geesteranus & Münzmay, 1997) and more are likely to be discovered in future.

Mycena flocculina (Kalamees) Villarreal, *comb. nov.* — Figs. 1–5

≡ *Hydropus flocculinus* Kalamees, Folia Cryptog. Eston. 26 (1987) 7.

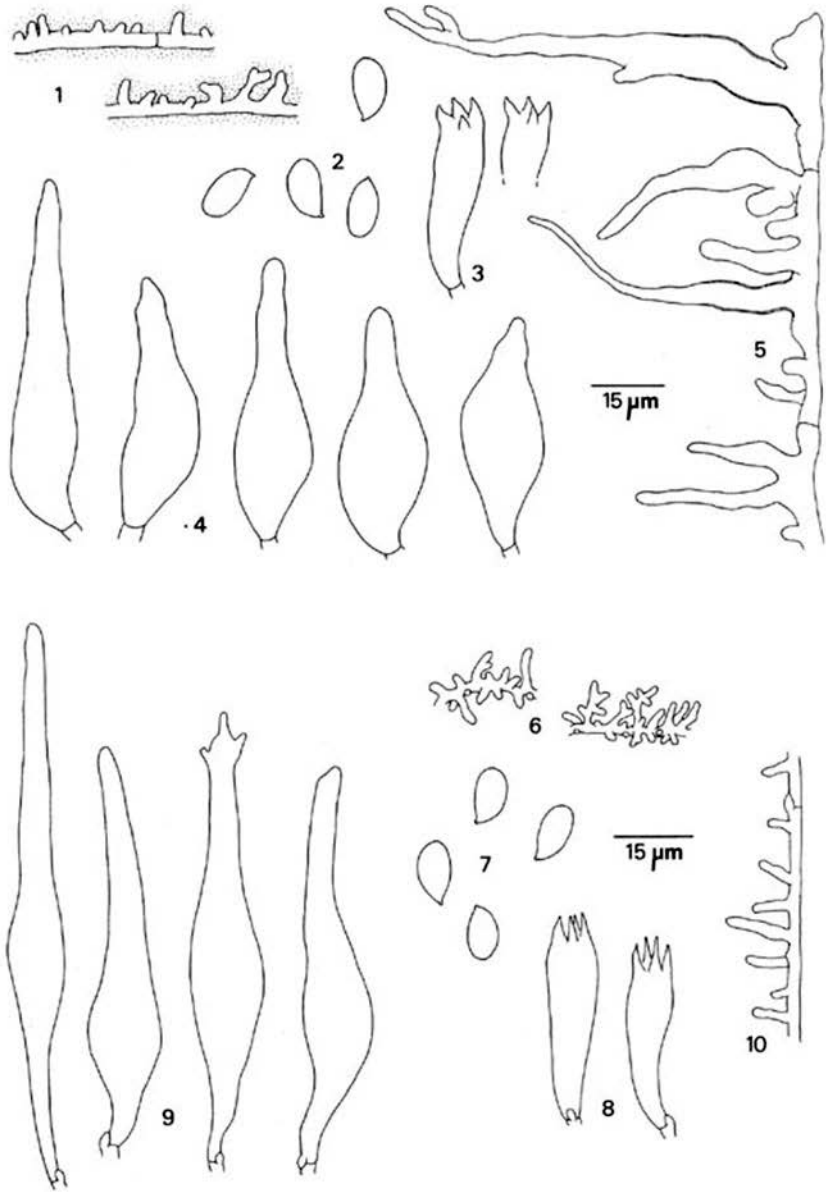
Original diagnosis:

Pileus ad usque 1 cm latus, hygrophanus, striatus, griseo-farinaceus, griseo-brunneus, campanulatus, umbonatus. Lamellae brunneo-griseae, ad aciem claro-griseae anastomosans, rugosae, distantes, adnexae. Stipes ad usque 5 cm longus, 1 cm crassus, griseo-brunneus, griseo-farinaceus. Odor alcalinus, sapor indistinctus. Sporae 6,5–11 × 5–6,5 µm, cylindricae, ellipsoideae, ovoideae vel guttiformes. Cheilocystidia 50–75 × 11–13 × 6,5 µm, numerosa, lageniformia. In juniperetis, ad lignum putridum.

Holotypus: URPSS, Uzbekistan, regio Dzhizak, distr. Zaamin, montes Pamiro-Alai, jugum Turkestan, Tujasai, in Junipereto, ad truncum *Juniperus sp.*, alt. 2500 m. s. m., 25. V. 1980, leg. K. Kalamees (TAA 121354).

Basidia 30–34 × 8–10 µm, clavate, 4-spored, rarely 2-spored, clampless, with sterigmata up to 6 µm long. Spores (8.50–)8.75–***11.11***–13 × 5.20–***6.23***–7.20 µm; Q = 1.61–***1.78***–1.94; (n = 21), ellipsoid to narrowly ellipsoid, smooth, weakly amyloid. Cheilocystidia 39–62 × 10–17.5 µm, hyaline, clampless, broadly lageniform, lageniform to fusiform, smooth, forming a sterile band (lamella-edge homogeneous). Pleurocystidia not observed. Hymenophoral trama slightly dextrinoid. Hyphae of the pileipellis 2.5–4 µm wide, clampless, vacuolar pigment absent, densely covered with simple or more rarely somewhat furcate excrescences 3–5(–14) × 2–4 µm, embedded in dense gelatinous matter. Hyphae of the pileitrama up to 45 µm wide. Hyphae of the stipitipellis 2–6 µm wide, clampless, covered with short or long excrescences 3–25 × 3–5 µm and caulocys-

1) Dpto. de Biología Vegetal, Univ. de Alcalá, E-28871 Alcalá de Henares, Spain.
2) Rijksherbarium/Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands.



Figs. 1– 5. *Mycena flocculina* (holotype). 1. Hyphae of the pileipellis; 2. spores; 3. basidia; 4. cheilocystidia; 5. stipitipellis. — Figs. 6 –10. *Mycena gilvipes* (holotype). 6. Hyphae of the pileipellis; 7. spores; 8. basidia; 9. hymenial cystidia; 10. hypha of the stipitipellis.

tidia up to 110 (or more) × 7–20 µm, versiform, usually tapering towards the apex, flexuose to straight, thin-walled but sometimes fairly thick-walled at their bases, not embedded in gelatinous matter.

Because of the fragmentary state of the holotype, we refrain from re-evaluating the macroscopic features described by Kalamees (1987) except for the width of the stipe which hardly could be 1 cm wide, as indicated by its author (in dried material not even 1 mm), and the colours mentioned for the gills which are stated to be grey-brown (in dried material pale cream). The microscopic details are based on re-examination of the holotype.

According to the following characters, i) the absence of any trace of vacuolar pigment in the hyphae of pileipellis, ii) the absence of oleiferous hyphae [more or less rare in *Mycena* (Kühner, 1938), and often present in *Hydropus* (Singer, 1986)], iii) the densely diverticulate hyphae of pileipellis embedded in gelatinous matter [according to Singer (1982), the epicutis in *Hydropus* is never gelatinized except in cases where the upper layer of the hypodermium is gelatinized, which is not the case in *M. flocculina*] and, iv) the dextrinoid hymenophoral trama (very rare in *Hydropus*), it becomes clear that this taxon should be placed in *Mycena*.

Mycena flocculina belongs to sect. *Fragilipedes* and is characterized by its long and peculiar caulocystidia throughout the stipe, its pileipellis embedded in gelatinous matter, its relatively large and amyloid spores, and the absence of clamp-connections. Within sect. *Fragilipedes* this species keys out close to *M. deceptor* Maas G. (Maas Geesteranus, 1988a), which is however completely different.

On the other hand, the stipitipellis of *M. flocculina* recalls that of *M. pilosella* Maas G., but the latter differs in having cylindrical and slender caulocystidia, smaller spores, presence of clamps, and a pileipellis without any trace of gelatinous matter.

Another species which recalls *M. flocculina* is *M. scirpicola* (described as new in this paper), both sharing the greyish brown colour of the pileus, the structure of the stipitipellis, and the presence of similar cheilocystidia. The latter can be separated by the absence of a nitrous odour, presence of clamps, smaller spores, pileipellis which is not embedded in gelatinous matter, and the very different habitat (fruiting on dead culms of *Scirpus holoschoenus* L.).

Mycena gilvipes Villarreal, Heykoop & Maas G., *spec. nov.* — Figs. 6–10

Basidiomata caespitosa. Pileus 15–17 mm latus, conico-campanulatus, glaber videtur, hygrophanus, striatus, obscure olivaceogriseus, pallescens. Caro tenuis, albida, odore nitroso. Lamellae 14–17 mm stipitem attingentes, usque ad 3,5 mm latae, molles, adscendentes, adnatae, albae vel pallide flavidae, margine convexae, concolores. Stipes –100 × 1,5–3 mm, cavus, cylindraceus, aequalis, fragilis, glaber videtur, nitens, olivaceus, deorsum flavo-tinctus, sursum flavus, basi fibrillis albidis vel flavidis munitus.

Basidia 26–33 × 7–9 µm, clavata, 4-sporigera, fibulata. Sporae 8,50–***9,84***–11,50(–13) × 4,50–***5,17***–6 µm, ellipsoideae vel subcylindraceae, leves, amyloideae. Cheilocystidia 80–110 × 6,5–12(–16) µm, hyalina, fibulata, fusiformia vel lageniformia, levia, apice raro subramosa, interdum paulo crasse-tunicata. Pleurocystidia crebra, similia. Trama hymenophori dextrinoidea. Hyphae pileipellis –5 µm latae, fibulatae, dense diverticulatae, haud in materiam gelatinosam immersae. Hyphae stipitipellis 2,5–4 µm latae, fibulatae, diverticulatae, haud in materiam gelatinosam immersae, cellulae terminales haud observatae.

