

## AURICULARIOPSIS AND THE SCHIZOPHYLLALES

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The cultural characters and the development of the basidiomes of *Auriculariopsis ampla* (Lév.) Maire are described and compared with those of *Schizophyllum commune* Fr.: Fr. It is concluded that *Auriculariopsis* is very close to *Schizophyllum* and that the existence of the order Schizophyllales is not justified.

*Auriculariopsis ampla* (Lév.) R. Maire is a rather rare species in Europe, but locally it can be quite common, for example in the more southern coastal sand dunes of the Netherlands. Its substrate is generally twigs of *Populus*, but occasionally *Salix*; there is one report from *Rubus* (Donk, 1959).

In the older literature this species occurs almost uniformly under the name *Cytidia flocculenta* (Fr.) Höhn. & Litsch. Donk (1959) doubted that *Thelephora flocculenta* Fr. really was this species and Eriksson & Ryvarden (1975) found that authentic material contained *Cylindrobasidium evolvens* (Fr.: Fr.) Jülich.

### METHODS

Isolates were grown in plastic Petri dishes on neutralized 2% malt agar (MEA) and cherry decoction agar (ChA) at room temperature (18–20°C) in diffuse daylight. Drop tests on laccase and tyrosinase were performed as described by Käärik (1965) and Stalpers (1978). Preparations for scanning electron microscopy were made according to Samson et al. (1979).

### CULTURAL CHARACTERS OF AURICULARIOPSIS AMPLA

Growth on MEA rather fast, reaching 25–35 mm radius in 2 weeks, on ChA up to 40 mm. Odour insignificant. Advancing zone appressed to submerged, with irregularly undulating outline; hyphae dense. Mycelial mat 1–2 mm from the margin, cottony-woolly to floccose, white. Around the inoculum the mycelium is thinner, cottony. After four weeks the colony is locally woolly to felty, or floccose-cottony, up to 2 mm high, in other spots appressed, occasionally zonate. After four weeks the colour is generally white, but the felty spots may show some light orange-brown tints. On these spots basidiomata may develop. Reverse unchanged. Reaction with  $\alpha$ -naphthol negative, with p-cresol positive. Cardinal temperatures for growth: minimum 4°C, optimum 26–27°C, maximum 34°C.

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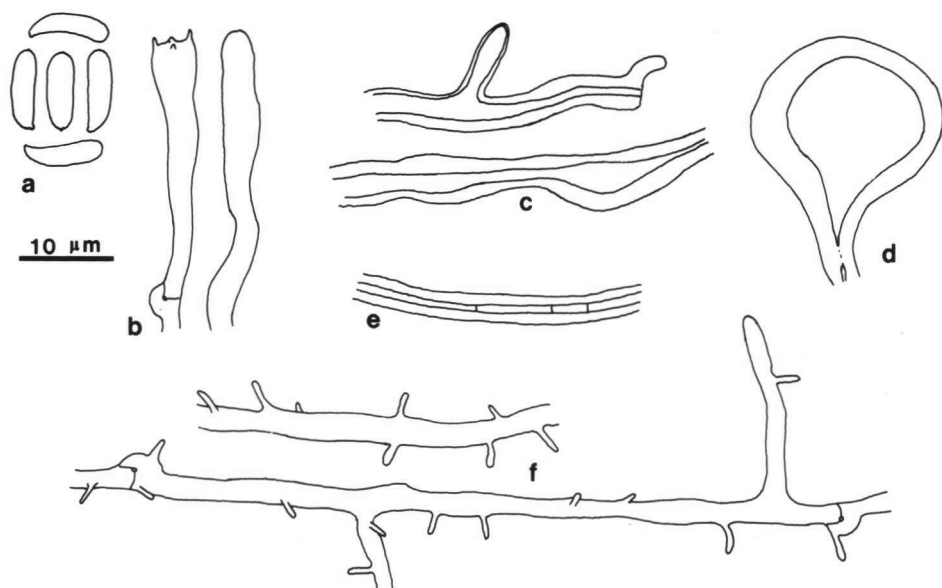


Fig. 1. *Auriculariopsis ampla*. — a. Basidiospores. — b. Basidia. — c. Thick-walled hyphae. — d. Hyphal swelling. — e. Thick-walled hypha with secondary septa. — f. Generative hyphae with spines.

