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Ectomycorrhizae of *Tomentella albomarginata* (Thelephoraceae) on Scots pine

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Abstract The ectomycorrhizae of *Tomentella albomarginata* are comprehensively described and compared to ectomycorrhizae of other *Tomentella* species and to ectomycorrhizae of some members of Thelephoraceae ss. Stalpers and Bankeraceae ss. Stalpers. The ectomycorrhizae of *T. albomarginata* are characterized by a hyphal net lying on a pseudoparenchymatous mantle surface, by tubular outgrowths of irregularly angular mantle cells, and by clamp-bearing emanating hyphae.

Key words Ectomycorrhizae · Characterization · Identification · *Pinus sylvestris* · *Tomentella albomarginata*

Introduction

Members of the Thelephorales form their fruitbodies either on the soil, and they may be connected to considerable amounts of ectomycorrhizae, as is true of several genera that form pileate fruitbodies, e.g. *Hydnellum* and *Sarcodon* (Agerer 1991a, 1993), or are wood-inhabiting fungi, at least as far as their fruitbodies are concerned, e.g. the genus *Tomentella* (Jülich 1984; Larsen 1974). Fruitbodies of *Tomentella* species are often found on pieces of rotten wood, twigs or leaves lying on the soil or occur in soil cavities (Agerer, unpublished work; Larsen 1974; Stalpers 1993). Since species of other genera that inhabit similar substrates have been shown to be ectomycorrhizal, e.g. *Piloderma croceum* (Brand 1991), *Amphinema byssoides* (Weiss 1990), *Tylospora fibrillosa* (Taylor and Alexander 1990), it is of great importance to focus mycorrhizal research on the widely distributed genus *Tomentella*, of which several dozen species are known (Jülich 1984; Stalpers 1993).

The Thelephorales are divided into the families Thelephoraceae ss. Stalpers and Bankeraceae (Stalpers 1993). According to Stalpers (1993), the Bankeraceae is comprised of the stipitate genera *Bankera* Cooker & Beers: Pouz., *Boletopsis* Fayod, *Hydnellum* P. Karst., *Phellodon* P. Karst., and *Sarcodon* Quél.: P. Karst. One species of each genus exept *Bankera* is sufficiently known by ectomycorrhizal characters: *Boletopsis leucomelaena* (Pers.: Fr.) Fayod (Agerer 1992a), *Hydnellum peckii* Banker apud Peck (Agerer 1993), *Phellodon niger* (Fr.: Fr.) Karst. (Agerer 1992b), and *Sarcodon imbricatus* (L.: Fr.) Karst. (Agerer 1991a). Only two species of the several Thelephoraceae ss. Stalpers genera are well known with respect to their ectomycorrhizal characters: *Thelephora terrestris* Pers.: Fr. (Agerer and Weiss 1989) and *Pseudotomentella tristis* (P. Karst.) M. J. Larsen (Agerer 1994). Stalpers (1993) also includes the genus *Tylospora* within the Thelephoraceae, based on its corticioid fruitbody forming rhizomorphs, on warty or similarly shaped but hyaline spores, and on its ability to form ectomycorrhizae. The ectomycorrhizae of *Tylospora fibrillosa* (Burt) Donk are well known (Taylor and Alexander 1990).

Whereas *Tomentella crinalis* is known by some features of synthesized ectomycorrhizae (Köljalg 1992), *Tomentella albomarginata* is the first species of the genus *Tomentella* to be comprehensively described. Whether the remaining genera of Thelephoraceae ss. Stalpers (*Botryohypochnus* Donk, *Lenzitopsis* Malencon & Bertault, *Polyozellus* Murr, *Tomentellago* Hjortstam, and *Tomentellopsis* Hjortstam) form ectomycorrhizae is still unknown.