Ad aciculas dejectas in silvis acerosis.

Holotypus: no. 19360 (AH); isotypus: no. 996.157-396 (L).

Etymology: from Latin gilvus = yellowish tan referring to the colour of the stipe.

Basidiomata cespitose. Pileus 15–17 mm in diam., conical-campanulate, apparently glabrous, hygrophanous, translucent-striate nearly to the centre of pileus, dark grey or olive grey (Munsell 5Y 3/1–2, Munsell, 1988) at centre, becoming paler towards the margin to pale olive (–5Y 6/3–4), remaining almost whitish (5Y 8/2), finally light olive-brown (2.5Y 5/4–6) when dry. Flesh thin and whitish. Smell strongly nitrous. Taste 'sweetish'. Gills 14–17 reaching the stipe, up to 3.5 mm broad, tender, ascending, adnate, white to pale yellow (2.5Y 7/4) when dry, lamella-edge convex and concolorous; lamellulae present. Stipe up to 100 × 1.5–3 mm, hollow, cylindrical, equal, becoming slightly wider at the base, fragile, appearing entirely glabrous, shiny, olive (5Y 5/6, 4/3–4), with more pronounced yellowish tinges towards the base (5Y 6/6, 6/8), becoming yellow to pale yellow (5Y 8/4, 8/6, 8/8) towards the apex and with a slightly pinkish tinge, dark yellowish brown (10YR 4/4, 4/6, 3/4 to 3/6) in dried material, the base covered with long, interwoven, whitish to pale yellowish fibrils.

Basidia 26–33 × 7–9 µm, clavate, 4-spored, clamped. Spores 8.50–***9.84***–11.50(–13) × 4.50–***5.17***–6 µm; Q = 1.50–***1.91***–2.41(–2.44); (n = 22), ellipsoid to subcylindrical, smooth, amyloid. Cheilocystidia 80–110 × 6.5–12(–16) µm, hyaline, clamped, fusiform to lageniform, smooth, rarely ramified at apex into two or three short excrescences, sometimes with slightly thick walls (less than 1 µm), forming a sterile band (lamella-edge homogeneous). Pleurocystidia abundant, similar to cheilocystidia in shape and size. Hymenophoral trama strongly dextrinoid. Hyphae of pileipellis –5 µm wide, clamped, densely diverticulate, with cylindrical excrescences 2–8(–15) × 1–3 µm, tending to grow out to much longer and profusely branched structures, not embedded in gelatinous matter. Hyphae of the stipitipellis 2.5–4 µm wide, covered with fairly numerous excrescences 2–17 × 1.5–3 µm, clamped, not embedded in gelatinous matter. Terminal cells of the cortical layer of the stipitipellis not observed.

Habitat — On needles of *Pinus pinaster* Aiton.

Material studied. SPAIN: Ávila, Casavieja, UTM 30TUK502665, alt. 1590 m, leg. M. Villarreal & M.A. Jiménez, 26 Nov. 1995, AH 19360 holotype; isotype: no. 996.157-396 (L).

Mycena gilvipes, a member of sect. *Fragilipedes*, possesses several characters similar to those of two other species of this section, such as clamped hymenial elements, densely diverticulate hyphae of the pileipellis with cylindrical excrescences which are not embedded in gelatinous matter, and a yellowish brown stipe. These are *M. alcaliniformis* (Murrill) Murrill and *M. citrinomarginata* Gillet, but both differ in lacking a strong nitrous smell and pleurocystidia.

Because of the unusual olivaceous tints of the stipe *Mycena gilvipes* may be thought to be similar to *M. cyrnea* Maas G. (Maas Geesteranus, 1993), a species described from Corsica. Moreover, both share the presence of long and lageniform cheilocystidia, which are nevertheless shorter in *M. cyrnea*. The differences between both species under discussion are tabulated below (Table I).

Table I. A comparison between *M. cyrnea* and *M. gilvipes*.

	cespitose habit	pileus margin	mean Q value	cheilocystidia length	hyphae of the stipitipellis
Mycena cyrnea	no	dingy pink	1.75	40–70 µm	very sparsely diverticulate
Mycena gilvipes	yes	without pink tinge	1.91	80–110 µm	densely diverticulate

Mycena scirpicola Villarreal, Heykoop, Esteve-Rav. & Maas G., *spec. nov.* — Figs. 11–15

Basidiomata gregaria. Pileus 6–20 mm latus, conicus vel conico-campanulatus, haud umbonatus, paulo hygrophanus, striatus, subsulcatus, siccus, palide brunneus vel griseus, centro obscure griseobrunneus, omnino albopulverulentus. Caro tenuis, pallide brunnea, odore saporeque nullis. Lamellae 15–27 stipitem attingentes, c. 2,5 mm latae, molles, adscendentes, adnatae vel dente decurrentes, albae vel griseae, margine convexae, concolores. Stipes 35–60 × 1–2,5 mm, cavus, cylindraceus, aequalis, fragilis, albo vel griseolo-pulverulentus, griseobrunneus, siccus, basi obscure griseus vel ater, basi fribrillis crassis albidis munitus.

Basidia 23–30 × 7–9 µm, clavata, 4-sporigera, fibulata, sterigmatibus usque ad 4,5 µm praedita. Sporae 7,70–***8,54***–9,50 × (4,20–)4,25–***4,90***–5,50 µm, ellipsoideae vel subcylindraceae, leves, amyloideae. Cheilocystidia 30–65 × 8–15 µm, hyalina, levia, fibulata, lageniformia, sublageniformia, fusiformia, interdum apice subcapitata. Pleurocystidia margine solum observata. Trama hymenophori paulo dextrinoidea. Hyphae pileipellis 2–4 µm latae, fibulatae, dense diverticulatae, haud in materiam gelatinosam immersae. Hyphae stipitipellis 1,5–3 µm latae, fibulatae, leves vel raro diverticulatae, caulocystidibus longis, interdum furcatis instructae.

Ad *Scirpi* folii vaginam putridam.

Holotypus: no. 20882 (AH); isotypus: no. 996.157-334 (L).

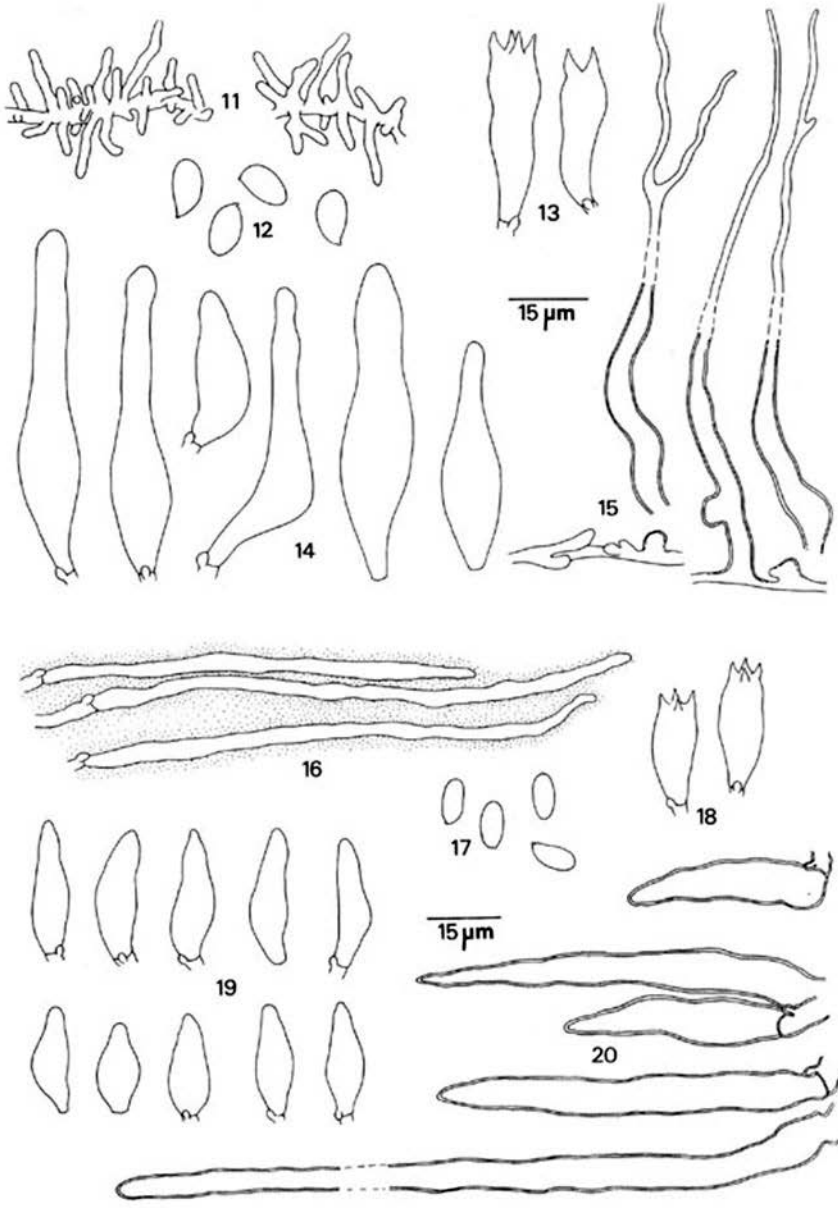
Etymology: because of its typical habitat on *Scirpus holoschoenus* L.