Marginal hyphae hyaline, thin-walled, often slightly wavy, 2–4.5  $\mu\text{m}$  wide. Clamps present at all primary septa, often sprouting. Diameter of clamp smaller than or equal to the hyphal width. Branching frequent, somewhat irregular; branches often rather short. Cells short, generally less than 70  $\mu\text{m}$  long, but occasionally in straight hyphae reaching up to 200  $\mu\text{m}$ . Crystals absent.

Aerial hyphae in floccose mycelium hyaline, thin- to slightly thick-walled, 2–3.5  $\mu\text{m}$  wide, with clamps at every septum, often covered with granular material. Later also wider hyphae occur, 3–6.5  $\mu\text{m}$  wide, with prominent clamps. Spinulose projections (Fig. 1f) are often present. Crystals present, long.

Submerged mycelium tough. Hyphae hyaline, thin- to thick-walled (wall up to 0.5  $\mu\text{m}$  thick, swelling in KOH), 2–6.5  $\mu\text{m}$  wide.

Young basidiomes produced in vitro are stalked, about 1 mm high, terminating with a cup-shaped structure, 0.3–0.6 mm diam. (Fig. 2a, b). Both stipe and cup are covered on the outer surface with slightly reflexed white to greyish hairs. The stipe stops growing, but the cup expands. Further development is dependent on the orientation. If the agar surface is not facing a light source, the cup will grow out to form a flattened disc, partly attached to the substratum, which may become up to 8 mm in diam. (Fig. 2c). The hymenial surface remains even, the margin of the disc is appressed or slightly recurved.