Considered as part LX of the series "Studies on ectomycorrhizae"; part LIX: Rauscher et al. (1995)

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Materials and methods

Only the reference specimen was studied. Identification was verified by tracing hyphae from mycorrhiza to fruitbodies, and by comparison of emanating hyphae, rhizomorph hyphae (of fruitbody and mycorrhiza), and subicular hyphae of the fruitbody.

Methods for characterizing ectomycorrhizae have been previously explained by Agerer (1986, 1987–1994, 1991b), and a glossary of terms has already been published (Agerer 1987–1994). Fresh material of ectomycorrhizae immediately after excavation was used to study those characteristics that are expected to change during fixation (Agerer 1987–1994, 1991b) and other features were examined using fixed material. Sections were studied after embedding in Historesin and cutting into 5-μm-thick sections. The term 'intrahyphal hypha' refers to a hypha of the same fungus (*Tomentella albomarginata*).

The fungus was identified from the descriptions of Stalpers (1993) and Jülich (1984). The dried fruitbody collection and fixed material of the reference specimen of the ectomycorrhizae are kept at the Botanische Staatssammlung, Munich.

Tomentella albomarginata (Bourd. & Galz.) M.J. Larsen

Reference specimen: Germany, Hessen, Offenbach rural district, gravel pit near Langen in the neighbourhood of Egelsbach, under 2- to 3-m-high *Pinus sylvestris*; fruitbody and soil collected by H. Zinth, 16 October 1993, RA 12051 (in M)

Morphological characters (Fig. 1a,b)

Mycorrhizae dichotomous, mycorrhizal systems up to 2.5 mm long, unramified ends straight or at most slightly bent, up to 1.3 mm long and 0.3–0.36 mm in diameter, often with a obclavate recently grown tip, axes 0.36–0.52 mm in diameter; surface of unramified ends shiny, in part glistening, mantle surface distinct, cortical cells not shining through, mostly smooth but sometimes loosely and even densely woolly; emanating hyphae often crowded at the basis of the mycorrhizal forks, sometimes also growing out from the very tip; rhizomorphs infrequent, only few hyphae bundled, thicker rhizomorphs occurring only close to the fruitbody; unramified ends dark brown, reddish tint lacking, sometimes with a slight bluish-black tint, older parts greyish rimy of sand particles.

Anatomical characters of mantle in plan views (Figs. 1c,d,e, 2a,b, 3a, 4, 5)

Outer surface of mantle (Figs. 1c, 2a,b, 3a, 4) pseudoparenchymatous, cells often irregularly angular or roundish, in part elongated, no pattern recognizable, covered by a gelatinous matrix and a hyphal net, but the latter often lacking in older parts, cells (11–)15–27(–45) μm long and 6–18(–21) μm in diameter, ca. (3–)8–12(–14) cells in a square of 20 × 20 μm, cell walls 0.5–1 μm thick, membranaceously brown, mostly smooth, but not infrequent with short thin and tubular outgrowths, 3–6 μm long and 1 μm in diameter, these often covered by gelatinous matrix with embedded soil particles. *Middle layers of mantle* (Fig. 1d) densely plectenchymatous, no pattern discernable, cells irregularly shaped, 7–25 μm long and 3.5–6.5(–10) μm in diameter, cell walls 0.5 μm thick, brown. *Inner surface of mantle* (Fig. 1e) plectenchymatous, clamps sometimes visible, no pattern recognizable, cells short, approximately cylindric, 5–35(–60) μm long and (2–)3–4(–5) μm in diameter, cell walls 0.5 μm thick, membranaceously brown. *Surface of very tip* (Fig. 1c) plectenchymatous, distinctly gelatinous, hyphal net prominent, cell walls of hyphal net still rather thin. *Rhizomorphs* (Figs. 2c, 5, 6f) only as bundles of few hyphae, rhizomorphs close to fruitbody thicker, hyphal characteristics identical to those of mycorrhiza, but walls thicker, hyphae loosely arranged (type A after Agerer 1987–1994; Agerer 1991b), hyphae 4–7 μm in diameter, cells 100–185 μm long, walls 0.5–1 μm thick, smooth, membranaceously brown, with clamps, very infrequent with one simple septum in between, intrahyphal hyphae frequently occurring. *Emanating hyphae* (Figs. 2a, 3a,b,c) of fruitbody rhizomorphs, subiculum-hyphae of fruitbody and of mycorrhiza rhizomorphs are identically shaped, smooth, with intrahyphal hyphae, brown, less coloured at the tips, with clamps, clamps without a hole, 0–1(–2) simple septa in between and approximately at equal distance, cells from clamp to clamp (10–)60–140 μm long, (3.5–)4.5–5(–6) μm in diameter, reversely oriented clamps occurring, no backwards oriented ramifications found, side branches often growing off at approximately right angles (Fig. 4), often originating at a considerable distance from clamps, hyphae often with elbow-like protrusions, sometimes in series, frequently with thicker walls, hyphal tips thin-walled, covered by soil particles, sometimes tortuous, emanating hyphae of thicker rhizomorphs and subiculum hyphae often very thick-walled and forming cap-like structures at their tips (Fig. 5) with hyphae slightly amyloid, slight amyloidity also present infrequently at septa, anastomoses simple. *Cystidia* not found.