Basidiomata gregarious. Pileus 6–20 mm in diam., conical to conical-campanulate, not umbonate, slightly hygrophanous, striate, dry, slightly sulcate, very pale brown to light grey (Munsell 10 YR 8/3, 8/4 to 10 YR 7/2, 7/3), dark grey or dark greyish brown (10 YR 4/1–2) at the disc, completely covered with a whitish powdery 'bloom' which is easily removed with the slightest contact. Flesh thin, very pale brown (10 YR 7/4). Smell and taste not characteristic (none). Gills 15–27 reaching the stipe, aprox. 2.5 mm broad, tender, ascending, adnate to decurrent with a small tooth, white to greyish (between 10 YR 8/1 and 10 YR 7/1), lamella-edge convex, concolorous; lamellulae present. Stipe 35–60 × 1–2.5 mm, cylindrical, hollow, equal, very slightly wider towards the base, fragile, completely covered with whitish-greyish powdery 'bloom' (similar to that in the pileus), greyish brown (10 YR 5/2) becoming very dark grey at the base (5 YR 3/1) to black (2.5 YR N 2/) when drying, the base covered with scarce, short, coarse, straight and appressed whitish fibrils.

Basidia 23–30 × 7–9 µm, clavate, 4-spored, but also 2-spored (presumably immature), clamped, sterigmata up to 4.5 µm long. Spores 7.70–***8.54***–9.50 × (4.20–)4.25–***4.90***–5.50 µm; Q = (1.37–)1.46–***1.74***–1.93; (n = 21); ellipsoid to subcylindrical, smooth, amyloid. Cheilocystidia 30–65 × 8–15 µm, hyaline, smooth, clamped, lageniform, sublageniform to fusiform, sometimes with subcapitate apex. Lamella-edge homogeneous and sterile. Pleurocystidia only observed with certainty near to the lamella-edge. Hymenophoral trama slightly dextrinoid. Hyphae of the pileipellis 2–4 µm wide, densely diverticulate with short to long excrescences up to 35 × 2–3 µm, clamped, not embedded in gelatinous matter. Hyphae of the stipitipellis 1.5–3 µm wide, clamped, smooth or with some isolated thick excrescences (3–10 × 2–3.5 µm), covered with long caulocystidia tapering towards the apex, –300 × 4–7 × 1–1.5 µm (length × width at base × width at apex), with slightly thickened walls at the base (up to 1.5 µm), and sometimes with apical furcations or lateral excrescences.

Habitat — On dead culms of *Scirpus holoschoenus* L.

Material studied. SPAIN: Ávila, Casavieja, UTM 30TUK512631, alt. 650 m, leg. M. Heykoop, F. Esteve-Raventós & M. Villarreal, 19 Nov. 1996, AH 20882 holotype; isotype: no. 996.157-334 (L).



Figs. 11–15. *Mycena scirpicola* (holotype). 11. Hyphae of the pileipellis; 12. spores; 13. ba: ia; 14. cheilocystidia; 15. stipitipellis. — Figs. 16–20. *Mycena rubescens* (holotype). 16. Terminal ce. of the pileipellis; 17. spores; 18. basidia; 19. hymenial cystidia; 20. caulocystidia.

Mycena scirpicola is a typical member of sect. *Fragilipedes* of which it is the only known species fruiting on *Cyperaceae* and, more specifically, on *Scirpus holoschoenus*, a mediterranean plant. Besides, it is characterized by the strong blackening of the stipe which is completely covered by long and very characteristic caulocystidia.

Mycena section **Rubescentes** Villarreal, Esteve-Rav., Heykoop & Maas G., *sect. nov.*

Basidiomata statura media. Pileus flavus, centro striisque pallide olivaceobrunneis, margine aetate rubroaurantiaca. Caro odore raphanoideo. Lamellae molles, adscendentes, albae, margine concolores. Stipes fragilis, siccus, pruinosus, flavus, basi fibrillis praeditus.

Basidia subfusiformia, 4-sporigera, fibulata. Sporae ellipsoideae, leves, inamyloideae. Cheilocystidia fusiformia vel subutriformia, levia, fibulata. Pleurocystidia nulla. Trama hymenophori dextrinoidea. Hyphae pileipellis fibulatae, leves, cellulis terminalibus elongatis instructae, in materiam gelatinosam immersae. Hyphae stipitipellis leves, fibulatae, haud in materiam gelatinosam immersae; caulocystidia subincrassata, elongata.

Humicola.

Species typica: *Mycena rubescens*.

Basidiomata medium-sized. Pileus yellow, translucent-striate, with the disc and striation light olive-brown, and the margin staining strongly reddish orange in mature specimens. Smell raphanoid. Gills tender, ascending, white, with convex and concolorous lamella-edge. Stipe fragile, dry, pruinose, yellow, and rooting.

Basidia subfusiform, 4-spored, clamped. Spores ellipsoid, smooth, non-amyloid. Cheilocystidia fusiform to subutriform, smooth, clamped. Pleurocystidia absent. Hymenophoral trama strongly dextrinoid. Hyphae of the pileipellis clamped, smooth, with elongate terminal elements, embedded in gelatinous matter. Hyphae of the stipitipellis clamped, not embedded in gelatinous matter. Caulocystidia narrowly fusoid to subcylindrical, with slightly thick walls.

Humicolous.

Type species: *Mycena rubescens*.

Mycena rubescens Villarreal, Esteve-Rav., Heykoop & Maas G., *spec. nov.* — Figs. 16–20

Basidiomata caespitosa. Pileus 4–6 mm latus, e hemisphaerico convexus, haud umbonatus, hygrophanus, sublubricus, esulcatus, striatus, flavus, disco striisque pallide olivaceobrunneis, margine aetate rubroaurantiaca. Caro tenuis albida, odore raphanoideo. Lamellae 18–22 stipitem attingentes, haud 1 mm latae, molles, adscendentes, adnatae, albae vel pallide flavae, margine convexae, concolores. Stipes 19–35 × –1 mm, cavus, radicans, cylindraceus, aequalis, fragilis, siccus, dense pruinosus, e pallide flavo olivaceobrunneus, basi fibrillis brunneis munitus.

Basidia (16–)20–23 × 8–10 µm, subfusiformia, 4-sporigera, fibulata, sterigmatibus 4 µm longis praedita. Sporae (6,50–)6,55–***8,08***–9,50 × 3,50–***4,20***–4,93(–5,10) µm, ellipsoideae vel subcylindraceae, leves, inamyloideae. Cheilocystidia (16–)20–30 × 6–7 µm, hyalina, levia, fibulata. Pleurocystidia nulla. Trama hymenophori dextrinoidea. Hyphae pileipellis 1,8–4 µm latae, fibulatae, leves, cellulis terminalibus –120 × 2–4 µm, elongatae, in materiam gelatinosam immersae. Hyphae stipitipellis 3–8 µm latae, leves, fibulatae, haud in materiam gelatinosam immersae; caulocystidia 30–215 × 9,5–12 µm, fusiformia vel subcylindracea.

Ad *Betula pendula* ssp. *fontqueri* folia decisa.

Holotypus: no. 22062 (AH).

Etymology: referring to the red-orange staining of the pileus margin.

Basidiomata cespitose. Pileus 4–6 mm in diam., at first hemispherical to paraboloid, finally becoming convex, not umbonate, glabrous, hygrophanous, somewhat lubricous when wet, not sulcate, translucent-striate nearly to the disc, yellow (between Munsell 2.5 Y 8/8 and 7/8), with the disc and striation light olive-brown (2.5 Y 5/4, 5/6), margin becoming strongly reddish orange in mature specimens. Flesh thin and whitish. Smell slightly raphanoid. Taste not recorded. Gills 18–22 reaching the stipe, less than 1 mm broad, tender, ascending, adnate, white to pale yellow (5Y 8/4–6) when dry, with convex and concolorous lamella-edge; lamellulae present. Stipe 19–35 × –1 mm, hollow, rooting, cylindrical, equal, fragile, dry, densely pruinose throughout, especially at the apex, at first pale yellow (2.5 YR 8/6) then olive-yellow (2.5 YR 6/6) to light olive-brown (2.5 YR 5/4) in dried material, the base extending into a dense 'brownish' network of mycelial cords.

Basidia (16–)20–23 × 8–10 µm, subfusiform, 4-spored, clamped, sterigmata up to 4 µm long. Spores (6.50–)6.55–***8.08***–9.50 × 3.50–***4.20***–4.93(–5.10) µm; Q = 1.62–***1.92***–2.25; (n = 24), ellipsoid, narrowly ellipsoid to subcylindrical, smooth, non-amyloid. Cheilocystidia (16–)20–30 × 6–7 µm, hyaline, smooth, clamped, fusoid to narrowly fusoid or narrowly utriform, short-stalked, with obtuse apex, forming a sterile band (lamella-edge homogeneous). Pleurocystidia absent. Hymenophoral trama strongly dextrinoid. Hyphae of the pileipellis 1.8–4 µm wide, clamped, smooth, with elongate terminal elements –120 × 2–4 µm, embedded in gelatinous matter. Hyphae of the stipitipellis 3–8 µm wide, smooth, clamped, not embedded in gelatinous matter. Caulocystidia present throughout the stipe, variable in size, 30–215 × 9.5–12 µm, narrowly fusoid to subcylindrical, short-stalked, with slightly thick walls (less than 1 µm).