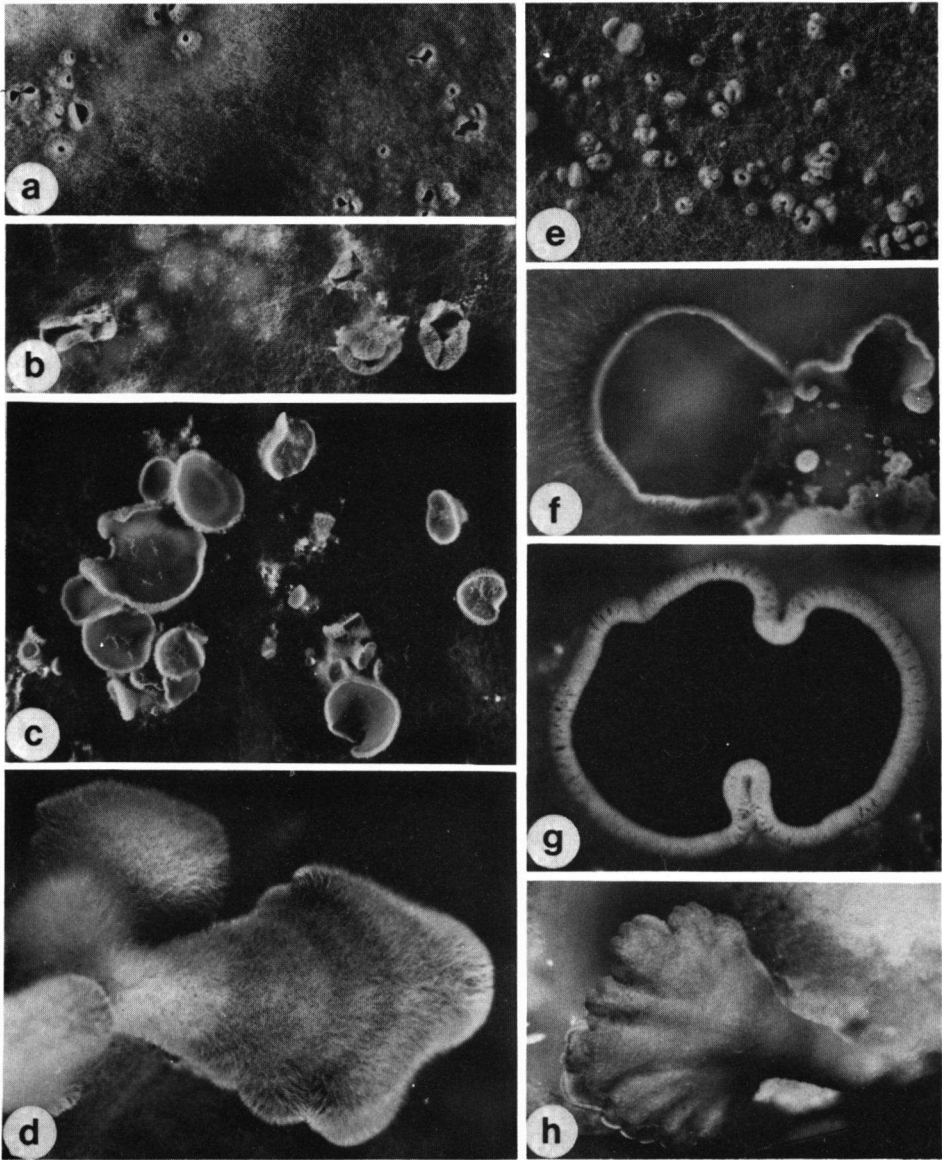


Fig. 2. Development of basidiomes of *Auriculariopsis ampla* (a–d) and *Schizophyllum commune* (e–h). — a, b, e. Young cup-shaped to cyphelloid basidiomes. — c, f, g. Young discoid basidiomes with already mature basidia. — d, h. Well-developed basidiomes.

If the agar surface is facing a light source (for example when the fungus grows on a vertical substrate), then the basidiome will become cupulate to semiglobose (Fig. 2d). The final result will always be a structure with a downward facing hymenium. In this type of basidiome the hymenium may become ridged. The tomentum of the abhymenial surface is white at first, but becomes greyish white or more rarely pale greyish brown. The hymenium is originally cream-coloured to light orange-yellow, but later it becomes pale ochraceous to cinnamon and even reddish brown, especially in dry conditions. In fresh condition the hymenium is ceraceous; when dry the basidiome shrinks strongly, curls inward (protecting the hymenium) and becomes paper-thin.

The abhymenial tomentum consists of hyaline to slightly yellowish thick-walled hyphae, 2–3.5  $\mu\text{m}$  wide, (wall up to 1  $\mu\text{m}$  thick), only occasionally branched, not gelatinized, septate. Clamps do occur, albeit rarely. Sometimes empty and collapsed clamps were observed on perfectly healthy hyphae. The tramal hyphae are thin- to slightly thick-walled, 2–4.5  $\mu\text{m}$  wide, gelatinized or embedded in mucus, in more or less parallel arrangement, septate, with clamps at nearly all septa. Branching is frequent, but the side-branches are usually rather short. Some hyphae display swellings, 5–8(–20)  $\mu\text{m}$  wide. The wall of the tramal hyphae swells strongly in KOH and then becomes up to 2.5  $\mu\text{m}$  thick.

The hymenium consists of cylindrical basidia, 22–40  $\times$  3.5–4.5  $\mu\text{m}$ , with four rather short sterigmata (0.8–2  $\mu\text{m}$  long) and with a clamp at the base. Brown resinous material often present between the basidia. The spores are hyaline, thin-walled, cylindrical and often slightly curved, somewhat attenuating towards the small apiculus, (6–)7.5–9(–10)  $\times$  (2–)2.5–3  $\mu\text{m}$ . The spore print is white at first, becoming cream coloured with age.

Hyphal cells and spores are binucleate. The species is tetrapolar (bifactorial).

Species code of Stalpers (1978): 2, (8), (11), 13, 15, (19), 21, 22, 25, (29), 30, (32), (34), 39, 42, (44), (45), 48, 52, 53, 54, 59, (80), 82, 88, 89, 94, 95, 98.

Species code of Nobles (1965): 1, 3, 20, 32, 36, 38, 43, 48, 54, 60.

**Material examined.** — Living strains: CBS 182.83, from branch of *Populus* lying in pear orchard, Netherlands, prov. Zeeland, Zeeuws Vlaanderen, leg. & det. J. A. Stalpers; CBS 285.88, from *Populus*, Netherlands, prov. Utrecht, Bunnik. — Herbarium material: NETHERLANDS: prov. Zuid-Holland: Wassenaar, 'Meijendel', 30 Oct. 1955, M. A. Donk 11055, on fallen rotten branches of *Populus* (L); Wassenaar, 'Bierlap', 23 Oct. 1952, R. A. Maas Geesteranus (Donk 11206), on fallen branches of *Populus tremula* (L); prov. Zeeland, 's Gravenpolder, Koedijk, date unknown, W. D. J. Kuijs (herb. Kuijs).