Anatomical characters, cross-section (Fig. 6a,b,e)

Mantle organization corresponding to longitudinal section; outer part hyphae tangentially (5–)10–20(–30) μm, radially 3–5 μm, walls 0.5–1 μm thick; middle layer pseudoparenchymatous, composed of hyphae tangentially 3–10(–20) μm, radially 3–7(–10) μm, walls 0.5 μm thick; inner layer plectenchymatous, hyphae tangentially 3–10(–20) μm, radially (2–)3(–4) μm, walls up to 0.5 μm thick; residues of calyptra cells close to tannin cells formed as long, strongly light-reflecting, wavy lines. *Tannin cells* irregularly tangentially oval, with some intracellular hyphae, tangentially (17–)25–80(–99) μm, radially 3–9(–12) μm, TCt = 47.4 μm, TCq = 6.9. *Cortical cells* tangentially oval to tangentially cylindric, tangentially (15–)20–75(–80) μm, radially (10–)18–35(–40) μm, CCt = 48.1 μm, CCq = 1.8. *Hartig net* of tannin cells 2.5–4 μm thick, composed of one row of hyphal cells, hyphal cells in section roundish to cylindrical and of cortical cells 2.5–3 μm thick, composed of

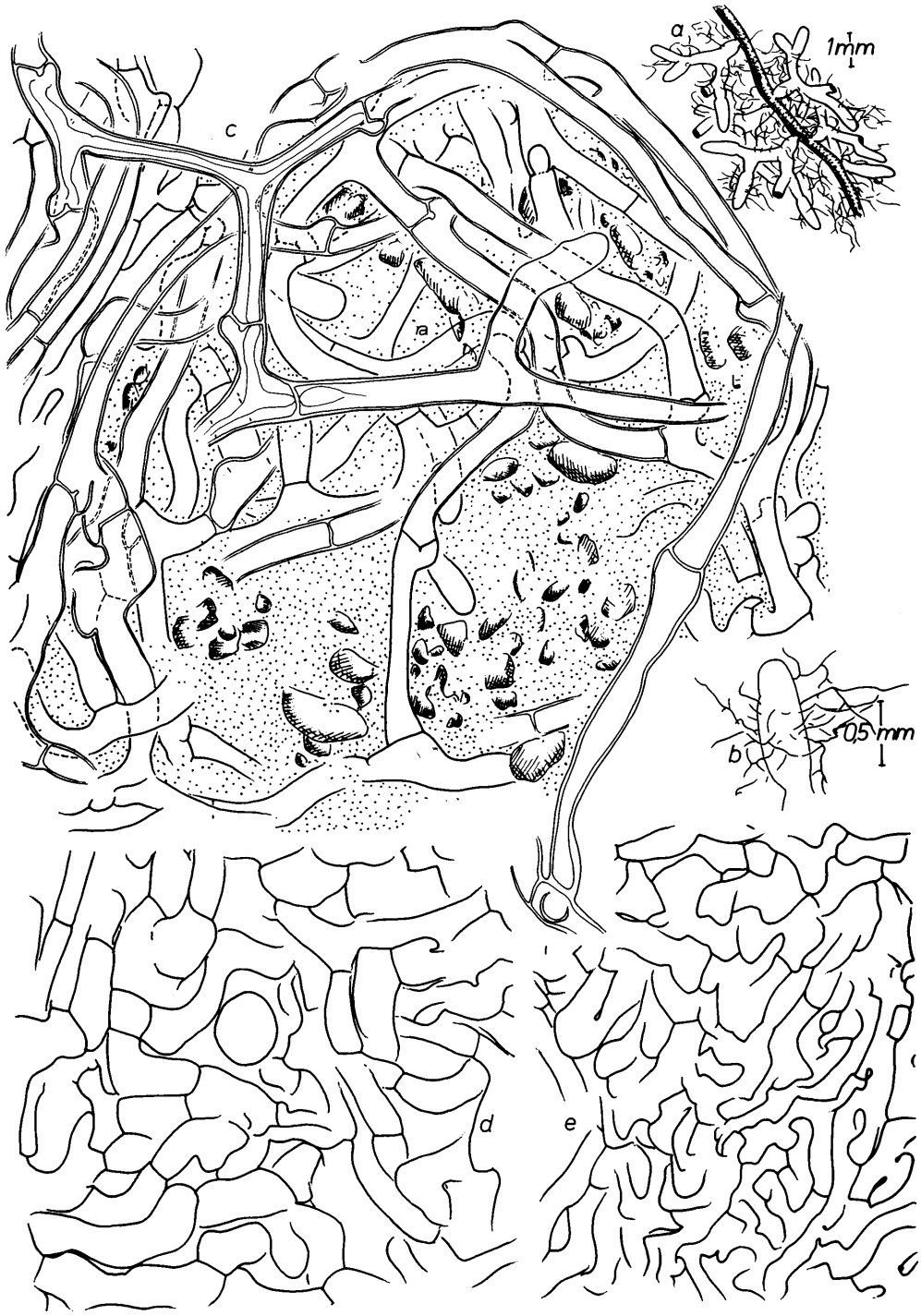


Fig. 1a,b Habit. **c** Plan view of mantle surface near mycorrhizal tip showing the hyphal net on the surface of a plectenchymatous mantle with intrahyphal hyphae, very distinct gelatinous