Habitat — On humus of *Betula pendula* ssp. *fontqueri* G. Moreno & Peinado.

Material studied. SPAIN: Madrid, Canencia, Pto. de Canencia, UTM 30TVL3425, alt. 1400 m, leg. F. Esteve-Raventós, C. Sánchez, J.N. Campoamor & M. Villarreal, 24 Oct. 1996, AH 22062 holotype.

In the key to the sections (Maas Geesteranus, 1992), *Mycena rubescens* would fit in key 4 and, more especially, in section *Adonideae* characterized by a brightly coloured pileus, smooth hyphae of the stipitipellis, inamyloid spores, and caulocystidia with colourless contents. However, several other features of *M. rubescens* produce a very different picture that does not agree with sect. *Adonideae*. *Mycena rubescens* constitutes the type species of a new section whose differential characters are tabulated below (Table II).

Table II. A comparison between sect. *Rubescentes* and sect. *Adonideae*.

	stipe	smell	hyphae of the pileipellis	hymenophoral trama	caulocystidia
section *Rubescentes*	rooting	raphanoid	smooth	strongly dextrinoid	subcylindrical with thickened cell walls
section *Adonideae*	not rooting	not distinctive	diverticulate	not dextrinoid	clavate to fusiform without thickened cell walls

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FIRST REPORT OF COPRINUS SPADICEISPORUS IN EUROPE

C.B. ULJÉ[1], A. GENNARI[2], F. DOVERI[3], G. CACIALLI[4] & V. CAROTI[5]

The first record of *Coprinus spadiceisporus* Van De Bogart in Europe is described. A study of the type and that of *Coprinus roseistipitatus* Van De Bogart revealed that both are conspecific, and accordingly the latter name is considered synonymous.

Some of us have spent many years studying the taxonomy and distribution of coprophilous fungi in Italy. So far 108 species, 19 belonging to the genus *Coprinus*, have been collected and described. Many of these species are quite common and widespread, others are typical of temperate climates, and some are undoubtedly rare. Among the rare ones is *C. spadiceisporus* Van De Bogart, an American species, which is here reported for the first time from Europe. The morphological characteristics of *Coprinus spadiceisporus* are described and compared with some related taxa, including *C. roseistipitatus,* another American species described by Van De Bogart (1976), but which we consider synonymous with *Coprinus spadiceisporus*.

In the following description the notation [100, 4, 2] stands for '100 spores from 4 basidiocarps in 2 collections'. L × B × W means: length × breadth in frontal view × width in side view. Q stands for 'length of the spores divided by breadth in frontal view'.

Coprinus spadiceisporus Van De Bogart — Fig. 1

Coprinus spadiceisporus Van De Bogart, Mycotaxon 4 (1976) 245.
Coprinus roseistipitatus Van De Bogart, Mycotaxon 4 (1976) 262.

Pileus up to 20 × 14 mm when still closed, 20–50 mm when expanded, ellipsoid-ovoid at first, later campanulate, finally applanate or even revolute at deliquescence. Cap cuticle whitish at first, soon with a brown or dark grey-brown disc, then cream-hazelnut coloured, progressively greying, pronouncedly grooved up to the centre, covered with a fibrous-woolly, whitish veil, which appears more crowded at the centre, split up toward the periphery in small upturned browning scales. Lamellae fully deliquescent, ascending, free, narrow, very crowded, 2–5 mm high, white at first, then grey and finally blackish, with a lighter, whitish but turning to pink, scurfy edge. Stipe 20–80 × 3–5 mm, up to 8 mm at the base, cylindrical or slightly tapering towards the apex, at first bulbous-clavate, later slightly bulbous, solid, becoming hollow, fully white, thinly striate and vaguely flocculose, provided with a thoroughly differentiated median annulus, white above, cream

1) Van Dijkstraat 21, 2405 XE Alphen aan den Rijn, The Netherlands.
2) Via Anconetana 35/A, 52100 Arezzo, Italy.
3) Via Baciocchi 9, 57126 Livorno, Italy.
4) Via Aloisi 3, 57128 Livorno, Italy.
5) Via Zola 51, 57122 Livorno, Italy.

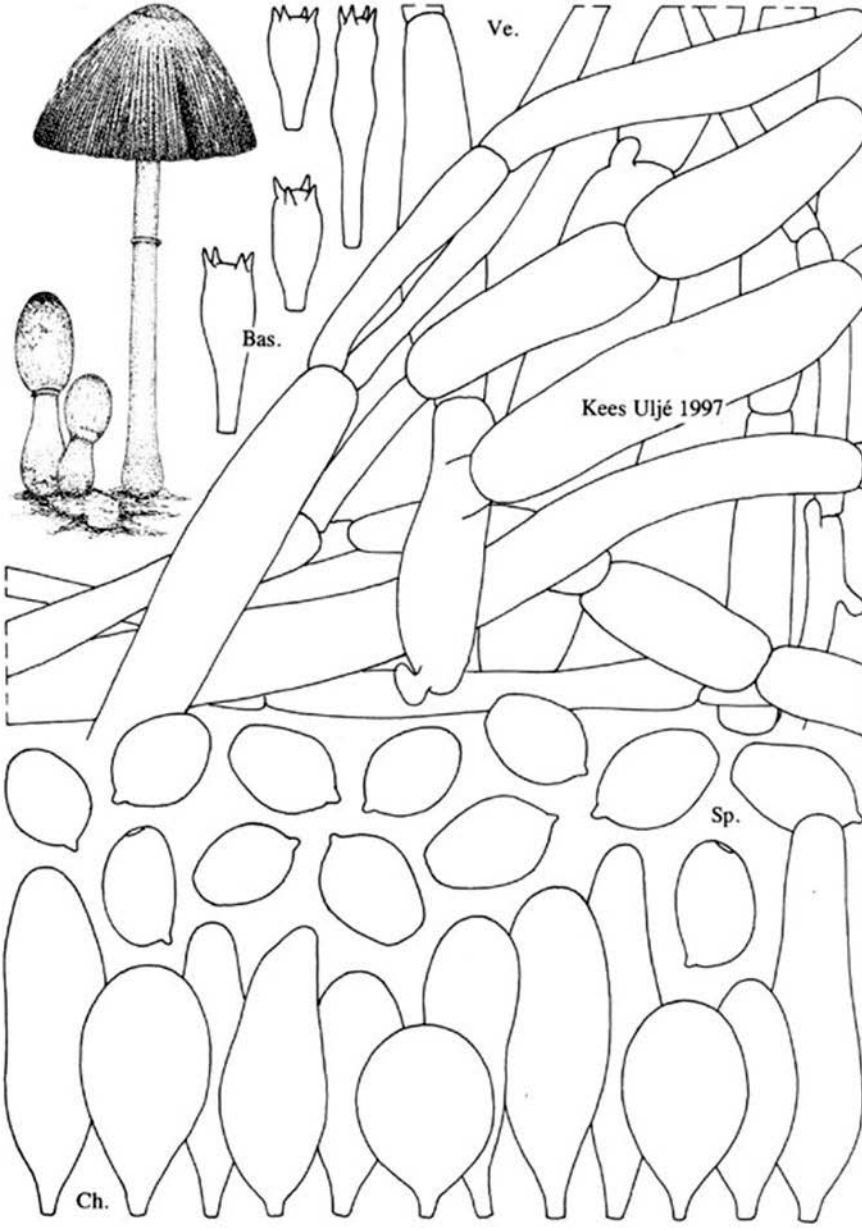

Fig. 1. *Coprinus spadiceisporus*. Sp. = spores, × 2000; Bas. = basidia; Ch. = cheilocystidia; Ve. = veil (Bas., Ch. & Ve. × 800).

ochraceous on the lower surface. Context white, quite firm, fibrous, devoid of particular smell and taste.

Spores [100, 5, 2] 6.7–9.3 × 5.3–6.8 × 4.7–5.4 µm (L × B × W); Q = 1.20–1.45; av. Q = 1.35; av. L = 8.0–8.4, av. B = 6.0–6.3 µm, submitriform, rhomboid or ovoid and somewhat truncate in frontal view, ellipsoid in side view, dark red-brown, with conspicuous hilar appendage and a distinct, central or slightly eccentric, 1.5–1.8 µm wide germ pore. Basidia 24–43 × 8–10 µm, 4-spored, present in three forms: claviform and short-stalked, cylindrical-clavate or elongated cylindrical with a distinct median narrowing. Each basidium is surrounded by (3–)4–6(–8) pseudoparaphyses. Pleurocystidia and caulocystidia absent. Cheilocystidia 30–65 × 15–23 µm, abundant (edge sterile), polymorphous: (sub)globose, ovoid, ellipsoid, oblong, utriform, subcylindrical. Veil made up of elongate elements in chains, cylindrical or somewhat inflated, often constricted at septum, 30–125 × 6–25 µm, with fusiform, ovoid or cylindrical terminal cells. Pileipellis a cutis, made up of cylindrical, more or less parallel, repent hyphae. Clamp-connections absent. Pseudoclamps present, difficult to observe because of the very thin walls.

Habitat & distribution — Several solitary or fasciculate specimens on dung of fallow deer. Only known from the type locality (State of Washington, USA) and from the finds described in this paper (Italy).