The data in the literature (Bourdot & Galzin, 1928; Donk, 1930; Møller, 1942; Eriksson & Ryvarden, 1975) agree with the present description of the basidiomes, but two remarks have to be made. In the Dutch material the spore length is in average shorter: the maximum size rarely exceeds 9.5  $\mu\text{m}$ , while in the literature a maximum of 10.5  $\mu\text{m}$  is normal and extremes up to 12  $\mu\text{m}$  are known. The descriptions in the literature always mention ridges on the hymenial surface, but some specimens had a perfectly even hymenium, while others displayed ridges.

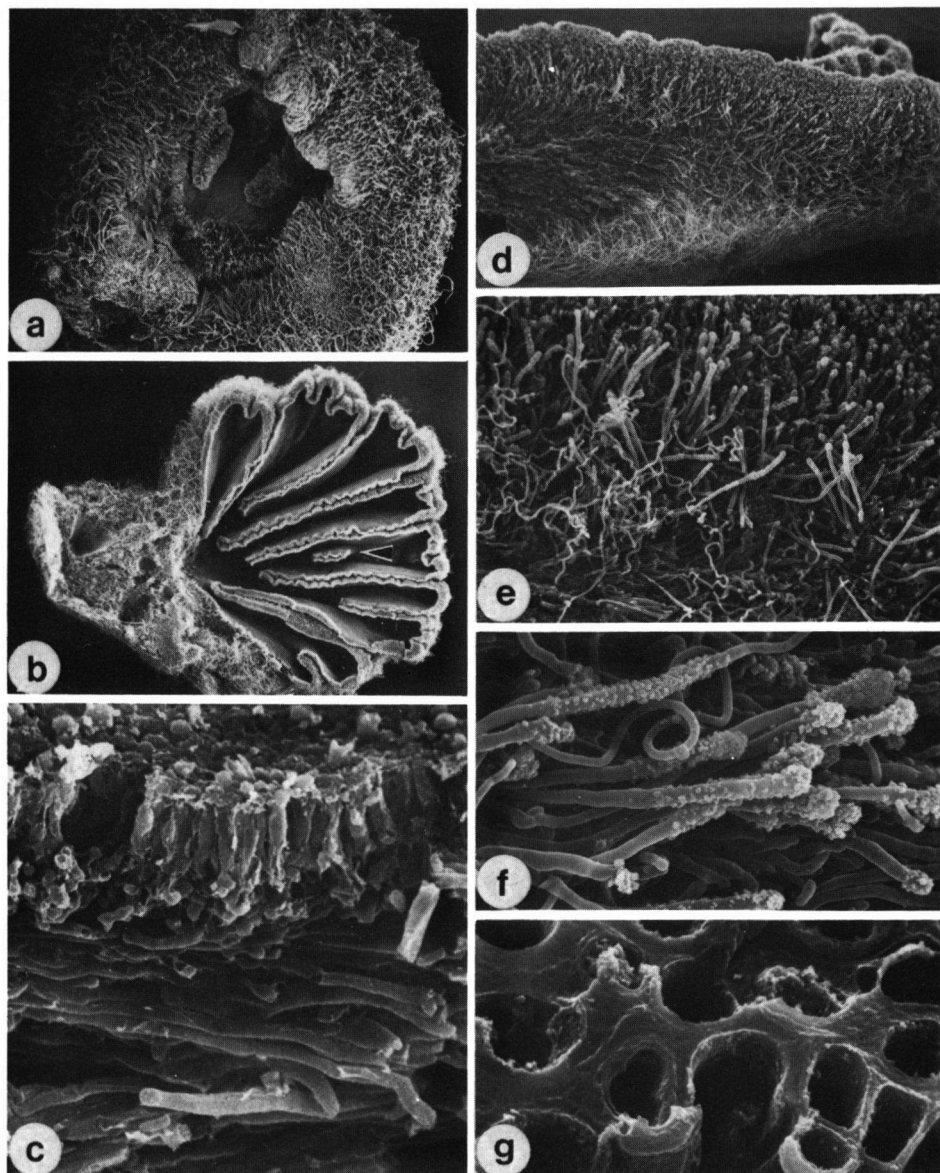


Fig. 3. *Schizophyllum commune*. — a. Basidiome with young, centrally developing gills. — b. Mature basidiome with invaginated and centrally developing (arrow) gills. — c. Section through hymenium, subhymenium and upper trama. — d–f. Basidiome sectioned through split lamella. — d. Overview, showing from top to bottom inner side of split, gelatinized hyphae and abhymenial hairs. — e. Detail of inner side of split with encrusted hyphae. — f. Detail of encrusted hyphae. — g. Gelatinized hyphae of basal layer.

Traditionally (Léveillé, 1848; Bourdot & Galzin, 1928; Donk, 1930) the relatives of *Auriculariopsis* are sought in the cyphelloid basidiomycetes. More recently this has been doubted: *Phlebia*-like genera, like *Merulius* s. str., are now considered as most closely related (Donk, 1959; Eriksson & Ryvarden, 1975).

Very closely related is certainly *Cytidiella* Pouzar, like *Auriculariopsis* a monotypic genus. Pouzar (1954) mentioned the absence of gelatinized hyphae in *Cytidiella melzeri* Pouzar as the only important difference between the genera, but Eriksson & Ryvarden (1975) demonstrated gelatinized hyphae in *C. melzeri*. They retain *Cytidiella* on the basis of another character, the presence of a basal layer parallel to the substratum consisting of non-gelatinized, thick-walled hyphae in *Auriculariopsis* and the absence of such a layer in *Cytidiella*. However, there are several natural genera within which both situations exist, for example *Amylostereum* Boidin or *Peniophora* Cooke. *A. chailletii* (Pers.) Boid. and *P. limitata* (Fr.) Cooke have a distinct parallel layer of often thick-walled hyphae, while *A. laevigatum* (Fr.) Boidin and *P. cinerea* (Fr.) Cooke lack such a layer completely. Moreover, *C. melzeri* displays a kind of rudimentary parallel layer in which the hyphae are less thick-walled than in *Auriculariopsis ampla*, have several clamps and are not gelatinized.