matrix, and embedded soil particles. **d** Plan view of middle layer of mantle near mycorrhizal tip, with a plectenchymatous arrangement of hyphae. **e** Plan view of inner layer of mantle remote from the tip (mycorrhizal flank). (From RA 12051)

one row of hyphal cells, hyphal cells in section rectangular to cylindrical.

Anatomical characters, longitudinal section (Fig. 6c,d)

Mantle 15–20(–25) μm thick, differentiated in distinct layers, outer part plectenchymatous, represented by the hyphal net, outer part hyphae tangentially 8–20(–30) μm, radially 2–5 μm, walls 0.5–1 μm thick; middle layer hyphae tangentially 6–8(–15) μm, radially 3–5(–6) μm, walls 0.5 μm thick; inner layer hyphae tangentially 3–10(–20) μm, radially 2–3 μm, walls 0.5 μm thick, residues of calyptra cells close to or integrated into inner part of mantle, visible as long, irregularly shaped, strongly light-reflecting lines; mantle of very tip 15 μm thick, differentiation, shape and size of cells as remaining part of the mantle, not so distinctly pseudoparenchymatous. *Tannin cells* in 1(–2) rows, irregularly oval, parallel or slightly oblique to root surface, tangentially (17–)22–60(–90) μm, radially 5–11 μm, TCt = 34.2 μm, TCq = 4.6. *Cortical cells* in (1–)2–3 rows (exclusive of