Collections examined. USA: State of Washington, no other annotations, *F. Van De Bogart 217* (holotype, WTU); Lewis, Cispus Centre, 25 Oct. 1975, *F. Van De Bogart 3369* (holotype of *C. roseistipitatus*, WTU). — ITALY: Grosseto, Ansedonia-Orbetello (Tombolo di Feniglia), 26 Dec. 1995, *A. Gennari, MCVE 571* (private herb.); 27 Nov. 1996, *A. Fani, L. Casetti, A. Gennari, MCVE 572* (private herb.).

The macroscopic and microscopic features of our collections were independently described by each of us before they were compared. It was immediately apparent that this taxon fitted none of the recognised European species.

To place what was a new European taxon or, possibly, a species known elsewhere, we searched the literature and found a fairly good structural likeness and similarity of habitat between our Italian specimens and the taxon described by Van De Bogart (1976).

To confirm the identification, the type material was studied by one of us (C.B. Uljé). The microscopic and macroscopic characters were indeed similar to those in our collections. The spores were slightly larger in the type material (8.2–10.3 × 5.8–7.3 µm; Q = 1.25–1.45; av. Q = 1.35; av. L = 9.1, av. B = 6.7 µm), but the quotient and shape were in good agreement. The study dispelled any doubt and confirmed that our species deserves the name *C. spadiceisporus*.

Coprinus spadiceisporus was described as a new species by Van De Bogart (l.c.) in the first part of a study devoted to the genus *Coprinus* in western North America. He placed the new taxon in section *Coprinus*, after acknowledging that he based his systematics on Kühner & Romagnesi (1953).

Uljé & Noordeloos (1997) divided section *Coprinus* in four subsections (based on Singer, 1986), mainly on the basis of characters in the veil: subsect. *Coprinus* (= *Annulati* J. Lange), subsect. *Atramentarii* (Fr.) Konr. & Maubl., subsect. *Alachuani* Sing., and subsect. *Lanatuli* Sing.

In this scheme, *Coprinus spadiceisporus* has to be placed in the subsection *Coprinus*, on account of the presence of a ring together with the adpressed, hyphoid veil.

The other species belonging to subsection *Coprinus* are *C. comatus* and *C. sterquilinus*. *Coprinus comatus* differs from *C. spadiceisporus* in having much larger basidiocarps, larger spores and a habitat on soil, while *C. sterquilinus*, though growing on dung, has larger basidiocarps and spores twice as large.

A second taxon, that was also first described and placed in sect. *Coprinus* by Van De Bogart (l.c.), is *C. roseistipitatus*. Its distinguishing characteristics were given as pink-coloured cystidia and stipe apex, basidia with a median grey pigment band and spores that are slightly larger than *C. spadiceisporus*. In the type collection of this species the spores were indeed found to be slightly larger (less than Van De Bogart found) than in *C. spadiceisporus* (8.7–10.7 × 6.3–7.9 µm), but of similar shape and quotient. The slight difference in spore size is not unusual in species of *Coprinus* and not sufficient in itself to maintain two species.

The pink colour of the cheilocystidia could not be not found in the dried material of the type collection, nor could the median grey band on the basidia. The pink-coloured apex of the stipe does not seem to us to be a usable macroscopic feature because of the fact that white colours often become pinkish in mushrooms, especially under wet conditions.

For these reasons we consider *C. roseistipitatus* synonymous with *C. spadiceisporus*.

Although *C. spadiceisporus* and *C. roseistipitatus* are each based upon only one collection, Van De Bogart described both species as occurring on rabbit and deer dung (no annotations were added to the type collections). For *C. spadiceisporus* he described clamp-connections as being present, for *C. roseistipitatus* as being absent. We found only pseudoclamps in both species.

ACKNOWLEDGEMENTS

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MARASMIUS CELTIBERICUS (TRICHOLOMATACEAE, AGARICALES) A NEW SPECIES FROM SPAIN

G. MORENO[1] & A. RAITVIIR[2]

Marasmius celtibericus G. Moreno & Raitviir, a new species from Spain, is described and illustrated. It is characterized by very small basidiocarps with a smooth hymenophore, somewhat resembling *Marasmius cornelii* Laessøe & Noordel. Microscopically, however, *Marasmius celtibericus* must be ranged in sect. *Hygrometrici* on account of the pileipellis.

In the autumn of 1996, which has been exceptionally rainy in the Iberian Peninsula, we carried out several forays to the autochtonous vegetation of *Kochia prostrata* (L.) Schrader in the stands of the association *Artemisio herba-albae-Salsoletum vermiculatae* (Br.-Bl. & O. Bolòs 1957) O. Bolòs 1967, a type of halonitrophilous brushwoods, which have a Saharian-Indian and Irano-Turanian optimum, but occur also all over Spain on clayey-marly miocenic sediments, particularly when these are rich in chlorides. In general, this association is a final state of the degradation of climax evergreen oak forests belonging to the association *Quercetum rotundifoliae* Br.-Bl. & O. Bolòs 1957. On dead branches of *Jasminus fruticans* L., a characteristic shrub in these associations, we collected an abundantly fruiting small *Marasmius* species, which we describe as new here.

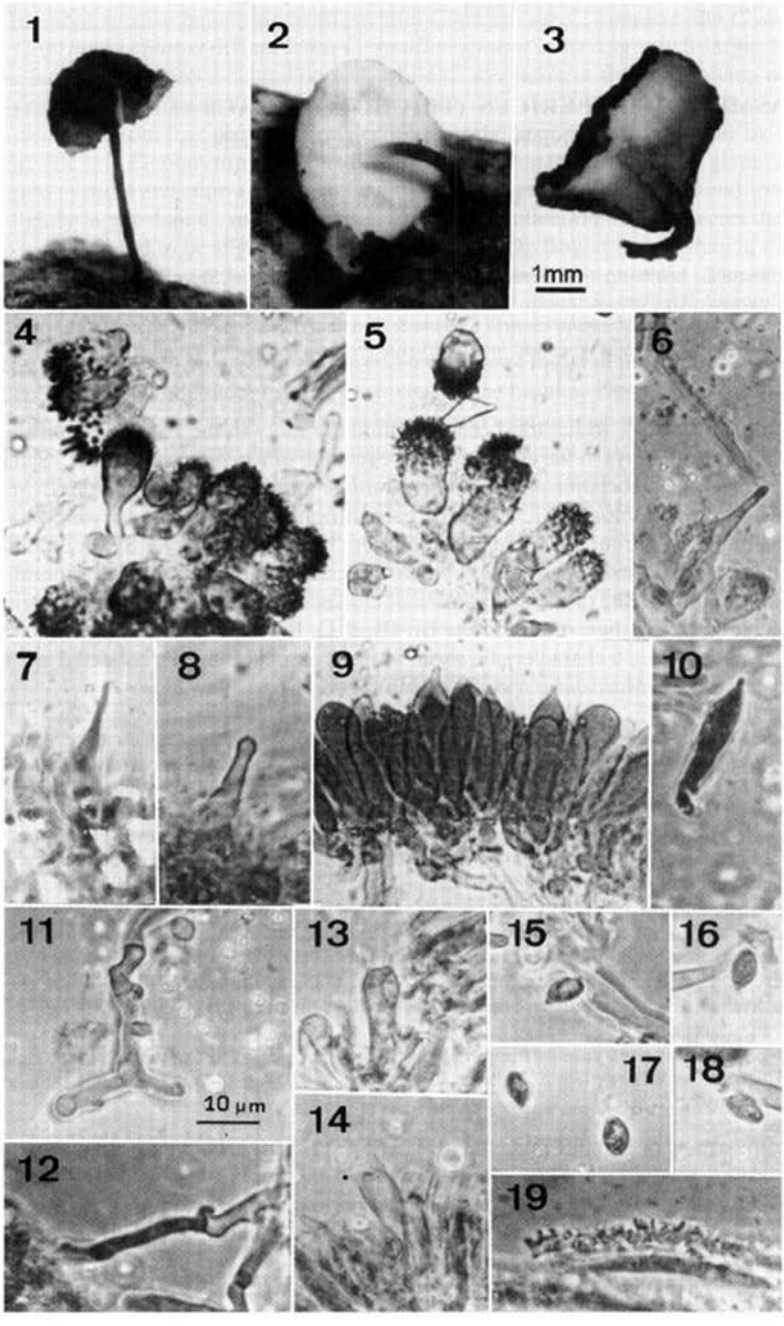
Marasmius celtibericus G. Moreno & Raitviir *spec. nov.* — Figs. 1–19

Pileus 0.3–1.2(–1.5) mm latus, convexus vel plano-convexus, rufo-brunneus, sicca rufus, minute granulosus, in vivo plicato-rugosus. Margine recto, concoloro. Hymenophorum laeve vel plicatum, raro 3–7 lamellas adnatas habentes, albidum vel albido-cremeum. Stipes 0.5–4 × 0.1 mm, teres, curvatus, centralis, obscure rufo-brunneus, velutino-furfuraceus, apice pallide stramineo. Pileipellis hymeniformis, cellulis fibulatis, globosis vel clavatis, apicibus crassiter brunneotunicatis, verrucosis. Pileocystidia fusiformia, 20–28 × 5–6 µm, hyalina. Basidia 24–33 × 7–9 µm, clavata, tetraspora, fibulata, hyalina. Sterigmata arcuata, ad usque 2 µm in longitudine. Sporae 8–10(–11) × 5–7 µm, amygdaliformes vel late ellipsoideae, non amyloideae, non dextrinoideae. Hymenocystidia numerosa, 27–40 × 6–9 µm, clavato-fusoideae, hyalina, apicibus papillatis. Stipes hyphis parallelis diverticulatis compositur. In ramis siccis Jasmini fruticans crescit.