The presence of a basal layer parallel to the substratum is connected with the potential of the basidiome to become partly free from the substratum. The above-mentioned species of *Amylostereum* and *Peniophora* with such a layer generally have a rolled-up or even reflexed margin, while the species without such a layer remain strictly effused and even have no loosening margins. The basidiomes of *Auriculariopsis* are distinctly cup-shaped, while those of *Cytidiella* are more disc-shaped with a revolute margin. These features do not seem sufficient to maintain *Cytidiella* as generically distinct from *Auriculariopsis*. (See note added to the Proof on p. 504.)

The observations made from pure cultures certainly do not exclude a relationship with *Merulius* s. str. and relatives, although these species show an astatocoenocytic behaviour and are unifactorial (bipolar), while *Auriculariopsis* has a normal behaviour and is bifactorial (tetrapolar).

The relationship of *Auriculariopsis* with *Schizophyllum commune* Fr.: Fr. is, however, much closer. Traditionally from Fries until Singer (1962), the genus *Schizophyllum* has been considered to belong to the Agaricales, simply because it has gills. In recent years its affinities were thought to be in the Aphyllophorales, because the ontogeny of the gills proved not to be homologous with the lamellae of the Agaricales, and the texture of the basidiome also was aberrant. So Donk in his conspectus of the families of the Aphyllophorales (1964), restored the family Schizophyllaceae Roze, and considered *Stromatoscypha* as possibly the closest relative, a view shared by Wessels (1965). The reason for this was, that a basidiome of *Schizophyllum* was thought to be composed of several cups, developing on a common stroma, an idea that may have originated from a cross section through a basidiome (cf. illustration of Watling, 1973).

Nuss (1980) correctly considered *Scytinostroma* as unrelated with *Schizophyllum* and erected the order Schizophyllales, with a single family and a single genus.