Fig. 2 a,a' Plan view of mantle surface with hyphal net and emanating hyphae which grow out of the net, part of an emanating hypha with an intrahyphal hypha; **a'** is the continuation of a (see *xx*). **b,b'** Plan view of mantle surface of mycorrhizal flank, showing hyphal net and tubular outgrowths of pseudoparenchyma cells of the mantle surface, cells covered by gelatinous matrix and by soil particles. **c** Part of a rhizomorph near fruitbody. (From RA 12051)

tannin cells), radially oval to tangentially oval, oriented obliquely to root surface, tangentially (16–)22–55(–63) µm, radially (13–)17–43(–50) µm, CCt = 37.3 µm, CCq = 1.5. *Hartig net in plan* view of palmetti type, lobes (1–)1.5–3 µm broad.

Colour reaction in different reagents

Mantle and rhizomorph preparations: KOH 10%: no reaction; Melzer's reagent: mostly no reaction, but hyphae rather dark, a reaction could perhaps have been obscured, but slightly amyloid at the cap-like thickenings of the emanating hyphae of rhizomorphs.

Autofluorescence

Whole mycorrhiza: UV 254 nm: –; UV 366 nm: –. *Mantle in section*: UV filter 340–380 nm: only outermost layer slightly yellow, between mantle hyphae in part

Fig. 3a Hyphal net of mantle surface with irregularly shaped cells and intrahyphal hyphae. **b,b'** Emanating hyphae originating from the hyphal net showing thin-walled hyphal ends covered by soil particles, as well as intrahyphal hyphae; in **b** serial elbow-like protrusions evident. **c** Part of emanating hyphae with intrahyphal hyphae and a hyphal bridge between formerly intrahyphal hyphae. (From RA 12051)

tangentially oriented, thin, blue lines; blue filter 450–490 nm: only outermost layer slightly yellow; green filter 530–560 nm: only outermost layer slightly reddish.

Staining of nuclei

Aceto-carmine: two nuclei per cell, mostly very close together (0–1 µm apart), in ramified cells sometimes more remote (up to 14 µm); nuclei round or ellipsoid, 1.5 µm in diameter or 1.5–2.5 × 1–1.5 µm. Siderophilous granulations lacking.

Discussion

The ectomycorrhizae of *Tomentella albomarginata* differ from those of *T. crinalis* synthesized on *Pinus sylvestris* (Köljalg 1992) by a pseudoparenchymatous man-

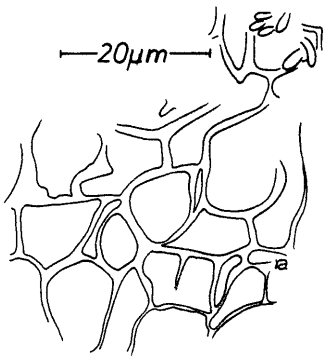


Fig. 4 Plan view of mantle surface with angular cells. (From RA 12051)