Holotypus: In ramis siccis Jasmini fruticans, Reservatum ecologicum Las Cuestas, Alcalá de Henares, Madrid, Hispania, 26.XI.1996, leg. A. Raitviir, M. Lizárraga & G. Moreno (holotypus: AH 18389); isotypus: TAA-137666.

Basidiocarps very small. Pileus 0.3–1.2(–1.5) mm broad, convex or plano-convex, reddish brown, reddish when dry, granular under lens, more or less folded or wrinkled when moist with straight, concolorous margin. Hymenophore smooth or slightly fold-like, rarely 3–7 adnate poorly developed lamellae present, whitish to whitish cream, con-

1) Dpto. de Biología Vegetal (Botánica), Univ. de Alcalá, 28871 Alcalá de Henares, Madrid, Spain.
2) Institute of Zoology and Botany, 181 Riia Street, EE 2400 Tartu, Estonia.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trasting with the colour of pileus; well-developed lamellae not observed. Stipe 0.5–4 × 0.1 mm, insititious, cylindrical, more or less central, curved, pale straw-yellow in the upper third, dark reddish brown in lower two thirds, darker towards base, velvety-scurfy.

Spores 8–10(–11) × 5–7 µm, almond-shaped to broadly ellipsoid, not amyloid, not dextrinoid. Basidia 24–33 × 7–9 µm, with curved, up to 2 µm long sterigmata, 4-spored, clavate, hyaline, clamped. Hymenial cystidia very abundant, 27–40 × 6–9 µm, clavate-fusiform with an apical papilla; more rarely hyaline cystidia similar to the pileocystidia are present.

Pileipellis hymeniform, made up of globose to clavate broom cells, 13–30 × 10–20 µm, having more or less thickened brown wall in upper half, remaining hyaline in lower half, covered with abundant cylindrical, nonramified, short projections (Rotalis-type); clamped. Pileocystidia 20–28 × 5–6 × 1–2 µm, fusiform to lentiform, rarely slenderly tibiiform, with a long, subcapitate neck, projecting for example 17 × 5 × 2 µm from pileipellis (similar to those observed in *Marasmius buxi*). Stipitipellis a cutis of thick-walled, brown, diverticulate hyphae. Stipititrama formed of cylindrical, parallel, hyaline hyphae.

Collections studied. SPAIN: Madrid, Alcalá de Henares, Reserva ecológica Las Cuestas, creciendo sobre ramas secas de *Jasminum fruticans* L., 24-XI-1996, leg. A. Raitviir, AH 18388; idem, 26-XI-1996, leg. A. Raitviir, M. Lizárraga & G. Moreno, AH 18389. Holotypus, ibidem AH 18390; idem, 18-XII-1996, leg. A. Raitviir, AH 18393.

Marasmius celtibericus is characterized by its small size, reddish colour which contrasts to the whitish-cream hymenium, the very dark central stipe, the hymeniform pileipellis composed of broom cells, the hymenial cystidia, and almond-shaped spores. In this combination of characters it differs clearly from the other species of *Marasmius* known in Europe. *Marasmius cornelii* Laessøe & Noordel. has similar small size, but its pileipellis is not made up of broom cells, its caulocystidia and cheilocystidia are different, its spores are narrowly ellipsoid, 12–18 × 3.5–6.5 µm, and it fruits on the leaves of *Cladium mariscum* (Antonín & Noordeloos, 1993). Singer (1976) has reported *Marasmius sphaerodermus* Spegazzini from Hawaii and Argentina with smooth or fold-like hymenium, but it differs by its smaller pileus (0.3–0.7 mm), longer stipe (3–15 × 0.08–0.12 mm) and absence of diverticulate hyphae in the stipe. Corner (1996) has described two species without lamellae from Malesia: *Marasmius patellula* Corner and *M. cyphella* Dennis & Reid. The first differs from the proposed new species in 1–3 mm broad, pale yellowish cream pileus, the short stipe of only 0.2 mm in length, absence of cystidia and nondiverticulate hyphae in stipe. *Marasmius cyphella* differs in the olivaceous brown pileus, lateral rudimentary stipe, tissue above the hymenium containing crystalline masses and absence of cystidia (cf. Dennis & Reid, 1957, fig. 2).

Marasmius celtibericus belongs to the section *Hygrometrici* Kühner according to the classification adopted by Antonín & Noordeloos (1993) on account of its pileipellis structure. It is related to *Marasmius buxi*, which clearly differs, however, by its well-developed lamellae, and habitat.

Figs. 1–19. *Marasmius celtibericum.* 1–3. Basidiocarps, showing hymenophore; 4, 5. elements of pileipellis; 6. fusiform-lageniform pileocystidium; 7, 8. hymenial cystidia similar to the pileocystidia; 9, 10. hymenial cystidia, clavate-fusiform with an apical mucro; 11, 12. clamp-connections; 13, 14. basidia; 15–18. spores; 19. diverticulate hyphae of stipitipellis (all from holotype).

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MYCENA CUPRESSINA, A NEW SPECIES OF SECTION SUPINAE FROM ITALY

V. ANTONÍN[1] & R.A. MAAS GEESTERANUS[2]

Mycena cupressina, found on bark of *Cupressus*, is proposed as a new species belonging to section *Supinae*. It is compared to *M. corticalis* which equally grows on bark of a coniferous tree in North America.

In a field excursion during the 4th Congress of C.E.M.M. (Confederatio Europaea Mycologiae Mediterraneensis) held in Poggibonsi near Siena (Tuscany, Italy), November 4–9, 1996, the first author found a small *Mycena* species predominantly growing on bark of *Cupressus*. It is distinguished especially in having ochraceous coloured carpophores, globose spores, and belongs to the section *Supinae*. However, it differs from other known taxa of that section by smooth stipitipellis hyphae. Therefore, the authors decided to describe it as a new species.

Mycena cupressina Antonín & Maas G., *spec. nov.* — Figs. 1–6

Basidiomata dispersa. Pileus usque ad 8 mm latus, e subhemisphaerico convexus, centro applanatus vel subdepressus, margine subcrenulatus, striatus, griseolo-ochraceus, centro potius brunneus, tenuiter albidofurfuraceus. Caro tenuis, sapore miti. Lamellae 7–10 stipitem attingentes, molles, subarcuatae, 1.5 mm latae, late adnatae, haud decurrentes, albido-ochraceae, aetate pileo pallidiores. Stipes usque ad 7 × 0.5 mm, centralis, cavus, fragilis, subaequalis, incurvus, levis, puberulus, ochraceoflavus, basi e disco albopubescenti natus.

Basidia c. 36 × 11–11.5 µm, clavata, 4-sporigera, fibulata. Sporae 9.5–9.8 × 9.0–9.5 µm, globosae, leves, amyloideae. Cheilocystidia 24–40 × 7–11.5 µm, clavata, fibulata, surculis 1.5–9 × 1–2 µm, simplicibus vel nonnullis furcatis vel ramosis instructa. Pleurocystidia nulla. Trama lamellarum iodi ope rubrobrunnescens. Hyphae pileipellis 1.5–2.5 µm latae, fibulatae, ramosae, surculis crebris munitae. Hyphae stipitis corticales 1.5–2.5 µm latae, fibulatae, leves, cellulae terminales (caulocystidia) 15–20 × 4.5–10 µm, clavatae, surculis 1–2 × 1 µm praeditae.

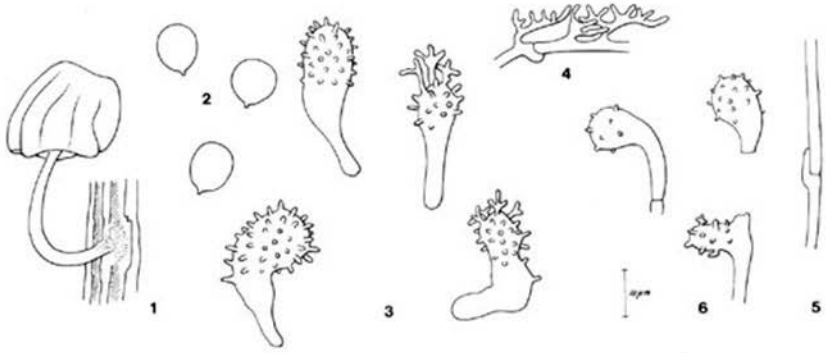
In Cupressi vel raro Arbuti corticem.

Holotypus: Italia, Castellina, leg. Antonín 96.263 (No. 993.342-032; L); isotypus: BRNM 612470.

Etymology: cupressina, of *Cupressus* trees.

Basidiomata scattered, single. Pileus up to 8 mm across, at first almost hemispherical, then more or less convex, centrally applanate to slightly depressed, somewhat crenulate at the margin, translucent-striate, greyish ochraceous (Kornerup & Wanscher 5B4–5B5), more distinctly brown at the centre, finely whitish-furfuraceous. Context thin. Taste mild. Lamellae 7–10 reaching the stipe, tender, somewhat arcuate, 1.5 mm broad, broadly adnate, not decurrent, whitish with an ochraceous tinge when young, later ochraceous-yellowish (paler than the pileus). Stipe up to 7 × 0.5 mm, central, hollow, fragile, terete but

[1]) Moravian Museum in Brno, Dept. of Botany, Zelný trh 6, CZ-659 37 Brno, Czech Republic.
[2]) Rijksherbarium / Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands.