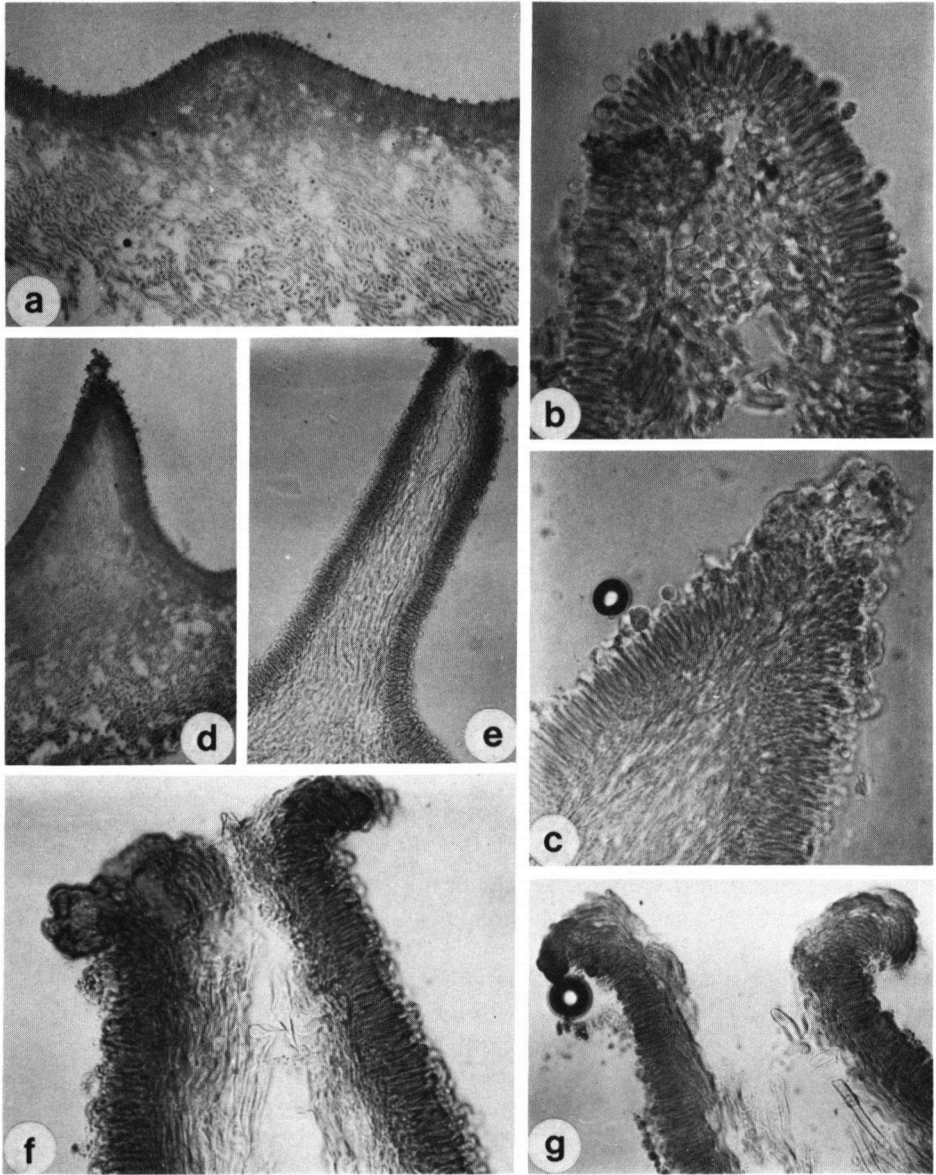


Fig. 4. *Schizophyllum commune*, development from ridge to gill. — a. Young ridge. — b. Older ridge. — c–d. Beginning of splitting, crystalline material present at apex. — e–f. Actual splitting. — g. Split gill with incrustated hyphae.

Jülich (1981) more or less adopted Donk's concept, but accepted the order Schizophyllales. On several places he mentioned a relationship with the Lentinaceae, but did not give any reasons. I am not aware of any argument in favour of this theory.