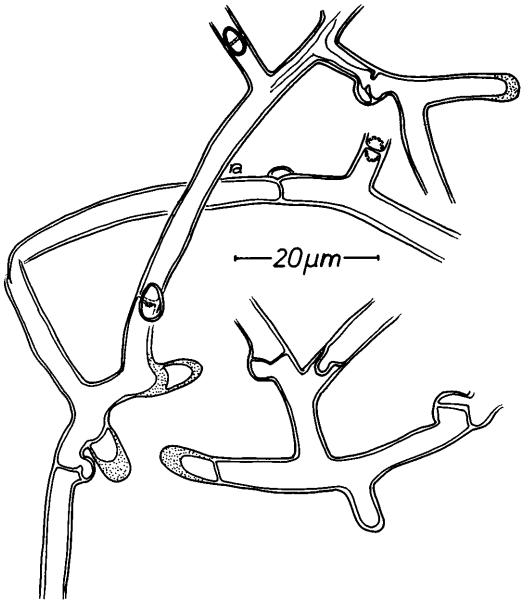
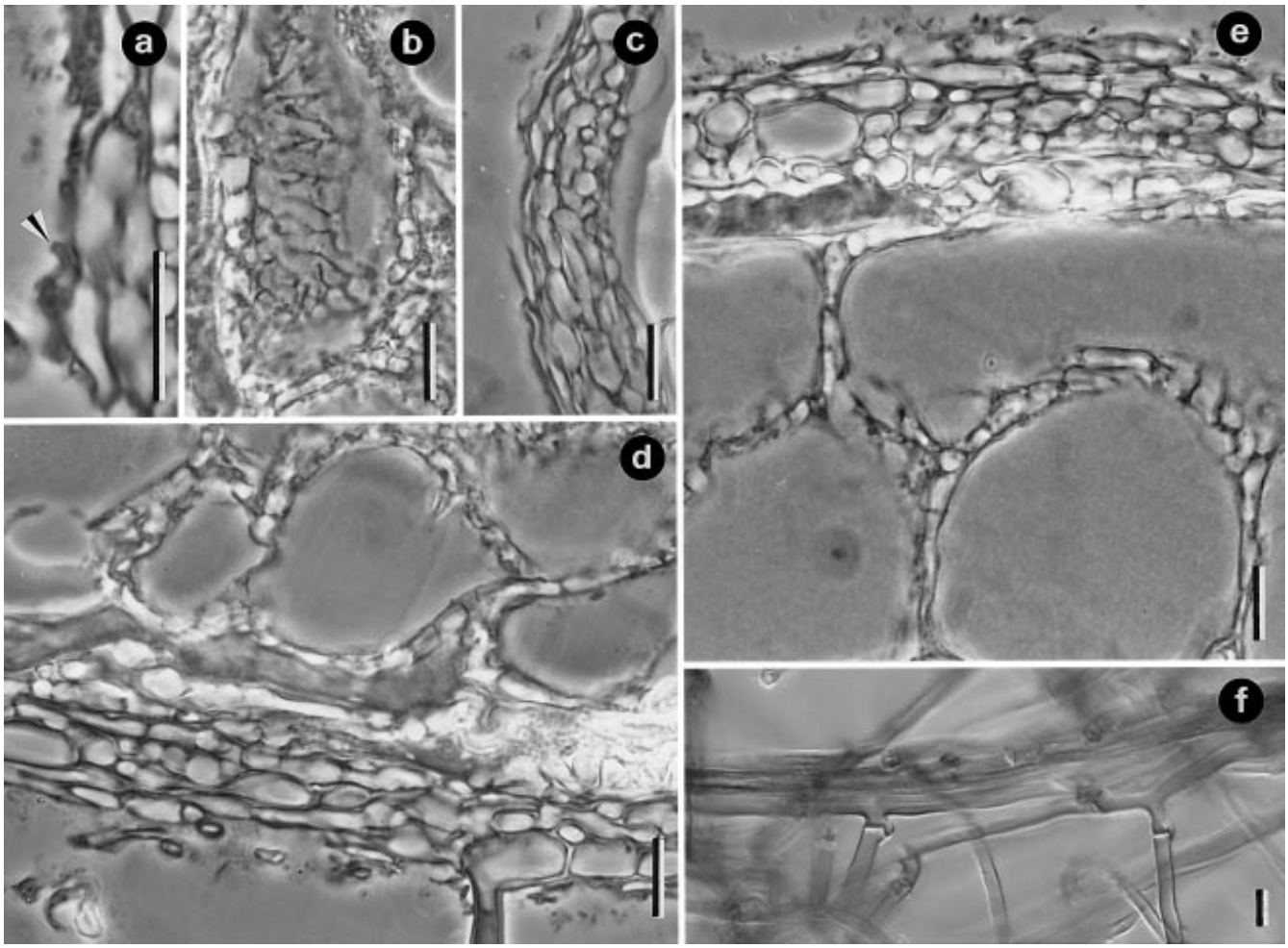