Figs. 1–6. *Mycena cupressina* (holotype). 1. Habitus (dried specimen); 2. spores; 3. cheilocystidia; 4. hypha of the pileipellis; 5. hypha of the cortical layer of the stipe; 6. caulocystidia. — Fig. 1, × 5; all others, × 700; bar = 10 µm.

slightly broadened at apex and base, curved, smooth, puberulous, ochraceous-yellowish (somewhat concolorous with the pileus), the base springing from a white-pubescent patch.

Basidia c. 36 × 11–11.5 µm, clavate, 4-spored, clamped. Spores 9.5–9.8 × 9.0–9.5 µm (Q = 1.1), globose, smooth, amyloid (blue-grey). Cheilocystidia 24–40 × 7–11.5 µm, clavate, clamped, covered with not very numerous, evenly spaced, fairly coarse, simple, cylindrical excrescences 1.5–4 × 1–1.5 µm, or sometimes (at the apex of the cheilocystidia) with much longer (up to 9 µm), furcate to branched excrescences. Pleurocystidia absent. Lamellar trama staining dark red-brown in Melzer's reagent. Pileipellis a cutis of repent, radiately aligned hyphae which are 1.5–2.5 µm wide, clamped, much branched, densely covered with simple to furcate, cylindrical excrescences. Hypoderm made up of moderately inflated hyphae 15–20 µm wide. Hyphae of the cortical layer of the stipe 1.5–2.5 µm wide, clamped, smooth, the terminal cells (caulocystidia) 15–20 × 4.5–10 µm, clavate, covered with comparatively few, generally simple excrescences.

On bark of dead and living *Cupressus*, rarely on bark of *Arbutus*; dominating *Cupressus sempervirens*, with *Quercus ilex, Q. pubescens, Arbutus unedo, Pinus pinaster*, on mostly calcareous soil.

Holotype: '*Mycena cupressina* Antonín & Maas G. / 6 Nov. 1996 / Italy, Province Tuscany, Castellina in Chianti, Cupresseta di St. Agnese / V. Antonín 96.263' (No. 993.342-032; L); isotype: BRNM 612470.

Mycena cupressina is a member of section *Supinae* Konr. & Maubl. and it is the only member known to have an overall ochraceous colour (Maas Geesteranus, 1984: 139). Also, *M. cupressina* is the only species known in Europe to grow on (predominantly) *Cupressus*. *Mycena corticalis* A.H. Smith (1947: 72), a North American species, equally grows on bark of a coniferous tree, but is easily separated from *M. cupressina* by its arcuate lamellae and the peculiar shape of its cheilocystidia.

Mycena cupressina moreover differs from all other species of the section on account of the smooth hyphae of the cortical layer of the stipe.

Yet another character, possibly overlooked in other members of the section, is the presence of a white-pubescent basal patch, from which the stipe develops.

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A NEW COPRINUS FROM PAPUA NEW GUINEA SPORULATING IN PURE CULTURE

C.B. ULJÉ[1], A. APTROOT[2] & A. VAN IPEREN[2]

From Papua New Guinea a new *Coprinus* is described, which forms basidiocarps and basidiospores in pure culture. It was isolated from material collected on two collecting trips to the area, in 1992 and 1995.

The basidiomycete flora from tropical areas is still very incompletely known, and Papua New Guinea is no exception. In 1992 and in 1995 collecting trips to Papua New Guinea were made by the second author, together with P. Diederich, P. Lambley, E. Sérusiaux, and H.J.M. Sipman. The aim of these trips was to collect ascomycetes (including their anamorphs) from undisturbed tropical forests at various altitudinal zones.

Among the pure isolates obtained from the collected material, two collections, from different years and localities, yielded fruit-bodies of a species belonging to the genus *Coprinus*. This species is characterized by a veil made up of globose, thick-walled and yellow-brown coloured sphaerocysts in combination with thin-walled setulae (= pileocystidia) with cylindrical neck and small, somewhat phaseoliform spores. These features indicate, with little doubt, that the species is new, even though only two collections exist. The species is now described.

Coprinus aureogranulatus Uljé & Aptroot, *spec. nov.* — Fig. 1

Pileus primo subglobosus, ad 20 × 15 mm demum expansus ad 50 mm, ovoideus vel campanulatus, sulcato-striatus, aureus vel flavo-brunneus, lanato-tomentosus; lamellae liberae, albae demum flavo-brunneae, obscure brunneae vel atrae; stipes usque ad 100 × 2–3 mm, albidus, sericeus, basim versus aureo-flavo tomentoso-hirsutus.

Sporae 6.2–7.8 × 4.1–5.1 × 3.7–4.3 µm, medio vel obscure rubro-brunneae, subcylindraceae-phaseoliformae apice truncatae, cum poro germinativo 1.4 µm lato; basidia 16–30 × 5.5–8 µm, tetrasporigera, 3–5 pseudoparaphysibus circumcincta; pleurocystidia absentes vel rara, cheilocystidia similia. Cheilocystidia 40–90 × 13–23 µm, lageniformia; velum e sphaerocystis crassitunicatis, flavis, incrustatis, ad 30 µm diam.; caulocystidia 60–125 × 14–22 × 10–16 µm, lageniformia; fibulae praesentes. Habitat ad ramos vel ad terram.

Holotypus: Madang Province, near Gogol river bridge, 17 km S of Madang along road to Lae, 5° 20' S, 145° 42' E, alt. 10 m, 15 Aug. 1992, no. 33271H. On branch in open forest on raised coral reef.

Type-material: cultivated in the Netherlands, prov. Utrecht, Baarn, 16 July 1996 (holotype: *Uljé 1295*, L; isotypes, CBS 753.96).

Pileus up to 20 × 15 mm when still closed, 50 mm wide when expanded, first (sub)-globose, woolly felty and golden-yellow (Mu. 10 YR 8/8) or yellow-brown to orange-brown, then ellipsoid or ovoid to campanulate and becoming smooth, sulcate striate and

1) Van Dijkstraat 21, 2405 XE Alphen a/d Rijn, The Netherlands.
2) Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands.

paler around centre (Mu. 10 YR 8/6), at margin cream (Mu. 10 YR 8/3). Lamellae, L = c. 26, l = 1–3, free, first white, then yellow-brown to dark brown, finally black; margin dentate. Stipe up to 100 × 2–3 mm, silky white; base slightly bulbous, up to 4 mm wide and densely covered with golden-yellow mycelium.

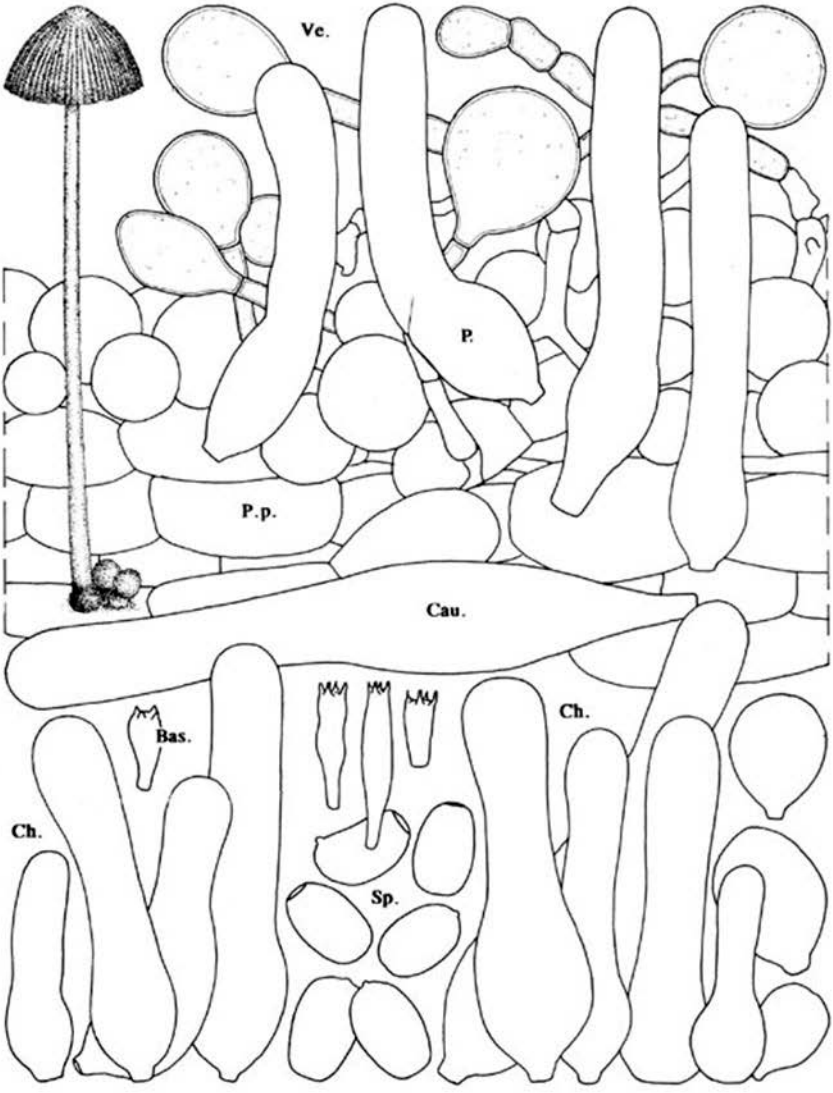

Fig. 1. *Coprinus aureogranulatus*. Sp. = spores, × 2000; P. p. = pileipellis, P. = pileocystidia, Ve. = veil, Cau. = caulocystidia, Ch. = cheilocystidia and Bas. = basidia, all × 800.