The development of a basidiome of *Schizophyllum*, however, does not support the theory of a common stroma, but points towards a close relationship with *Auriculariopsis*. In fact it is identical with it, up to the formation of ridges (Fig. 2e–h). Figs. 3a, b clearly show, that there are two ways in which a split lamella can be formed. As already indicated by Donk (1964) the first consists of a marginal invagination (Fig. 2h), or the development of lobes, which elongate with further growth, resulting in a lamella. The other mode in which a gill can be formed starts with a central development of a ridge somewhere on the existing hymenium. This ridge grows — at first mainly by expansion of the subhymenium, later there is also tramal growth — until it reaches a certain height and then splits. The series of sections illustrated in Fig. 4 shows what happens during the splitting process. A peculiar type of hyphae with terminal encrustations occurs on the surface of the exposed split (Fig. 3e, f; Fig. 4g), which was earlier reported by van der Valk & Marchant (1978) from the edge of the basidiome initial. This observation may serve as an argument, that the hyphae of the split lamellae are homologous with the abhymenial hairs.

It is considered unlikely that both ways of the gill development are fundamentally different (they cannot be distinguished morphologically). The early development of the latter type agrees completely with ridge formation as seen in for example *Phlebia*, *Merulius* and *Auriculariopsis*, up to the actual splitting of the ridge. The idea of non-homology of *Schizophyllum* gills with ridges as defended by Watling (1973), has to be abandoned.

Colony morphology and microscopical characters are similar in *Schizophyllum* and *Auriculariopsis*. Like *Schizophyllum*, *Auriculariopsis* has cylindrical binucleate basidiospores; as both species are tetrapolar (bifactorial), the two nuclei are genetically identical. Colonies of the various mating types are morphologically distinguishable as was already known from *Schizophyllum* (Wessels, 1965) and they display a similar unpleasant odour. The occurrence of the peculiar hyphae with spines was so far only known in *Schizophyllum* and a sterile culture displaying them was automatically identified as *Schizophyllum commune*. This view is no longer true as the same kind of spinose hyphae may also occur in *Auriculariopsis*. Like *Schizophyllum*, *Auriculariopsis* is very resistant against drought. The capacity of reviviscence lays in the tramal hyphae which are slightly gelatinized or embedded in gelatinous material, which prevents drought damage. Normally, however, basidiomes of both species live long, but not more than a year.

In fact the only difference between the genera is the more pronounced development and splitting of the gills in *Schizophyllum*.

In the literature there is one further fact, that may point towards a possible relative of *Schizophyllum*. Breitenbach & Kränzlin (1986, Fig. 230) illustrate a specimen of *Lachnella alboviolascens* (Alb. & Schw.: Fr.) Fr., showing 'insel-artige Wucherungen' that are strikingly similar to developing lamellae of *Schizophyllum* as illustrated in Fig. 3a). The microscopical details given by the authors exclude a misidentification with this



species. However, a specimen of the closely related *L. villosa* (Pers.: Fr.) Gillet, collected and isolated from the Netherlands, showed neither the spines nor other *Schizophyllum*-like characters.

The evidence presented here indicates that *Schizophyllum commune* can no longer be seen as an outsider in the basidiomycete system. The evidence for a developmental line from *Cyrtidiella* to *Auriculariopsis* and finally *Schizophyllum* is too convincing. A separate order Schizophyllales is not considered justified, and the family Schizophyllaceae should also include *Auriculariopsis* (and, if retained, *Cyrtidiella*). The family Auriculariopsidaceae proposed by Jülich (1981) is certainly superfluous. If *Merulius* is ever considered to belong to the same family as *Auriculariopsis*, Schizophyllaceae Roze (1876) has priority above Meruliaceae Rea (1922).

#### ACKNOWLEDGEMENT

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## NOTE ADDED TO THE PROOF

After the submission of the manuscript additional material of *Cytidiella melzeri* from Sweden could be examined. This fully supported the close relationship with *Auriculariopsis ampla* and thus a new combination is proposed: *Auriculariopsis melzeri* (Pouzar) *comb. nov.* (basionym: *Cytidiella melzeri* Pouzar, Česká Mykol. 8: 129. 1954).

**Material examined.** — SWEDEN: Upland, Svartsjölandet, Hillersjö, 30 Apr. 1905, L. Romell 2872½, on *Pinus silvestris*; Södermanland, Södertälje, 1 June 1949, Ph. Johansson (Fungi suecici 8720), on dry branch of *Quercus robur*.