Fig. 5 Marginal hyphae of fruitbody rhizomorphs, showing slightly amyloid, cap-like hyphal ends, a slightly amyloid septum, and an intrahyphal hypha. *Dots* indicate slight amyloidity. (From RA 12051)

Fig. 6a Cross-section, outer mantle cells with tubular outgrowths (*arrowhead*). **b** Cross-section, Hartig net in plan view. **c** Longitudinal section, mantle of very tip of mycorrhiza. **d** Longitudinal section, from mantle with a basis of an emanating hypha to cortex cells with Hartig net. **e** Cross-section, from mantle to cortex cells with Hartig net. **f** Distal end of a rhizomorph. *Bars* 10 µm. (From RA 12051)



tle. At least in these synthesis experiments, *T. crinalis* invaded cortical cells, but it remains to be shown that such hyphal invasions also occur in naturally grown ectomycorrhizae. Rhizomorphs emanate from the mantle of *T. crinalis* and correspond to those formed by the fruitbodies. Since the synthesized mycorrhizae were only very young, a more detailed comparison with *T. albomarginata* cannot be given.

Another *Tomentella* ectomycorrhiza, not identified to the species level, was described by Danielson et al. (1984). It was characterized as having a pseudoparenchymatous mantle with awl-shaped cystidia and rather small epidermoid cells up to 6 μm in diameter. Two ectomycorrhizae designated as *Tomentella*–like with cystidia and pseudoparenchymatous mantles with angular and epidermoid cells were described by Danielson and Pruden (1989). In the same article, a further *Tomentella*–like mycorrhiza was described with a pseudoparenchymatous mantle forming both epidermoid and angular cells, with clamped emanating hyphae of 4–5 μm diameter and lacking cystidia. The latter ectomycorrhiza has some similarities to those of *T. albomarginata*, but more detailed comparison is not possible.

The very tips of *T. albomarginata* mycorrhizae possess a plectenchymatous organization, which becomes more and more pseudoparenchymatous behind the tip. This change in structure differs from that of *Piceirhiza nigra* (Gronbach 1988) on *Picea abies*, possibly also formed by a member of the genus *Tomentella* (Agerer et al. 1995), where already the very tip is pseudoparenchymatous. The earliest ontogenetical steps in *Piceirhiza nigra* are plectenchymatous (Weiss 1990) but, in contrast to *T. albomarginata*, its organization changes even at the tip to pseudoparenchymatous during mantle differentiation. In contrast to *T. albomarginata*, *Piceirhiza nigra* forms mounds of cells on its mantle surface, and hollow warts are lacking (Gronbach 1988).

Pseudotomentella tristis (Agerer 1994) differs completely from *T. albomarginata* and the *Tomentella*–like mycorrhizae described by Danielson et al. (1984) and Danielson and Pruden (1989) by a plectenchymatous mantle in plan view. In addition, *Pseudotomentella tristis* ectomycorrhizae have blue patches or are completely blue. *Thelephora terrestris* mycorrhizae have typical awl-shaped cystidia (Agerer and Weiss 1989), which originate from a hyphal net.

All these genera belong to the family Thelephoraceae ss. Stalpers and a variety of mantle features seems to have been verified. To date, the lack of chlamydospores is common to all known ectomycorrhizae of this family.

In contrast to the Thelephoraceae ss. Stalpers, ectomycorrhizae of the family Bankeraceae ss. Stalpers (see Introduction) all possess plectenchymatous mantles and characteristically shaped chlamydospores (Agerer 1991a, 1992a,b, 1993). This is also valid for *Bankera fuligineo-alba* (Danielson 1984). At present, it is impossible even to hypothesize whether the two families can be separated with respect to their ectomycorrhizal features, as too few species have been studied.

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