Spores [60, 3, 1] 6.2–7.8 × 4.1–5.1 × 3.7–4.3 µm, subcylindrical ellipsoid in frontal view, phaseoliform in side-view, medium to rather dark red-brown with rounded, convex or flattened base and truncate apex, with central, c. 1.4 µm wide germ pore; apiculus very small and difficult to see; Q = 1.40–1.70, av. Q = 1.50–1.55; av. L = 6.8–7.0 µm, av. B = 4.5–4.7 µm, av. W = c. 4.0 µm. Basidia 16–30 × 5.5–8 µm, 4-spored, surrounded by 3–5 pseudoparaphyses. Pleurocystidia absent or very rare and similar to cheilocystidia. Cheilocystidia 40–90 × 13–23 µm, lageniform with 8–16 µm wide, cylindrical neck, in tufts on lamellae edge; in young basidiocarps intermixed with (sub)globose cells (probably from veil). Pileipellis covered with a thick layer of globose cells intermixed with hyphoid elements and pileocystidia 60–90(–110) × 12–20 µm with cylindrical, 6–15 µm wide neck. Veil consisting of sphaerocysts, abundant, up to 30 µm in diam., thick-walled, with walls up to 1.2 µm thick, yellow-coloured and slightly incrusted, often as end cells in chains of elongate elements. Caulocystidia 60–125 × 14–22 × 10–16 µm, lageniform, in small tufts scattered on stipe. Clamp-connections absent.

Habitat & distribution — Subfasciculate on dead branches, sometimes seemingly on soil. Only known from Papua New Guinea (north-east).

Collections examined. PAPUA NEW GUINEA: Madang Province, near Gogol river bridge, 17 km S of Madang along road to Lae (5° 20' S, 145° 42' E, alt. 10 m), on branch in open forest on raised coral reef, 15 Aug. 1992, *Aptroot no. 33271H* (CBS).

Type-material cultivated in the Netherlands, prov. Utrecht, Baarn, 16 July 1996 (holotype: *Uljé 1295*, L), living culture, CBS 753.96; Madang Province, S side of Ramu valley, 11 km W of Brahman Mission. Logging site in lowland forest remnant (5° 44.9' S, 145° 19.7' E, alt. 100 m), 30 Oct. 1995, *Aptroot no. A404* (CBS). Isolated from soil in tropical forest, living culture, CBS 973.95.

Coprinus aureogranulatus belongs to subsection *Setulosi* because of the presence of setulae on stipe and pileus. On account of the structure of the veil and the shape of the pileocystidia, *C. aureogranulatus* seems rather similar to *C. disseminatus* (Pers.: Fr.) S.F. Gray (descr. Uljé & Bas, 1991: 290), but that species has darker brown sphaerocysts, longer setulae, and spores that are slightly smaller and of different shape. The spores of *C. disseminatus* are ovoid with conical base (submitriform) in frontal view and ellipsoid in side-view, whereas in *C. aureogranulatus* the spores are ellipsoid or ovoid with rounded to convex or even almost flattened base in frontal view and slightly phaseoliform in side-view. In addition, cheilocystidia are abundant in *C. aureogranulatus*, but lacking in *C. disseminatus*. In this species only the pileocystidia continue along the edge of the lamellae over a short distance near the margin of the pileus. Another neighbouring species is *C. pyrrhantes* Romagn. (Romagnesi, 1951: 128; Uljé & Bas, 1991: 288), which can have the same golden-brown colour, but the pileocystidia in that species have a much smaller, tapering neck, spores that are never phaseoliform and subglobose cheilocystidia.

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R. Agerer (Ed.). *Colour atlas of ectomycorrhizae, Issue 10.* (Einhorn Verlag, Eduard Dietenberger GmbH, Schwäbisch Gmünd. 1996.) ISSN 1431-4819. Pp. 40, 23 col. pls. Price: DM 140.-.

The tenth issue of this colour atlas of ectomycorrhizae comprises new keys to determination of ectomycorrhizae on *Nothofagus*, *Populus*, *Tetraberlina*, and *Tsuga*, as well as revised keys to those on *Alnus*, *Carpinus*, *Picea*, *Pinus*, *Pseudotsuga*, and *Salix*. Together with the already published keys in the former issues of this very valuable series, altogether 269 species of ectomycorrhizae can be determined. A computer checklist with 337 characters and up to 20 character states is included. The colour plates in this tenth issue deal with fifteen identified and eight unidentified ectomycorrhizae on various economically important forest trees such as *Picea abies*, *Pinus sylvestris*, and *Nothofagus pumilo*.

E.J.H. Corner. *The agaric genera Marasmius, Chaetocalathus, Crinipellis, Heimiomyces, Resupinatus, Xerula, and Xerulina in Malesia.* (Nova Hedwigia, Beiheft 111, J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin, Stuttgart, 1996.) ISBN 3-443-51033-7. Pp. 175, 23 col. pls., 46 text-figs, 1 table. Price: DM 150.-.

This book is the last scientific paper of the late Prof. Corner, one of the most famous mycologists of this century. It contains descriptions of about 100 species belonging to the genera mentioned in the title, many of them being new to science. The author also presents his views on generic concepts in the marasmioid fungi, which deviates in some respects from the currently accepted delimitation of Singer, mainly because of the importance paid to characters such as presence or absence of acerosa basidioles and formation of secondary septa in the stipitetrama. The fine colour plates add much to the value of this book.

M. Duenas (Ed.). *Bases chorologicas de Flora Micologica Iberica, vol. 11.* (Real Jardin Botanico, Madrid, Spain. 1996.) Pp. 99. Price: Ptas 1700.-.

The series publishes papers on basic mycological information which is needed for the realisation of a mycological flora of the Iberian Peninsula. This eleventh issue gives chorological and ecological information on 109 taxa in the orders Tremellales, Auriculariales, Septobasidiales, Exobasidiales, Dacrymycetales and Tulesnales.

L. Hansen & H. Knudsen (Eds.). *Nordic Macromycetes, vol. 3.* (Nordsvamp, Gothersgade 13, DK-1123, Copenhagen, Denmark, 1997.) Pp. 444, 775 text-figs. Price: DEK 350 (about US$ 50.-).

In the third volume of this flora (vol. 2, containing agarics and boletes, appeared in 1992), the remaining basidiomycetes are treated, including about 1200 species of heterobasidioid, aphyllophoroid, and gasteroid basidiomycetes. Natural and artificial keys lead the user to families, and within families keys lead to genera. The keys to the species are hybrid, i.e. they contain key-characters as well as additional diagnostic characters. For each species the ecology, distribution and known frequency in the Nordic Countries is given. Much care has been taken to present these fungi in a modern taxonomic system and updated nomenclature. Numerous text-figures and a glossary of terms facilitate identification. This flora is rather unique in its kind, and will find a wide usage, both by professional and amateur mycologists. It is hoped that the final volume, treating the higher ascomycetes, will follow soon.

Y.-M. Ju & J.D. Rogers. *A revision of the genus Hypoxylon. Mycological Memoir 20.* (American Phytopathological Society Press, St. Paul, MN, USA. 1996.) ISBN 0-89054-214-7. Pp. iv + 365, 22 text-figs. Price: US$ 68.- (in USA: US$ 54.-).

This work is the result of many years of taxonomic study and critical revision of authentic material of most previously described taxa of *Hypoxylon*. It provides a modern treatment of the genus world-wide. The chapters in the general part contain surveys of all aspects of *Hypoxylon*: history; nomenclature; morphology; teleomorphs and anamorphs; structure of perithecia, ostioles, asci, and ascospores; evolution; speciation; development, including important criteria in taxonomy and delimitation. The special part includes: keys to the genera of the Xylariaceae, keys to sections and species of *Hypoxylon*, and detailed descriptions of the 118 species and 10 varieties included. No less than 48 new names for species and varieties are proposed. Illustrations are provided of stromata, ascospores, conidiophores, and conidia. Special attention is paid to comparison of the results of the present study with those of an important earlier monograph of *Hypoxylon* by J.H. Miller in 1961. The book ends with a valuable annotated list (101 pages) of accepted names with most of their synonyms and homonyms, together with a great number of names that are excluded from *Hypoxylon*, or that are doubtful. This work is of great importance to anyone dealing with the taxonomy or identification of *Hypoxylon* and related fungi.

T.W. May & A.E. Wood. *Fungi of Australia, Vol. 2A.* (CSIRO Publishing, P.O. Box 1139, Collingwood 3066, Victoria, Australia. 1997.) ISBN 0-643-05929-6 (hardcover), 0-643-05930-X (softcover). Pp. 347, 1 col. pl. Price: hardcover US$ 69.95, softcover US$ 54.95.

Fungi 2A is the first of two volumes providing bibliographic information on Macrofungi recorded from Australia. This volume contains such information on Agaricales, Boletales, Cantharellales, Gauthierales, Hymenogastrales, Melanogastrales, Phallales p.p., Podaxales, Russulales and Miscellaneous Aphyllophorales, as well as a list of extra-Australian species associated with *Eucalyptus*. As such the book is a valuable tool only for those wanting to contribute to the Australian Flora project in future, but of little or no use for others interested in the mycoflora of Australia.

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New names are in bold-face type. Subdivisions of genera are indicated by the sign §, illustrations by an asterisk (*) added to the page number.

N. B. — The paper by Senn-Irlet on *Crepidotus* in Part 1 is separately indexed on page 80.